

# Endometriosis: updates on the etiology, pathophysiology, measurements and therapeutics

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# Endometriosis: updates on the etiology, pathophysiology, measurements and therapeutics

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# Endometriosis in transgender men: recognizing the missing pieces

Alexandre Vallée<sup>1\*</sup>, Anis Feki<sup>2</sup> and Jean-Marc Ayoubi<sup>3,4</sup>

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Endometriosis, traditionally associated with cisgender women, should be recognized as a significant issue for transgender men. This perspective highlights the need to address the unique experiences and challenges faced by transgender men with endometriosis. Diagnostic difficulties arise due to hormone therapy and surgical interventions, which can alter symptoms. Limited research in transgender men undergoing hysterectomy further complicates the understanding of endometriosis in this population. Healthcare providers must be aware of these challenges and adapt the diagnostic approaches accordingly. Education and inclusive care are essential to ensure timely and appropriate management of endometriosis in transgender men, ultimately improving their quality of life.

## KEYWORDS

transgender, endometriosis, healthcare, diagnosis, hormones

## Introduction

Endometriosis is a complex and debilitating condition that has long been associated with cisgender women (1). However, as we strive for inclusivity in healthcare, it is crucial to shed light on the experiences of transgender men, who also face the challenges of endometriosis. Few studies have provided the prevalence of endometriosis among transgender men. However, the pooled prevalence of endometriosis could be estimated at 25.14, 95% CI (17.24%–33.94%) and the frequency of patients using testosterone without other medications and presenting dysmenorrhea was 70.58, 95% CI (63.87%–80.91%) (2–4). Furthermore, stage 1 (40%) and 2 (32%) endometriosis were the most reported findings (2). Among transgender men who underwent hysterectomy, 89.5% were on testosterone, 59.7% were amenorrheic, 43.2% had dysmenorrhea, 17.9% reported heavy menses, and 14.9% had irregular menses, 50.7% complained of pelvic pain (35.3% constant, 64.7% cyclic) (4). Moreover, endometriosis was found in 32% of patients who reported pelvic pain at the preoperative consultation and in 22% of patients who did not complain of pain (4, 5). This perspective aims to raise awareness about endometriosis in transgender men, emphasizing the need for research, education, and comprehensive care tailored to their unique circumstances (Figure 1).

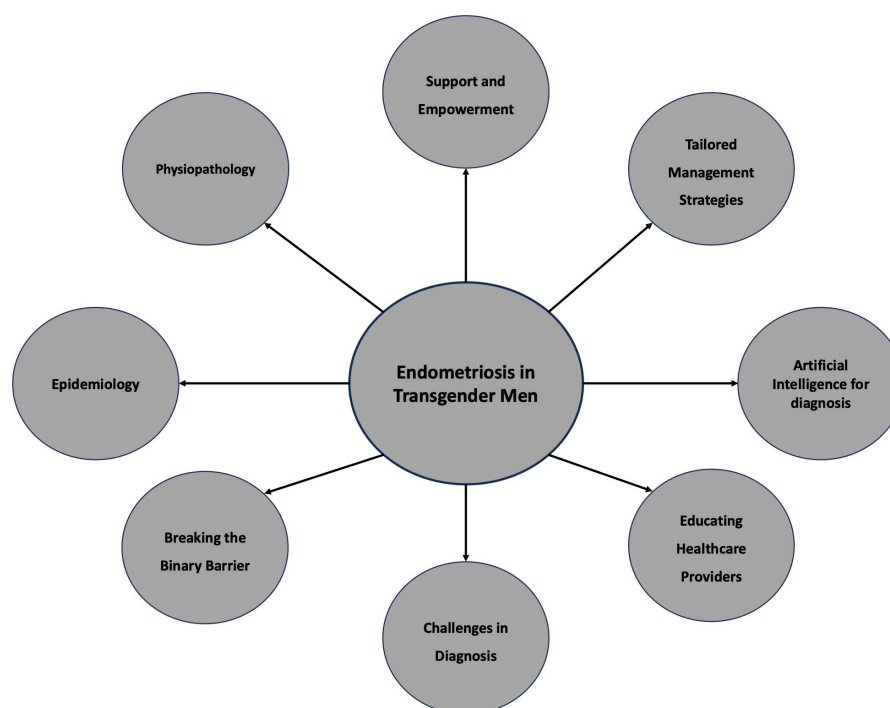


FIGURE 1  
Raise awareness about endometriosis in transgender men.

## Physiopathology of endometriosis among transgender men

Only a limited number of studies have investigated pelvic organ pathology among transgender men who undergo hysterectomy, and the existing reports present conflicting results. For instance, Grimstad et al. (6) examined uterine pathology in transgender men undergoing hysterectomy as part of their gender affirmation process while on testosterone. Interestingly, most of the pathology reports from these individuals displayed active endometrial tissue. In contrast, Khalifa et al. (7) studied a similar group of patients and found that most specimens they assessed exhibited endometrial changes consistent with inactive endometrium. These mixed findings make it difficult to definitively determine the impact of testosterone on the endometrium. However, the presence of active endometrium in some patients suggests that complete cessation of ovarian function and/or endometrial activity might not occur for all individuals on testosterone.

These contrasting results could potentially be linked to the conversion of exogenous testosterone to estradiol in peripheral tissues, a process known as aromatization (1). While there are no studies specifically investigating trends in estradiol levels among transgender individuals on long-term testosterone therapy, it is plausible that elevated androgen levels could be transformed into estrogen in this clinical context, potentially resulting in a state of heightened estrogenic activity (1). As endometriosis is regarded as an estrogen-driven condition, this could contribute to its development. Although this hypothesis does not elucidate the precise mechanism underlying the increased occurrence of endometriosis in transgender individuals compared to their cisgender counterparts, it does explain their

potential symptomatic presentation of the condition, even in cases where menstruation has ceased (1). To date, the risk of endometrial disease in transgender men using testosterone is unclear, and expert opinion recommendations for routine endometrial surveillance (ultrasound or biopsy) or primary surgical prevention (hysterectomy) of endometrial pathology are not evidence based (5, 8). The effect of gender-affirming hormone therapy with testosterone therapy on the endometrium is incompletely characterized, and the etiology of this distribution of endometrial findings is unknown (5). Moreover, the proliferative endometrium in transgender men could be explained by the persistent elevated serum estrogen observed in patients who retain their ovaries while using testosterone (9).

## Breaking the binary barrier

Transgender men assigned female at birth but identifying as male often find themselves navigating a healthcare system that fails to adequately address their specific needs (10). Endometriosis, primarily viewed as a “women’s issue”, is a prime example of this oversight (3). By recognizing and studying endometriosis in transgender men, we can dismantle the binary understanding of this condition and pave the way for more inclusive healthcare practices.

## Challenges in diagnosis

Diagnosing endometriosis in transgender men presents unique challenges. Given their hormone therapy (testosterone) and potential surgical interventions such as hysterectomy and oophorectomy, the

symptoms and manifestations of endometriosis may differ from those experienced by cisgender women (11). Healthcare providers must be vigilant in considering endometriosis as a potential cause of pelvic pain, even in transgender men, and adapt the diagnostic approaches accordingly (4).

Endometriosis is often perceived as a condition exclusive to cisgender women (1). This limited understanding can result in healthcare providers overlooking endometriosis as a potential diagnosis in transgender men. It is essential to raise awareness and to educate healthcare professionals about the possibility of endometriosis in this population.

Testosterone therapy, which is commonly used during gender transition, can influence the symptoms and presentation of endometriosis in transgender men (12). The hormonal changes brought about by testosterone can mask or alter typical symptoms, such as changes in menstrual patterns or pelvic pain (13). Healthcare providers must be knowledgeable about these potential variations to ensure accurate diagnosis.

There is a scarcity of studies examining pelvic organ pathology in transgender men undergoing hysterectomy, and the existing reports are limited and present controversial findings (14). A previous study examined the characteristics of uterine pathology in 94 transgender men receiving testosterone treatment who underwent hysterectomy as part of their gender affirmation process (6). Interestingly, most of the pathology reports indicated the presence of an active endometrium in these patients. In contrast, other studies have investigated similar groups of patients and reported that most of the evaluated specimens showed endometrial changes consistent with an inactive endometrium (7, 15). These mixed findings make it challenging to definitively determine the effects of testosterone on the endometrium. However, considering the report of an active endometrium in some patients, it can be inferred that certain individuals do not experience complete cessation of ovarian function and/or endometrial activity while on testosterone therapy. This implies that transgender men predisposed to endometriosis may still have active disease, even when undergoing testosterone treatment.

Due to the lack of awareness and altered symptoms, there can be delays in diagnosing endometriosis in transgender men. Patients may experience dismissive attitudes or have their symptoms attributed to other causes, leading to a prolonged period of suffering and reduced quality of life. Overcoming diagnostic delays requires a proactive and open-minded approach from healthcare providers (16).

There is a dearth of research specifically focused on endometriosis in transgender men. The absence of comprehensive guidelines and evidence-based practices tailored to this population further hinders accurate diagnosis. More research is needed to understand the prevalence, pathophysiology, and optimal diagnostic approaches for endometriosis in transgender men. Traditional diagnostic modalities, such as imaging like ultrasound or magnetic resonance imaging (MRI), may not provide definitive results to diagnose endometriosis in transgender men (17). These imaging techniques may not effectively capture the presence of endometrial lesions in neovaginas or residual endometrial tissue in transgender men who have undergone hysterectomy (17).

Consequently, a more nuanced approach could be used, such as laparoscopy or specialized imaging techniques [MRI using artificial intelligence (18)] for accurate diagnosis. AI models using biomarkers could be accurate with investigations focused on protein ratios (19), metabolites (20) and miRNAs (21). Moreover, other predictive models could use protein spectra (22) in association with neural networks algorithms (23), and large protein-coding gene datasets from transcriptomics and methylomics data coupled with machine learning models (24, 25). Healthcare providers must approach the diagnostic process with sensitivity and open communication. Creating a safe and inclusive environment enables transgender men to discuss their symptoms openly, allowing for a more accurate assessment (26). Healthcare providers should proactively inquire about the gender transition history, hormonal therapies, and any complications related to gender-affirming surgeries that may contribute to endometriosis-like symptoms.

## Educating healthcare providers

Healthcare providers must receive adequate training and education on transgender healthcare and endometriosis management. Many medical professionals may lack knowledge in both areas, resulting in misdiagnoses, delayed interventions, or the dismissal of symptoms (27). By bridging this educational gap, we can ensure that transgender men receive competent and compassionate care from healthcare providers who understand the nuanced intersection of their gender identity and endometriosis.

## Tailored management strategies

Effective management of endometriosis in transgender men requires tailored approaches (1). Hormone therapy, the cornerstone of gender transition, may have an impact on the growth and symptoms of endometriosis (28). Healthcare providers should be knowledgeable about the potential interactions between testosterone therapy and endometriosis, ensuring that treatment plans strike a balance between gender-affirming care and mitigating endometriosis-related symptoms. The prevalence of endometriosis in transgender men is higher than the female cisgender population (2). Thus, surgeons should perform a careful intraoperative assessment of endometriotic foci within transgender men. But, to date, very few data are available and future prospective studies are needed.

Furthermore, a comprehensive biopsychosocial approach that encompasses various factors contributing to everyone's situation is essential. This approach may involve medical treatments, addressing sexual function, dealing with pain hypersensitivity, and considering psychological aspects like post-traumatic stress disorder (29).

A significant number of transgender individuals seek hysterectomy and/or oophorectomy as part of their gender affirmation process or due to persistent pelvic pain or abnormal bleeding. Among those who underwent hysterectomy, 72% reported experiencing relief from pelvic pain symptoms following the procedure (13). This surgery effectively stops ongoing menstruation, which is particularly prevalent in those experiencing pain after beginning testosterone therapy.

Although further research is required to explore the potential link between elevated pelvic floor muscle tension and pelvic pain in transgender individuals undergoing testosterone therapy for gender affirmation, a recent systematic review of pelvic floor physical therapy aimed at releasing myofascial trigger points demonstrated positive and beneficial outcomes, especially for individuals dealing with chronic pelvic pain and dyspareunia (30). Considering the limited available options to alleviate often incapacitating pelvic pain in transgender individuals undergoing testosterone therapy, pelvic floor physical therapy emerges as a viable and low-risk treatment strategy (29). A program focused on reducing pelvic floor muscle tension, emphasizing improved muscle function quality and the relaxation phase of contractions, holds promise in this clinical setting.

## Support and empowerment

Support networks and advocacy groups play a crucial role in empowering transgender men with endometriosis. By fostering a sense of community, raising awareness, and providing access to resources, these organizations can offer the much-needed support that helps transgender men navigate the challenges of endometriosis. Moreover, incorporating the voices of transgender men in policymaking and healthcare guidelines will ensure that their unique experiences and needs are considered (31).

## Conclusion

Endometriosis does not discriminate based on gender identity, and it is high time we recognized the existence and impact of this condition on transgender men. By promoting research, education, and comprehensive care, we can break down the barriers that hinder effective diagnosis and management of endometriosis in this marginalized population. It is our responsibility as healthcare providers, researchers, and advocates to address this gap in understanding and to provide equitable healthcare for all individuals, irrespective of their gender identity.

## References

- Ferrando CA. Endometriosis in transmasculine individuals. *Reprod Fertil*. (2022) 3:C7–C10. doi: 10.1530/RAF-21-0096
- Okita F, Abrao M, Lara LAS, Andres MP, Cunha C, LGO B. Endometriosis in transgender men—a systematic review with-metanalysis. *J Minim Invasive Gynecol*. (2021) 28:S103–4. doi: 10.1016/j.jmig.2021.09.140
- Shim JY, Laufer MR, Grimstad FW. Dysmenorrhea and endometriosis in transgender adolescents. *J Pediatr Adolesc Gynecol*. (2020) 33:524–8. doi: 10.1016/j.jpog.2020.06.001
- Ferrando CA, Chapman G, Pollard R. Preoperative pain symptoms and the incidence of endometriosis in transgender men undergoing hysterectomy for gender affirmation. *J Minim Invasive Gynecol*. (2021) 28:1579–84. doi: 10.1016/j.jmig.2021.01.018
- Hawkins M, Deutsch MB, Obedin-Maliver J, Stark B, Grubman J, Jacoby A, et al. Endometrial findings among transgender and gender nonbinary people using testosterone at the time of gender-affirming hysterectomy. *Fertil Steril*. (2021) 115:1312–7. doi: 10.1016/j.fertnstert.2020.11.008
- Grimstad FW, Fowler KG, New EP, Ferrando CA, Pollard RR, Chapman G, et al. Uterine pathology in transmasculine persons on testosterone: a retrospective multicenter case series. *Am J Obstet Gynecol*. (2019) 220:257.e1–7. doi: 10.1016/j.ajog.2018.12.021
- Khalifa MA, Toyama A, Klein ME, Santiago V. Histologic features of hysterectomy specimens from female-male transgender individuals. *Int J Gynecol Pathol*. (2019) 38:520–7. doi: 10.1097/PGP.0000000000000548
- Unger CA. Care of the transgender patient: the role of the gynecologist. *Am J Obstet Gynecol*. (2014) 210:16–26. doi: 10.1016/j.ajog.2013.05.035
- Deutsch MB, Bhakri V, Kubicek K. Effects of cross-sex hormone treatment on transgender women and men. *Obstet Gynecol*. (2015) 125:605–10. doi: 10.1097/AOG.0000000000000692
- Gonzales G, Henning-Smith C. Barriers to care among transgender and gender nonconforming adults. *Milbank Q*. (2017) 95:726–48. doi: 10.1111/1468-0009.12297
- Grimstad FW, Boskey E, Grey M. New-onset abdominopelvic pain after initiation of testosterone therapy among trans-masculine persons: a community-based exploratory survey. *LGBT Health*. (2020) 7:248–53. doi: 10.1089/lgbt.2019.0258
- T'Sjoen G, Arcelus J, Gooren L, Klink DT, Tangpricha V. Endocrinology of transgender medicine. *Endocr Rev*. (2019) 40:97–117. doi: 10.1210/er.2018-00011
- Zwickl S, Burchill L, Wong AFQ, Leemaqz SY, Cook T, Angus LM, et al. Pelvic pain in transgender people using testosterone therapy. *LGBT Health*. (2023) 10:179–90. doi: 10.1089/lgbt.2022.0187

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

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14. Carbonnel M, Karpel L, Cordier B, Pirtea P, Ayoubi JM. The uterus in transgender men. *Fertil Steril*. (2021) 116:931–5. doi: 10.1016/j.fertnstert.2021.07.005
15. Cao CD, Amero MA, Marcinkowski KA, Rosenblum NG, Chan JSY, Richard SD. Clinical characteristics and histologic features of hysterectomy specimens from transmasculine individuals. *Obstet Gynecol*. (2021) 138:51–7. doi: 10.1097/AOG.0000000000004421
16. Safer JD, Coleman E, Feldman J, Garofalo R, Hembree W, Radix A, et al. Barriers to health care for transgender individuals. *Curr Opin Endocrinol Diabetes Obes*. (2016) 23:168–71. doi: 10.1097/MED.0000000000000227
17. Asseler JD, Caanen MR, Verhoeven MO, Huirne JAF, Goddijn M, van Dulmen-den BE, et al. Endometrial thickness assessed by transvaginal ultrasound in transmasculine people taking testosterone compared with cisgender women. *Reprod Biomed Online*. (2022) 45:1033–8. doi: 10.1016/j.rbmo.2022.06.012
18. Sivajohan B, Elgendi M, Menon C, Allaire C, Yong P, Bedaiwy MA. Clinical use of artificial intelligence in endometriosis: a scoping review. *NPJ Digit Med*. (2022) 5:109–17. doi: 10.1038/s41746-022-00638-1
19. Knific T, Fishman D, Vogler A, Gstöttner M, Wenzl R, Peterson H, et al. Multiplex analysis of 40 cytokines do not allow separation between endometriosis patients and controls. *Sci Rep*. (2019) 9:16738. doi: 10.1038/s41598-019-52899-8
20. Domínguez F, Ferrando M, Díaz-Gimeno P, Quintana F, Fernández G, Castells I, et al. Lipidomic profiling of endometrial fluid in women with ovarian endometriosis. *Biol Reprod*. (2017) 96:772–9. doi: 10.1093/biolre/iox014
21. Vodolazkaia A, El-Aalamat Y, Popovic D, Mihalyi A, Bossuyt X, Kyama CM, et al. Evaluation of a panel of 28 biomarkers for the non-invasive diagnosis of endometriosis. *Hum Reprod*. (2012) 27:2698–711. doi: 10.1093/humrep/des234
22. Braga DPAF, Montani DA, Setti AS, Turco EGL, Oliveira-Silva D, Borges E. Metabolomic profile as a noninvasive adjunct tool for the diagnosis of grades III and IV endometriosis-related infertility. *Mol Reprod Dev*. (2019) 86:1044–52. doi: 10.1002/mrd.23221
23. Ghazi N, Arjmand M, Akbari Z, Mellati AO, Saheb-Kashaf H, Zamani Z. 1H NMR-based metabolomics approaches as non-invasive tools for diagnosis of endometriosis. *Int J Reprod Biomed*. (2016) 14:1–8. doi: 10.29252/ijrm.14.1.1
24. Akter S, Xu D, Nagel SC, Bromfield JJ, Pelch KE, Wilshire GB, et al. GenomeForest: an ensemble machine learning classifier for endometriosis. *AMIA Jt Summits Transl Sci Proc*. (2020) 2020:33–42.
25. Akter S, Xu D, Nagel SC, Bromfield JJ, Pelch K, Wilshire GB, et al. Machine learning classifiers for endometriosis using transcriptomics and methylomics data. *Front Genet*. (2019) 10:766. doi: 10.3389/fgene.2019.00766
26. Cooper KM, Auerbach AJ, Bader JD, Beadles-Bohling AS, Brashears JA, Cline E, et al. Fourteen recommendations to create a more inclusive environment for LGBTQ+ individuals in academic biology. *CBE Life Sci Educ*. (2020) 19:es6. doi: 10.1187/cbe.20-04-0062
27. van Heesewijk J, Kent A, van de Grift TC, Harleman A, Muntinga M. Transgender health content in medical education: a theory-guided systematic review of current training practices and implementation barriers & facilitators. *Adv Health Sci Educ*. (2022) 27:817–46. doi: 10.1007/s10459-022-10112-y
28. Glinborg D, T'Sjoen G, Ravn P, Andersen MS. Management of endocrine disease: optimal feminizing hormone treatment in transgender people. *Eur J Endocrinol*. (2021) 185:R49–63. doi: 10.1530/EJE-21-0059
29. Grinberg K, Sela Y, Nissanholtz-Gannot R. New insights about chronic pelvic pain syndrome (CPPS). *Int J Environ Res Public Health*. (2020) 17:3005. doi: 10.3390/ijerph17093005
30. van Reijn-Baggen DA, Han-Geurts IJM, Voorham-van der Zalm PJ, Pelger RCM, Hagenaars-van Miert CHAC, Laan ETM. Pelvic floor physical therapy for pelvic floor hypertonicity: a systematic review of treatment efficacy. *Sex Med Rev*. (2022) 10:209–30. doi: 10.1016/j.sxmr.2021.03.002
31. Reisner SL, Poteat T, Keatley J, Cabral M, Mothopeng T, Dunham E, et al. Global health burden and needs of transgender populations: a review. *Lancet*. (2016) 388:412–36. doi: 10.1016/S0140-6736(16)00684-X





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# Application of the #Enzian classification for endometriosis on MRI: prospective evaluation of inter- and intraobserver agreement

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**Objectives:** The purpose of this investigation was to evaluate the inter- and intraobserver variability of the updated #Enzian classification of endometriosis on MRI and to evaluate the influence of reader experience on interobserver concordance.

**Methods:** This was a prospective single-center study. All patients were included who received an MRI of the pelvis for evaluation of endometriosis between March and July 2023 and who have provided written informed consent. Images were reviewed independently for endometriosis by three radiologists, utilizing the MRI-applicable categories of the #Enzian classification. Two radiologists had experience in pelvic MRI and endometriosis imaging. One radiologist had no specific experience in pelvic MRI and received a one-hour briefing beforehand.

**Results:** Fifty consecutive patients (mean age, 34.9 years  $\pm$  8.6 [standard deviation]) were prospectively evaluated. Interobserver agreement was excellent for diagnosis of deep infiltrating endometriosis (Fleiss' kappa: 0.89; 95% CI 0.73–1.00;  $p < 0.001$ ) and endometriomas (Fleiss' kappa: 0.93; 95% CI 0.77–1.00;  $p < 0.001$ ). For the experienced readers, interobserver agreement in the assessment of compartments A, B and C was excellent ( $\kappa_w$  ranging from 0.84; 95% CI 0.71–0.97;  $p < 0.001$  to 0.89; 95% CI 0.82–0.97;  $p < 0.001$ ). For the pairings of the experienced readers to the reader without specific experience in pelvic MRI, agreement was substantial to excellent ( $\kappa_w$  ranging from 0.64; 95% CI 0.44–0.85;  $p < 0.001$  to 0.91; 95% CI 0.84–0.98;  $p < 0.001$ ). Intraobserver variability was excellent for compartments A, B and C ( $\kappa_w$  ranging from 0.85; 95% CI 0.73–0.96;  $p < 0.001$  to 0.95; 95% CI 0.89–1.00;  $p < 0.001$ ).

**Conclusion:** With sufficient experience, the #Enzian classification enables the achievement of excellent inter- and intraobserver agreement in MRI-based diagnosis of deep infiltrating endometriosis and endometriomas.

## KEYWORDS

pelvis, deep infiltrating endometriosis, endometrioma, magnetic resonance imaging, interobserver variability

## 1 Introduction

MRI is widely used and recommended in the diagnosis of deep infiltrating endometriosis (DIE) (1–3). Multiple attempts have been made to describe the extent of endometriosis, but to date no universally accepted classification system exists (4–6). In 2021, the #Enzian classification has been published to provide a comprehensive resource for the description and staging of endometriosis (7). The classification has been created to overcome limitations of the Enzian classification (established in 2003 and revised in 2011) (8) and the revised American Society for Reproductive Medicine classification of endometriosis (rASRM) and allows a complete description of superficial and deep infiltrating endometriosis, ovarian endometriosis, and uterine adenomyosis (9). Application of the #Enzian classification is intended for both surgical and diagnostic specialties and aims to enable communication and documentation of findings of surgery, ultrasound, and MRI clearly and objectively.

Reports on the applicability of the upgraded #Enzian classification for MRI examinations are promising (10, 11), but data on inter- and intraobserver variability are scarce. In one retrospective study, Manganaro et al. have reported overall good interobserver agreement (Cohen's kappa 0.73) of the #Enzian classification when applied to MRI (12). However, further studies are warranted as existing data are limited. A prospective evaluation of the interobserver variability of the updated #Enzian classification on MRI has not been reported yet. Furthermore, a detailed analysis of all MRI-applicable categories of the classification is pending, including evaluations of the assignment of lesions to the left and right body side (categories B, O) and evaluations of ordinal scaled data. Additionally, the evaluation of the influence of reader experience on interobserver agreement is of interest. Saba et al. found a significant increase in the accuracy of endometriosis diagnosis on MRI with experience (13), but studies on the #Enzian classification in this regard are not yet available.

The purpose of this investigation was therefore to evaluate the inter- and intraobserver variability of the MRI-applicable categories of the updated #Enzian classification and to evaluate the influence of reader experience on interobserver concordance.

## 2 Materials and methods

Ethical approval for this prospective, non-interventional study was obtained from the local institutional review board (IRB) and written informed consent from all participants was received (German Clinical Trials Register ID DRKS00031403).

### 2.1 Patients

We prospectively included 50 consecutive patients aged 18 years or older who were scheduled to undergo a pelvic MRI scan for suspected endometriosis at our tertiary care center from March 2023 to July 2023. The indications for the MRI examinations were established after clinical gynecological examination and transvaginal ultrasound. Exclusion criteria were pregnancy and inability or unwillingness to consent. MRI scans were conducted at two 1.5 Tesla scanners (Avanto, Siemens Healthcare,  $n = 40$ ; Espree, Siemens Healthcare,  $n = 10$ ). No adverse events were

encountered in the course of the MRI examinations. All patients have provided written informed consent.

### 2.2 MRI protocol for endometriosis

Patients were examined with an MRI protocol that is used in clinical practice and includes commonly recommended sequences for the evaluation of endometriosis (14, 15): Axial, sagittal, and coronal T2-weighted FSE (fast spin echo), axial T1-weighted FSE and axial fat-suppressed T1-weighted FSE.

According to current guidelines, MRI examinations were scheduled independently of the menstrual cycle (14). The preparation of the patients included rectal contrast with water and vaginal contrast with ultrasound gel when consent was given (48/50 and 44/50, respectively) (14, 16). An anti-peristaltic agent was administered in most patients (intravenous hyoscine butylbromide 20 mg, Carinopharm GmbH, 48/50). To achieve moderate filling and good assessability of the urinary bladder, care was taken to ensure that patients did void their bladder approximately 1 h before the examination and did not void their bladder afterwards until the completion of the MRI examination.

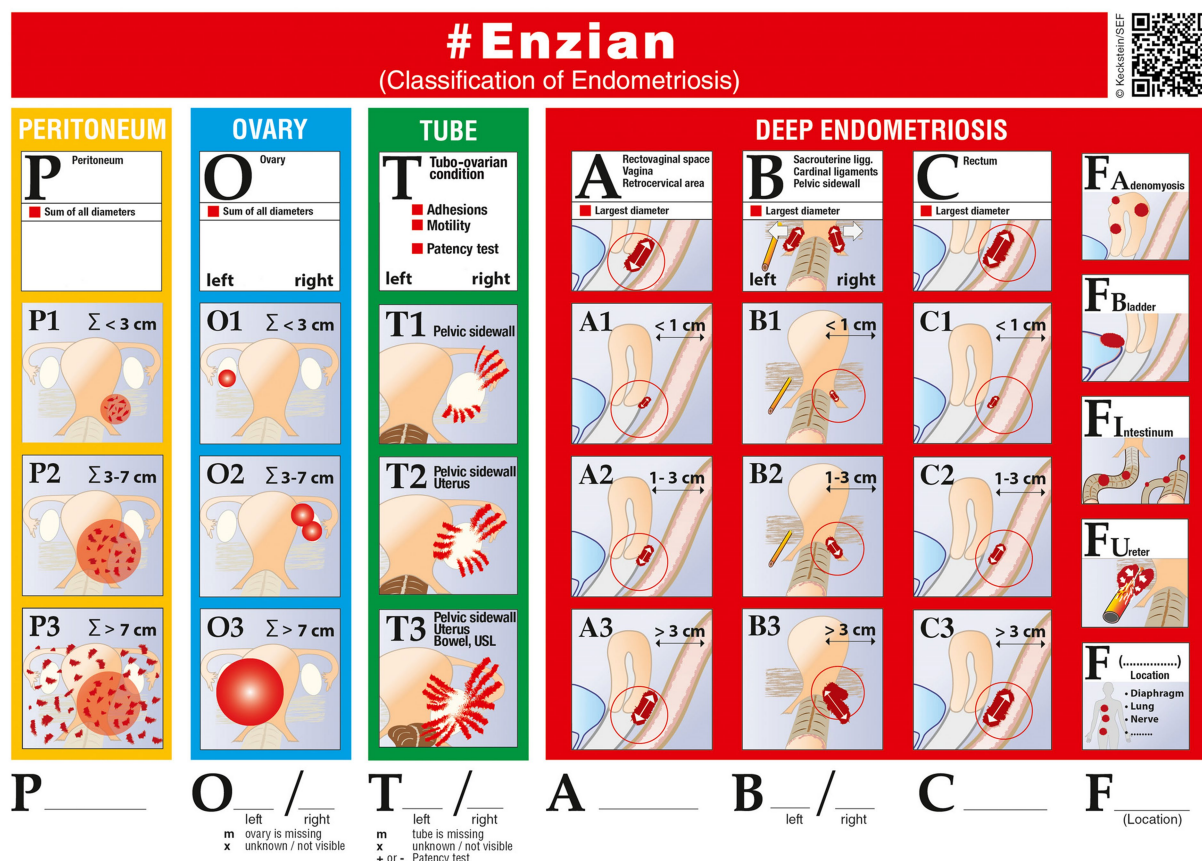
Intravenous administration of gadolinium based contrast agents (GBCAs) was performed optionally, depending on additional questions and the findings of the non-contrast images (11). For 40/50 (80.0%) patients, it was decided that contrast administration was not necessary. In 10/50 (20.0%) patients, GBCAs were administered (Gadoteridol, ProHance, 0.1 mmol/kg, Bracco Imaging s.p.a.) for the following reasons: indeterminate ovarian lesion (5/50), suspicion of pelvic venous congestion syndrome (3/50), indeterminate uterine mass (2/50).

### 2.3 MRI image analysis

All images were reviewed independently by three senior radiologists from two different medical centers on Picture Archiving and Communication System (PACS) workstations. Two radiologists (S.H., F.C.R.) had experience in pelvic MRI and endometriosis imaging (7 and 5 years, respectively). The third radiologist (H.E.K.) was a musculoskeletal radiologist without specific experience in pelvic MRI. The latter reader received a one-hour briefing by the radiologist with 7 years' experience with the following content: demonstration of the #Enzian classification based on the publications by Keckstein et al. (7) and Harth et al. (3); discussion of four exemplary cases that were not drawn from the collective of the present study (Case 1: #Enzian(m) A2, B2/2, C3, FA(external), FU(I); Case 2: #Enzian(m) A1, B2/2, C1, O1/0; Case 3: #Enzian(m) A1, B2/3, FA(external), FI(Sigma); Case 4: No endometriosis); discussion of different forms (internal, external) and diagnostic criteria of adenomyosis uteri (17); discussion of uterine contractions as mimickers of adenomyosis (18). Figures from the 2021 publication by Keckstein et al. and the 2023 publication by Harth et al. were made available to guide all readers (3, 7). Images of cases used for training were not included in later image analysis.

The radiologists evaluated each MRI for evidence of endometriosis independently. For this purpose, the categories of the #Enzian classification applicable in MRI were taken into





Three to seven months after completion of the first assessment, all 50 cases were assessed again by one of the experienced readers (S.H.) in a blinded evaluation without access to MRI reports, clinical data, or the results of the initial evaluation.

## 2.4 Statistical analysis

Statistical analyses were performed utilizing IBM SPSS Statistics 29.0.

Sample size estimation was performed to detect statistically significant Cohen's kappa coefficients ( $\kappa$ ) ( $p \leq 0.05$ ) on dichotomous and dichotomized variables, following the recommendations by Sim and Wright (19): With 80% power, expecting a proportion of positive ratings in the range of 35–45% (3, 11), expecting a minimum value for Cohen's kappa coefficient of 0.60, and assuming the null-hypothesis kappa to be 0.00, a minimum sample size of 22 was determined for a two-tailed-test.

Cohen's kappa coefficients ( $\kappa$ ) were computed for dichotomous variables (DIE all locations, FA, FB, FI, F(...), FU) and dichotomized variables (O both sides, O left side, O right side, A, B both sides, B left side, B right side, C) to assess agreement for pairs of two raters (reader 1 and 2, reader 1 and 3, reader 2 and 3, reader 1 and 1). For ordinal scaled variables (O0-3 left side, O0-3 right side, A0-3, B0-3 left side, B0-3 right side, C0-3), quadratic weighted kappa coefficients ( $\kappa_w$ ) were computed.

To assess agreement of all three raters, Fleiss' kappa was calculated for dichotomous variables (DIE all locations, FA, FB, FI, F(...), FU) and dichotomized variables (O both sides, O left side, O right side, A, B both sides, B left side, B right side, C), and Kendall's W was calculated for ordinal scaled variables (O0-3 left side, O0-3 right side, A0-3, B0-3 left side, B0-3 right side, C0-3).

Reader agreement was assessed using the following range definitions of kappa values: 0.81 and 1.00, excellent ('almost perfect'); 0.61–0.80, substantial; 0.41–0.60, moderate; 0.21–0.40, fair; 0.00–0.20 slight (20).

### 3 Results

Fifty consecutive patients (mean age, 34.9 years  $\pm$  8.6 [standard deviation]) were prospectively evaluated for endometriosis by three readers on MRI, utilizing the #Enzian classification. Table 1 summarizes the characteristics of the study population.

#### 3.1 MRI image analysis

The percentages of positive #Enzian categorizations assigned in this study among all readers were 24.0% (O), 36.0% (A), 40.0% (B),

33.3% (C), 18.0% (FA), 2.7% (FB), 8.0% (FI), 2.7% (FU) and 6.7% (F(...)).

The agreement between pairs of two readers each are listed in [Supplementary Tables S1–S36](#). An exemplary case of a patient with typical DIE on MRI is shown in [Figure 2](#). Calculations of Cohen's kappa coefficients ( $\kappa$ ) and quadratic weighted kappa coefficients ( $\kappa_w$ ) for pairs of two raters each are presented in [Table 2](#). For the two readers with experience in pelvic MRI (reader 1 and 2), agreement in the assessment of #Enzian categories A, B and C varied from  $\kappa=0.87$  (95% CI 0.72–1.00) to  $\kappa=0.96$  (95% CI 0.87–1.00) (dichotomized data) and from  $\kappa_w=0.84$  (95% CI 0.71–0.97) to  $\kappa_w=0.89$  (95% CI 0.82–0.97) (ordinal data). For the pairings of the readers with experience in pelvic MRI to the reader without specific experience in pelvic MRI (reader 1 and 3, reader 2 and 3), agreement in the assessment of #Enzian categories A, B, and C varied from  $\kappa=0.62$  (95% CI 0.39–0.84) to  $\kappa=0.96$  (95% CI 0.87–1.00) (dichotomized data) and from  $\kappa_w=0.64$  (95% CI 0.44–0.85) to  $\kappa_w=0.91$  (95% CI 0.84–0.98) (ordinal data).

Calculations of Fleiss' kappa and Kendall's W for the ratings of all three readers are shown in [Table 3](#). For #Enzian categories A, B, and C, Fleiss' kappa varied from 0.72 (95% CI 0.56–0.88) to 0.94 (95% CI 0.78–1.00) (dichotomized data) and Kendall's W from 0.84 to 0.96 (ordinal data).

Findings for category F(...) were concordantly noted by three readers in one case, where DIE was located in the anterior abdominal wall (intramuscular). Two of three readers reported DIE in single cases in the sciatic nerve, inguinal canal, and sacral plexus, respectively. In one case, only one of the three readers diagnosed DIE affecting the anterior abdominal wall (subcutaneous).

Calculations of Cohen's kappa coefficients ( $\kappa$ ) and quadratic weighted kappa coefficients ( $\kappa_w$ ) for the two assessments of reader 1 are presented in [Table 4](#).

### 4 Discussion

In our study, we prospectively evaluated inter- and intraobserver agreement of the MRI-applicable categories of the 2021 #Enzian-classification for endometriosis through a total of 50 MRI cases assessed by three readers from two different institutions. Our study demonstrated overall excellent interobserver agreement of the assessments of three independent readers for the diagnosis of deep infiltrating endometriosis on MRI with a Fleiss' kappa of 0.89 (95% CI 0.73–1.00), and for the diagnosis of endometriomas on MRI with a Fleiss' kappa of 0.93 (95% CI 0.77–1.00). Only moderate interobserver agreement was found in the evaluation of uterine adenomyosis, with a Fleiss' kappa of 0.46 (95% CI 0.30–0.62). Intraobserver agreement was excellent for all evaluated categories of the #Enzian classification. Our study indicated that radiologists without specific experience in pelvic MRI can achieve substantial to excellent agreement with experienced radiologists in the application of the #Enzian classification on MRI after only a short training and with guidance from explanatory illustrations.

To our knowledge, this is the first study to prospectively evaluate interobserver agreement of the MRI-based application of the 2021 #Enzian classification, in which endometriomas (O0-3, separately for the left and right body side) and separate category B values (B0-3) for the left and right body side were included. In addition, and in

TABLE 1 Patient demographics and clinical characteristics.

| Characteristic                    | N/total (%) unless shown otherwise |
|-----------------------------------|------------------------------------|
| Age (years), mean $\pm$ SD, range | 34.9 $\pm$ 8.6, 18–55              |
| BMI (kg/m <sup>2</sup> ) $\pm$ SD | 24.7 $\pm$ 4.8                     |
| Prior abdominal surgery           | 31/50 (62.0)                       |
| Laparoscopy for endometriosis     | 21/50 (42.0)                       |
| Cesarean section                  | 9/50 (18.0)                        |
| Appendectomy                      | 6/50 (12.0)                        |
| Laparoscopy for ovarian mass      | 4/50 (8.0)                         |
| Laparoscopy for adhesions         | 4/50 (8.0)                         |
| Total laparoscopic hysterectomy   | 4/50 (8.0)                         |
| Inguinal hernia repair            | 2/50 (4.0)                         |
| Rectum resection with anastomosis | 2/50 (4.0)                         |
| Psoas hitch                       | 2/50 (4.0)                         |
| Laparoscopic myomectomy           | 2/50 (4.0)                         |
| Neurostimulator implantation      | 2/50 (4.0)                         |
| Other surgical procedures         | 7/50 (14.0)                        |
| Prior vaginal delivery            | 4/50 (8.0)                         |
| Clinical symptoms                 |                                    |
| Chronic pelvic pain               | 47/50 (94.0)                       |
| Dysmenorrhea                      | 27/50 (54.0)                       |
| Dyspareunia                       | 15/50 (30.0)                       |
| Dyschezia                         | 14/50 (28.0)                       |
| Abnormal uterine bleeding         | 10/50 (20.0)                       |
| Dysuria                           | 6/50 (12.0)                        |
| Obstipation                       | 6/50 (12.0)                        |
| Infertility                       | 5/50 (10.0)                        |
| Leg pain                          | 5/50 (10.0)                        |
| Lower back pain                   | 4/50 (8.0)                         |
| Rectal bleeding                   | 3/50 (6.0)                         |
| Diarrhea                          | 2/50 (4.0)                         |
| Fatigue                           | 2/50 (4.0)                         |
| Foot drop                         | 2/50 (4.0)                         |
| Leg paresthesia                   | 2/50 (4.0)                         |
| Abdominal muscle fasciculations   | 1/50 (2.0)                         |

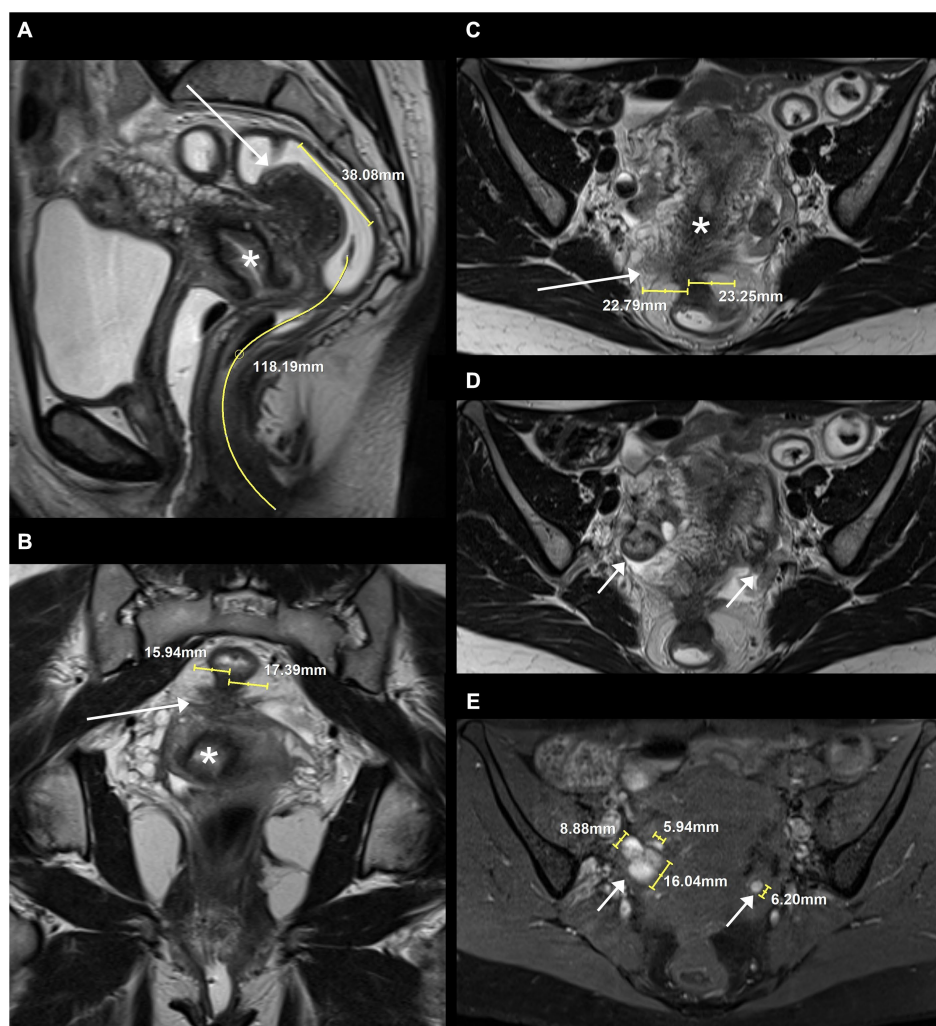


FIGURE 2

Example of typical deep infiltrating endometriosis (DIE), focal adenomyosis of the outer myometrium and endometriomas on MRI, categorized by two readers as #Enzian(m) O1/2, A2, B2/2, C3, FA and by one reader as #Enzian(m) O1/1, A2, B2/2, C3, FA (due to a borderline summed size of endometriomas on the right side between O1 and O2): (A) Sagittal, (B) coronal and (C) axial T2 FSE (fast spin echo) showing a hypointense mass containing hyperintense foci (long arrows) with extension to rectum, vaginal vault, parametria, and posterior outer myometrium. (D) Axial T2 FSE and (E) axial fat suppressed T1 FSE demonstrating characteristic bilateral endometriomas with T1w-hyperintensity and T2w-hypointensity (short arrows). Asterisks: cervix uteri.

contrast to previous studies, we performed analyses of the non-dichotomized, ordinal scaled data as specified in the classification. The only other study to date on interobserver variability of the updated #Enzian classification is the 2021 study by Manganaro et al. In their retrospective analysis of 60 cases, excellent interobserver agreement was stated for the diagnosis of endometriomas ( $\kappa$ : 0.8153) and good agreement for the assessments of compartments/categories A ( $\kappa$ : 0.7645), B ( $\kappa$ : 0.74023), C ( $\kappa$ : 0.7932) and F (extragenital deep infiltrating endometriosis,  $\kappa$ : 0.6349) (12). However, results of a separate evaluation of endometriomas and compartment B by body side and individual results for categories FA, FB, FI, FU and F(...) were not reported. In addition, no weighted kappa values were reported for the ordinal scaled data in categories A, B, C, and O. However, this detailed information is of importance because a difference of one grade (e.g., B3 versus B2) is less significant in practice than a difference of several grades (e.g., B3 versus B0). This is taken into account in our study

with the analysis of quadratically weighted kappa values. Finally, as mentioned above, it is also important in practice whether the intended separate description of category B and O findings by body side can be correctly performed on MRI images using the #Enzian classification. Our results suggest that the side-separated description of findings is useful and feasible, but also confirm the observation of other authors that assessment in category B can be challenging on MRI (10). However, it is inherent in the design of the classification that it is not a matter of an exact size measurement, but rather of a category assignment (1: < 1 cm, 2: 1–3 cm, 3: > 3 cm; see exemplary Figures 2B, C).

Several studies retrospectively evaluated interobserver agreement of the 2011 Enzian classification for the MRI-based diagnosis of DIE, obtaining varying results. Thomassin-Naggara et al. reported excellent agreement for category C ( $\kappa$  0.88, 95% CI 0.82–0.94), good agreement for category A ( $\kappa$  0.79, 95% CI 0.67–0.9) and poor agreement for category B ( $\kappa$  0.41, 95% CI 0.26–0.56) ( $n$  = 150) (21). Thus, greater

**TABLE 2 Agreement of two raters each for the assignment of the #Enzian classification on MRI.**

|                                          | Reader 1 and 2, 95% CI | Reader 1 and 3, 95% CI | Reader 2 and 3, 95% CI |
|------------------------------------------|------------------------|------------------------|------------------------|
| DIE, all locations <sup>a</sup>          | 0.92 (0.81–1.00)*      | 0.88 (0.75–1.00)*      | 0.88 (0.75–1.00)*      |
| O, dichotomized, both sides <sup>a</sup> | 0.95 (0.84–1.00)*      | 0.94 (0.83–1.00)*      | 0.89 (0.74–1.00)*      |
| O, dichotomized, left side <sup>a</sup>  | 1.00 (1.00–1.00)*      | 0.86 (0.68–1.00)*      | 0.86 (0.68–1.00)*      |
| O, dichotomized, right side <sup>a</sup> | 0.85 (0.65–1.00)*      | 1.00 (1.00–1.00)*      | 0.85 (0.65–1.00)*      |
| A, dichotomized <sup>a</sup>             | 0.96 (0.87–1.00)*      | 0.91 (0.80–1.00)*      | 0.96 (0.87–1.00)*      |
| B, dichotomized, both sides <sup>a</sup> | 0.92 (0.80–1.00)*      | 0.75 (0.56–0.94)*      | 0.75 (0.56–0.94)*      |
| B, dichotomized, left side <sup>a</sup>  | 0.87 (0.74–1.00)*      | 0.65 (0.44–0.87)*      | 0.62 (0.39–0.84)*      |
| B, dichotomized, right side <sup>a</sup> | 0.87 (0.72–1.00)*      | 0.69 (0.48–0.90)*      | 0.74 (0.55–0.93)*      |
| C, dichotomized <sup>a</sup>             | 0.87 (0.73–1.00)*      | 0.73 (0.53–0.93)*      | 0.77 (0.58–0.96)*      |
| FA <sup>a</sup>                          | 0.61 (0.36–0.86)*      | 0.39 (0.05–0.74)**     | 0.37 (0.09–0.64)*      |
| FB <sup>a</sup>                          | 0.66 (0.03–1.00)*      | 1.00 (1.00–1.00)*      | 0.66 (0.03–1.00)*      |
| FU <sup>a</sup>                          | 1.00 (1.00–1.00)*      | 0.00 (0.00–0.00)       | 0.00 (0.00–0.00)       |
| FI <sup>a</sup>                          | 1.00 (1.00–1.00)*      | 0.55 (0.10–0.99)*      | 0.55 (0.10–0.99)*      |
| F(...) <sup>a</sup>                      | 0.88 (0.64–1.00)*      | 0.31 (−0.16–0.78)**    | 0.38 (−0.15–0.91)*     |
| O, 0–3, left side <sup>b</sup>           | 1.00 (1.00–1.00)*      | 0.91 (0.82–0.99)*      | 0.91 (0.82–0.99)*      |
| O, 0–3, right side <sup>b</sup>          | 0.96 (0.89–1.00)*      | 0.96 (0.91–1.00)*      | 0.92 (0.84–1.00)*      |
| A, 0–3 <sup>b</sup>                      | 0.84 (0.71–0.97)*      | 0.89 (0.80–0.97)*      | 0.91 (0.84–0.98)*      |
| B, 0–3, left side <sup>b</sup>           | 0.88 (0.79–0.97)*      | 0.66 (0.47–0.84)*      | 0.64 (0.44–0.85)*      |
| B, 0–3, right side <sup>b</sup>          | 0.89 (0.80–0.98)*      | 0.67 (0.49–0.85)*      | 0.70 (0.53–0.87)*      |
| C, 0–3 <sup>b</sup>                      | 0.89 (0.82–0.97)*      | 0.83 (0.70–0.97)*      | 0.89 (0.78–1.00)*      |

DIE, deep infiltrating endometriosis. <sup>a</sup>Cohen's kappa. <sup>b</sup>Weighted kappa, quadratic weights. \* $p < 0.001$ . \*\* $p = 0.002$ .

Readers 1 and 2 had 5–7 years' experience in endometriosis MRI, reader 3 had no experience and received a 1 h training.

**TABLE 3 Agreement of three raters for the assignment of the #Enzian classification on MRI.**

|                                          | Reader 1, 2 and 3, 95% CI |
|------------------------------------------|---------------------------|
| DIE, all locations <sup>a</sup>          | 0.89 (0.73–1.00)*         |
| O, dichotomized, both sides <sup>a</sup> | 0.93 (0.77–1.00)*         |
| O, dichotomized, left side <sup>a</sup>  | 0.91 (0.75–1.00)*         |
| O, dichotomized, right side <sup>a</sup> | 0.90 (0.74–1.00)*         |
| A, dichotomized <sup>a</sup>             | 0.94 (0.78–1.00)*         |
| B, dichotomized, both sides <sup>a</sup> | 0.81 (0.65–0.97)*         |
| B, dichotomized, left side <sup>a</sup>  | 0.72 (0.56–0.88)*         |
| B, dichotomized, right side <sup>a</sup> | 0.77 (0.61–0.93)*         |
| C, dichotomized <sup>a</sup>             | 0.79 (0.63–0.95)*         |
| FA <sup>a</sup>                          | 0.46 (0.30–0.62)*         |
| FB <sup>a</sup>                          | 0.74 (0.58–0.90)*         |
| FU <sup>a</sup>                          | 0.49 (0.33–0.65)*         |
| FI <sup>a</sup>                          | 0.73 (0.57–0.89)*         |
| F(...) <sup>a</sup>                      | 0.57 (0.41–0.73)*         |
| O, 0–3, left side <sup>b</sup>           | 0.95*                     |
| O, 0–3, right side <sup>b</sup>          | 0.95*                     |
| A, 0–3 <sup>b</sup>                      | 0.96*                     |
| B, 0–3, left side <sup>b</sup>           | 0.84*                     |
| B, 0–3, right side <sup>b</sup>          | 0.86*                     |
| C, 0–3 <sup>b</sup>                      | 0.90*                     |

DIE, deep infiltrating endometriosis. <sup>a</sup>Fleiss' kappa. <sup>b</sup>Kendall W. \* $p < 0.001$ .

**TABLE 4 Intraobserver agreement for the assignment of the #Enzian classification on MRI.**

|                                          | Reader 1, 95% CI  |
|------------------------------------------|-------------------|
| DIE, all locations <sup>a</sup>          | 0.96 (0.88–1.00)* |
| O, dichotomized, both sides <sup>a</sup> | 1.00 (1.00–1.00)* |
| O, dichotomized, left side <sup>a</sup>  | 1.00 (1.00–1.00)* |
| O, dichotomized, right side <sup>a</sup> | 0.92 (0.77–1.00)* |
| A, dichotomized <sup>a</sup>             | 0.86 (0.72–1.00)* |
| B, dichotomized, both sides <sup>a</sup> | 0.96 (0.88–1.00)* |
| B, dichotomized, left side <sup>a</sup>  | 0.87 (0.73–1.00)* |
| B, dichotomized, right side <sup>a</sup> | 0.91 (0.79–1.00)* |
| C, dichotomized <sup>a</sup>             | 0.96 (0.87–1.00)* |
| FA <sup>a</sup>                          | 0.82 (0.63–1.00)* |
| FB <sup>a</sup>                          | 1.00 (1.00–1.00)* |
| FU <sup>a</sup>                          | 1.00 (1.00–1.00)* |
| FI <sup>a</sup>                          | 0.88 (0.64–1.00)* |
| F(...) <sup>a</sup>                      | 0.88 (0.64–1.00)* |
| O, 0–3, left side <sup>b</sup>           | 1.00 (1.00–1.00)* |
| O, 0–3, right side <sup>b</sup>          | 0.95 (0.90–1.00)* |
| A, 0–3 <sup>b</sup>                      | 0.89 (0.80–0.97)* |
| B, 0–3, left side <sup>b</sup>           | 0.85 (0.73–0.96)* |
| B, 0–3, right side <sup>b</sup>          | 0.90 (0.84–0.97)* |
| C, 0–3 <sup>b</sup>                      | 0.95 (0.89–1.00)* |

DIE, deep infiltrating endometriosis. <sup>a</sup>Cohen's kappa. <sup>b</sup>Weighted kappa, quadratic weights. \* $p < 0.001$ .



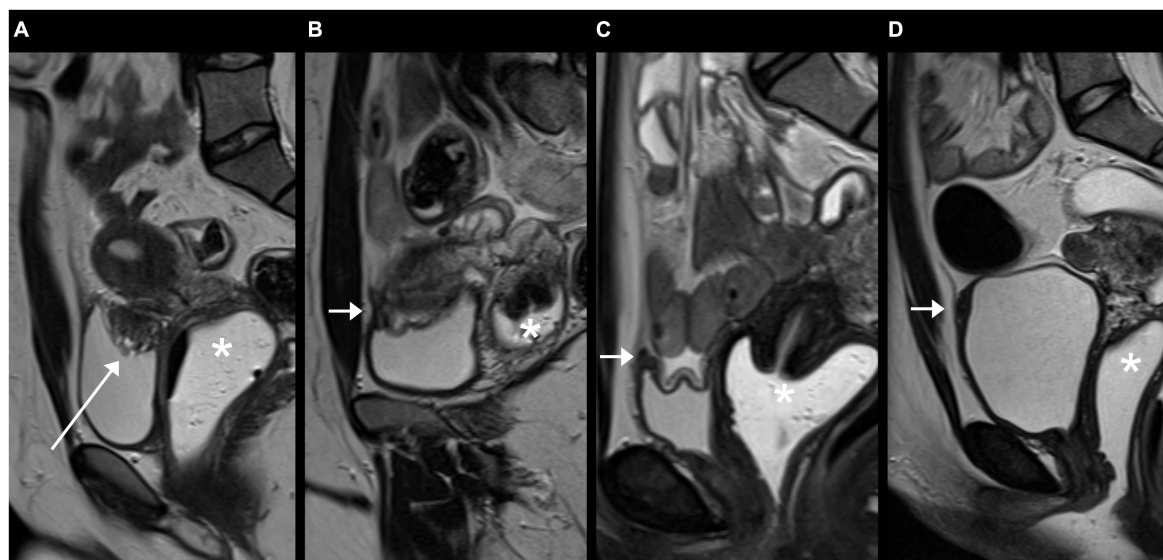


FIGURE 3

Sagittal T2 FSE (fast spin echo) slices demonstrating bladder findings of different patients on MRI: (A) Typical deep infiltrating endometriosis (DIE) of the bladder dome (hypointense mass with hyperintense foci, long arrow), rated #Enzian FB by all three readers, (B) focal thickening of the anterior bladder wall (short arrow), scored #Enzian FB by one of three readers due to off-midline location and central T2w-hyperintensity (no endometriosis on laparoscopy), (C) and (D) focal thickening of the anterior bladder wall (short arrows), interpreted as prominent urachal remnant by all three readers due to midline location on the serosal surface and the presence of a thin band, extending from the bladder dome toward the umbilicus. Asterisks: vaginal vault.

difficulties were noted by the authors in the assessment of Enzian category B on MRI compared to categories A and C. In contrast to Thomassin-Naggara et al. we found excellent interobserver variability for the experienced readers as well as excellent intraobserver variability for category B.

In a previous study (3), we also found excellent agreement for category C ( $\kappa_w$  0.89, 95% CI: 0.75–1.00), but moderate agreement for category A ( $\kappa_w$  0.57, 95% CI: 0.13–1.00) and category B ( $\kappa_w$  0.44, 95% CI: 0.11–0.76) ( $n=20$ ), although the smaller number of cases and also the adjustment effect in the application of the classification by two of the readers from the previous to the present study must be taken into account. No more than fair agreement of three radiologists was found by Burla et al. in their 2021 study ( $\kappa$  0.255 for category A, 0.146 for category B,  $-0.263$  for category C) ( $n=23$ ) (22). Previous studies also concluded that agreement in the detection of DIE at the uterosacral ligaments between different readers is not optimal (23, 24), an observation that we cannot currently confirm when considering the agreement of the two experienced readers.

Various groups have provided definitions of the appearance of endometriosis on MRI (25–27) and recently, a structured report template based on the #Enzian classification has been provided by Maciel et al. (10). Figure 3 demonstrates on the example of the urinary bladder how certain discrepancies in the agreement of several readers can occur on the verge of normal and pathological (28). Similar diagnostic challenges have led to the only moderate agreement in the diagnosis of adenomyosis (#Enzian FA) in our study, which can be mimicked by uterine contractions and for which diagnostic criteria on MRI are not without controversy (17, 29).

Further efforts to educate radiologists in endometriosis diagnostics are desirable to improve reliability of readings, as

several studies underscore: Saba et al. found that the accuracy of MRI diagnosis of endometriosis increased with radiologist experience when the same cases were reanalyzed after 12 and 24 months by the same reader (13). Jaramillo-Cardoso stated in their 2019 study that a structured expert-read outperformed routine reads and structured reported reads of pelvic MRIs for endometriosis, considering sensitivity and specificity and using surgical staging as reference (30). A 1 h training session and the provision of explanatory illustrations enabled a previously inexperienced radiologist to achieve remarkable agreement to experienced radiologists in our study.

Despite the explained strengths of this study, the conduction in a single tertiary care center might be a limitation of our study, whereby radiologists from two different institutions performed the analysis. When viewed in conjunction with our previous and other studies, the study population is typical of an endometriosis center, with relatively high rates of patients who had prior surgeries and patients presenting with infertility. Further studies on the reliability and validity of the #Enzian classification are desirable. The comparison of MRI assessments using the #Enzian classification with results of surgical procedures was not the subject of this study but should also be prospectively investigated in further studies to expand on the literature in this regard (31), considering a separate analysis by body side.

In conclusion, the #Enzian classification enables the achievement of excellent inter- and intraobserver agreement in MRI-based diagnosis of deep infiltrating endometriosis and endometriomas with sufficient reader experience. The #Enzian classification could be recommended for routine use by radiologists in daily pelvic MRI scans for endometriosis.

# Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

# Ethics statement

The studies involving humans were approved by Institutional Review Board of the Faculty of Medicine, Justus-Liebig-University Giessen. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

# Author contributions

SH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft. HK: Data curation, Investigation, Writing – review & editing. FZ: Conceptualization, Methodology, Resources, Writing – review & editing. IM-H: Conceptualization, Methodology, Resources, Writing – review & editing. JK: Conceptualization, Supervision, Writing – review & editing. SY: Data curation, Methodology, Writing – review & editing. EN: Data curation, Methodology, Writing – review & editing. GK: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. FR: Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

# References

1. Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, Kiesel L, et al. ESHRE guideline: endometriosis. *Hum Reprod Open*. (2022) 2022:hoac009. doi: 10.1093/hropen/hoac009
2. Harth S, Hoopmann M, Merz E, Grab D, Weichert J, Helmy-Bader S, et al. Expert opinion on the use of transvaginal sonography for presurgical staging and classification of endometriosis. *Arch Gynecol Obstet*. (2023) 307:5–19. doi: 10.1007/s00404-022-06766-z
3. Harth S, Roller FC, Zeppernick F, Meinhold-Heerlein I, Krombach GA. Deep infiltrating endometriosis: diagnostic accuracy of preoperative magnetic resonance imaging with respect to morphological criteria. *Diagnostics*. (2023) 13:1794. doi: 10.3390/diagnostics13101794
4. Hudelist G, Valentin L, Saridogan E, Condous G, Malzoni M, Roman H, et al. What to choose and why to use - a critical review on the clinical relevance of rASRM, EFI and Enzian classifications of endometriosis. *Facts Views Vis Obgyn*. (2021) 13:331–8. doi: 10.520254/FVVO.13.4.041
5. Zondervan KT, Missus S, Abrao MS, Einarsson JI, Horne AW, Johnson NP, et al. Endometriosis classification systems: an international survey to map current knowledge and uptake. *J Minim Invasive Gynecol*. (2022) 29:716–725.e1. doi: 10.1016/j.jmig.2022.01.014
6. Vermeulen N, Abrao MS, Einarsson JI, Horne AW, Johnson NP, Lee TTM, et al. Endometriosis classification, staging and reporting systems: a review on the road to a universally accepted endometriosis classification. *J Minim Invasive Gynecol*. (2021) 28:1822–48. doi: 10.1016/j.jmig.2021.07.023
7. Keckstein J, Saridogan E, Ulrich UA, Sillem M, Oppelt P, Schweppe KW, et al. The #Enzian classification: a comprehensive non-invasive and surgical description system for endometriosis. *Acta Obstet Gynecol Scand*. (2021) 100:1165–75. doi: 10.1111/aogs.14099
8. Tuttlies F, Keckstein J, Ulrich U, Possover M, Schweppe KW, Wustlich M, et al. ENZIAN-Score, eine Klassifikation der tief infiltrierenden Endometriose. *Zentralbl Gynakol*. (2005) 127:275–81. doi: 10.1055/s-2005-836904
9. American Society for Reproductive Medicine. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril*. (1997) 67:817–21. doi: 10.1016/s0015-0282(97)81391-x

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# Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2023.1303593/full#supplementary-material>

10. Maciel C, Ferreira H, Djokovic D, Kyaw Tun J, Keckstein J, Rizzo S, et al. MRI of endometriosis in correlation with the #Enzian classification: applicability and structured report. *Insights Imaging*. (2023) 14:1–12. doi: 10.1186/s13244-023-01466-x
11. Harth S, Roller FC, Zeppernick F, Meinhold-Heerlein I, Krombach GA. Feasibility of periprocedural decision on the administration of intravenous contrast media in MRI for endometriosis. *Eur J Radiol*. (2023) 165:110949. doi: 10.1016/j.ejrad.2023.110949
12. Manganaro L, Celli V, Dolciami M, Ninkova R, Ercolani G, Ciulla S. Can new ENZIAN score 2020 Represent a staging system improving MRI structured report? *Int J Environ Res Public Health*. (2021) 18:9949. doi: 10.3390/ijerph18199949
13. Saba L, Guerriero S, Sulis R, Pilloni M, Ajossa S, Melis G, et al. Learning curve in the detection of ovarian and deep endometriosis by using magnetic resonance: comparison with surgical results. *Eur J Radiol*. (2011) 79:237–44. doi: 10.1016/j.ejrad.2010.01.019
14. Bazot M, Bharwani N, Huchon C, Kinkel K, Cunha TM, Guerra A, et al. European society of urogenital radiology (ESUR) guidelines: MR imaging of pelvic endometriosis. *Eur Radiol*. (2017) 27:2765–75. doi: 10.1007/s00330-016-4673-z
15. Schneider C, Oehmke F, Tinneberg H-R, Krombach GA. MRI technique for the preoperative evaluation of deep infiltrating endometriosis: current status and protocol recommendation. *Clin Radiol*. (2016) 71:179–94. doi: 10.1016/j.crad.2015.09.014
16. Tong A, VanBuren WM, Chamié L, Feldman M, Hindman N, Huang C. Recommendations for MRI technique in the evaluation of pelvic endometriosis: consensus statement from the society of abdominal radiology endometriosis disease-focused panel. *Abdom Radiol*. (2020) 45:1569–86. doi: 10.1007/s00261-020-02483-w
17. Bazot M, Darai E. Role of transvaginal sonography and magnetic resonance imaging in the diagnosis of uterine adenomyosis. *Fertil Steril*. (2018) 109:389–97. doi: 10.1016/j.fertnstert.2018.01.024
18. Agostinho L, Cruz R, Osório F, Alves J, Setúbal A, Guerra A. MRI for adenomyosis: a pictorial review. *Insights Imaging*. (2017) 8:549–56. doi: 10.1007/s13244-017-0576-z
19. Sim J, Wright CC. The kappa statistic in reliability studies: use, interpretation, and sample size requirements. *Phys Ther*. (2005) 85:257–68. doi: 10.1093/ptj/85.3.257



20. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. (1977) 33:159–74. doi: 10.2307/2529310
21. Thomassin-Naggara I, Lamrabet S, Crestani A, Bekhouche A, Wahab CA, Kermarrec E, et al. Magnetic resonance imaging classification of deep pelvic endometriosis: description and impact on surgical management. *Hum Reprod*. (2020) 35:1589–600. doi: 10.1093/humrep/deaa103
22. Burla L, Scheiner D, Hötter AM, Meier A, Fink D, Boss A, et al. Structured manual for MRI assessment of deep infiltrating endometriosis using the ENZIAN classification. *Arch Gynecol Obstet*. (2021) 303:751–7. doi: 10.1007/s00404-020-05892-w
23. Bazot M, Gasner A, Ballester M, Daraï E. Value of thin-section oblique axial T2-weighted magnetic resonance images to assess uterosacral ligament endometriosis. *Hum Reprod*. (2011) 26:346–53. doi: 10.1093/humrep/deq336
24. Saba L, Guerriero S, Sulcis R, Ajossa S, Melis G, Mallarini G. Agreement and reproducibility in identification of endometriosis using magnetic resonance imaging. *Acta Radiol*. (2010) 51:573–80. doi: 10.3109/02841851003657343
25. Bazot M, Daraï E. Diagnosis of deep endometriosis: clinical examination, ultrasonography, magnetic resonance imaging, and other techniques. *Fertil Steril*. (2017) 108:886–94. doi: 10.1016/j.fertnstert.2017.10.026
26. Feldman MK, VanBuren WM, Barnard H, Taffel MT, Kho RM. Systematic interpretation and structured reporting for pelvic magnetic resonance imaging studies in patients with endometriosis: value added for improved patient care. *Abdom Radiol*. (2020) 45:1608–22. doi: 10.1007/s00261-019-02182-1
27. Rousset P, Florin M, Bharwani N, Touboul C, Monroc M, Golfier F, et al. Deep pelvic infiltrating endometriosis: MRI consensus lexicon and compartment-based approach from the ENDOVALIRM group. *Diagn Interv Imaging*. (2023) 104:95–112. doi: 10.1016/j.diii.2022.09.004
28. Shinagare AB, Sadow CA, Sahni VA, Silverman SG. Urinary bladder: normal appearance and mimics of malignancy at CT urography. *Cancer Imaging*. (2011) 11:100–8. doi: 10.1102/1470-7330.2011.0017
29. Loring M, Chen TY, Isaacson KB. A systematic review of Adenomyosis: it is time to reassess what we thought we knew about the disease. *J Minim Invasive Gynecol*. (2020) 28:644–55. doi: 10.1016/j.jmig.2020.10.012
30. Jaramillo-Cardoso A, Shenoy-Bhangle A, Garces-Descovich A, Glickman J, King L, Morteale KJ. Pelvic MRI in the diagnosis and staging of pelvic endometriosis: added value of structured reporting and expertise. *Abdom Radiol*. (2020) 45:1623–36. doi: 10.1007/s00261-019-02199-
31. Enzelsberger S-H, Oppelt P, Nirgianakis K, Seeber B, Drahoňovský J, Wanderer L, et al. Preoperative application of the Enzian classification for endometriosis (the cEnzian study): a prospective international multicenter study. *BJOG*. (2022) 129:2052–61. doi: 10.1111/1471-0528.17235



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# Prediction of adenomyosis according to revised definitions of morphological uterus sonographic assessment features

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**Objectives:** This study aimed to predict the diagnosis of adenomyosis by revised definitions of morphological uterus sonographic assessment (MUSA) features in individuals who had hysterectomy.

**Methods:** This was retrospective cohort research conducted at a tertiary facility. Between January 2022 and January 2023, 196 individuals who had hysterectomy were analyzed in the research. The revised definitions of MUSA features of the adenomyosis approach were used to record the direct and indirect results of the sonography. The cases were classified as Group 1 (adenomyosis;  $n = 40$ , 20.4%) and Group 2 (control;  $n = 156$ , 79.6%) according to histopathology reports.

**Results:** Hyperechogenic islands and echogenic subendometrial buds and lines were the most predictive direct features ( $p = 0.02$ ). Globular uterus and irregular junctional zone were the most predictive indirect features ( $p = 0.04$ ;  $p = 0.03$ , respectively). Among all indirect features, the globular uterus was the most predictive ( $p = 0.02$ ). Total feature  $>4$  was determined as the significant cutoff value to predict adenomyosis ( $p < 0.001$ ).

**Conclusion:** This study shows that combinations with a total number of features  $>4$  can be practically used in the evaluation of adenomyosis using the revised definitions of MUSA features.

## KEYWORDS

adenomyosis, direct feature, indirect feature, 3D transvaginal ultrasonography, 2D transvaginal ultrasonography

## 1 Introduction

The benign uterine condition known as adenomyosis is identified by the existence of stroma and endometrial glands in the myometrium (1). Whether or not there is a hypertrophic myometrium nearby, it may appear as a localized or widespread lesion in the inner or outer myometrium (2). The overall prevalence of histopathologically confirmed adenomyosis was reported as between 20.9 and 36.4% (3, 4). It is also stated that adenomyosis peaks between the ages of 40 and 59 (3).

While the diagnosis of adenomyosis is definitively made histopathologically, it can only be predicted by non-invasive imaging methods. Nowadays, transvaginal ultrasonography (TVS) is the first-line imaging technique in the diagnosis of adenomyosis (5). In a meta-analysis in which the diagnosis of adenomyosis was confirmed histopathologically, the sensitivity and specificity of preoperative TVS for predicting adenomyosis were found to

be both 78% (6). It has been noted that three-dimensional (3D) TVS increases the accuracy of adenomyosis diagnosis (6).

Diagnostic sonographic characteristics of adenomyosis were examined in many studies in the literature (4, 7, 8). Van Den Bosch et al. reported MUSA features to optimize and standardize sonographic markers of adenomyosis (9). After that, MUSA features were revised and updated to define adenomyosis by a modified Delphi procedure study (5). Everyone agreed that the ultrasonographic signs of adenomyosis in the MUSA features should be classified as either indirect (asymmetrical myometrial thickening, globular uterus, fan-shaped shadowing, translesional vascularity, inconsistent junctional region, and interrupted junctional region) or direct (myometrial cysts, hyperechogenic islands, echogenic subendometrial buds, and lines) (5). The most recent study on this subject, revised definitions of MUSA features of adenomyosis, highlighted a gap in the literature as further investigation of the accuracy of the existence of one or more indirect and/or direct features to diagnose adenomyosis (5).

Based on this perspective, we aimed to predict the diagnosis of histopathologically confirmed adenomyosis by utilizing the revised and updated MUSA ultrasonographic features (one or more indirect and/or direct features) in patients who underwent hysterectomy.

## 2 Materials and methods

This was retrospective cohort research carried out at a tertiary center. Informed consent was obtained from all participants in this research. The research was performed in compliance with the principles of the Helsinki Declaration. Institutional ethics committee approval was provided (File number: 7737-GOA, Registration number: 2023/02-13). Between January 2022 and January 2023, 214 patients who underwent hysterectomy were included in the research. Individuals with indications of cervical, uterine, and adnexal malignancy were excluded from the study ( $n = 18$ ).

Data from 196 patients were analyzed.

During the preoperative period within the last week, the patients were examined with 3D TVS (General Electric® Voluson E8 with a 4–9 MHz 3D transvaginal probe). Sonographic evaluations were performed by three gynecologists working at our institution whose special interest is in endometriosis and adenomyosis ultrasonography. The sonographic examinations were performed by two gynecologists, 30 and 10 years old (MG and OY, respectively). In cases of discrepancies, a third gynecologist with 6 years of experience (MEÖ) ensured consensus. The presence of leiomyoma (location, site, number, and maximum diameter) was recorded. The location of leiomyoma was defined as the anterior and posterior sides of the uterus. The site of leiomyoma was classified as type 0–7, in accordance with the current literature that defines the classification of leiomyoma (10). If more than one leiomyoma was detected, the characteristics of the largest myoma were used as the basis. To predict adenomyosis, the revised definitions of MUSA features, including direct (myometrial cysts, hyperechogenic islands, echogenic subendometrial buds, and lines), indirect (asymmetrical myometrial thickening, globular uterus, fan-shaped shadowing, translesional vascularity, irregular

junctional zone, and interrupted junctional zone), and the total number of signs (direct + indirect), were accepted as a reference, and the findings were documented (5). The demographic characteristics, clinical findings, surgery indications, and surgery type of the patients were recorded. Following the surgeries, the pathology materials were analyzed by a single experienced gynecopathologist (EÇU). Macroscopically, an enlarged uterus, a spherical and/or asymmetrical uterus, and a thick, irregularly fasciculated myometrium with tiny gaps were used to diagnose adenomyosis. When an adenomyoma resembles an intramural myoma or when the adenomyotic lesions are limited to the uterine wall, it is referred to as focal adenomyosis (11). Histologically, the existence of ectopic endometrial glands and/or stroma linked to neighboring smooth muscle hypertrophy and hyperplasia located 2.5 mm past the endometrial–myometrial interface when seen via a low-power microscope established the diagnosis of adenomyosis (11). The histopathological diagnosis of endometrioma was reported in patients who underwent unilateral or bilateral salpingo-oophorectomy along with hysterectomy. The characteristics of the leiomyomas assessed preoperatively were confirmed histopathologically. The cases were classified as Group 1 (adenomyosis;  $n = 40$ , 20.4%) and Group 2 (control;  $n = 156$ , 79.6%) according to histopathology reports.

Analyses were performed with SPSS version 26.0 (IBM Inc., Chicago, IL, USA). Normality analysis was performed according to the Kolmogorov–Smirnov test. Not normally distributed variables were analyzed with the Mann–Whitney  $U$ -test. These results were expressed as median (minimum–maximum) values for each group. The chi-square test and Fisher's precision test were used in the analysis of categorical data. These were presented as counts and percentages (%). An inter-rater reliability analysis was performed for direct and indirect ultrasonography findings. For this purpose, Cohen's Kappa was calculated and categorized as follows:  $k = 0$ – $0.20$ , slight agreement;  $k = 0.21$ – $0.40$ , fair agreement;  $k = 0.41$ – $0.60$ , moderate agreement;  $k = 0.61$ – $0.80$ , substantial agreement; and  $k = 0.81$ – $1.00$ , almost perfect agreement. Logistic regression models were used to analyze features that may be effective in predicting adenomyosis. Receiver operating characteristic (ROC) analysis was performed to determine the area under the curve (AUC), which indicates the average sensitivity of features. The appropriate cutoff value, indicating the sum of the highest sensitivity and specificity, was calculated for the most predictive feature. The results were a 95% confidence interval (CI). The  $p$ -value considered statistically significant was  $<0.05$ .

## 3 Results

Demographic characteristics and clinical findings of groups are listed in Table 1. The groups were similar with regard to age, gravity, parity, body mass index (BMI), menarche age, menopausal status, and smoking habit. There was no significant difference between the groups in terms of the history of myomectomy surgery, cesarean section, curettage, oral progesterone treatment, or levonorgestrel intrauterine device treatment. Although the history of dyspareunia and chronic pelvic pain was detected at a higher rate in the adenomyosis group, none of the clinical symptoms showed statistically significant differences between the groups.

TABLE 1 Demographic characteristics and clinical findings of groups.

| Variables                                    | All patients<br><i>n</i> = 196 (100%) | Group 1 (adenomyosis)<br><i>n</i> = 40 (20.4%) | Group 2 (control)<br><i>n</i> = 156 (79.6%) | <i>p</i> -value |
|----------------------------------------------|---------------------------------------|------------------------------------------------|---------------------------------------------|-----------------|
| Age (years)                                  | 49 (34–80)                            | 48 (35–75)                                     | 49 (34–80)                                  | 0.1             |
| Gravidy                                      | 2 (0–13)                              | 3 (0–7)                                        | 2 (0–13)                                    | 0.4             |
| Parity                                       | 2 (0–11)                              | 2 (0–7)                                        | 2 (0–11)                                    | 0.4             |
| Body mass index (%)                          | 28 (18–43.1)                          | 28 (20–40)                                     | 28 (18–43.1)                                | 0.9             |
| Menarche age (years)                         | 13 (11–17)                            | 13 (11–17)                                     | 13 (11–17)                                  | 0.7             |
| Menopausal status                            |                                       |                                                |                                             | 0.2             |
| Premenopausal                                | 56.1% (110/196)                       | 65% (26/40)                                    | 53.8% (84/156)                              |                 |
| Postmenopausal                               | 43.9% (86/196)                        | 35% (14/40)                                    | 46.2% (72/156)                              |                 |
| Smoking habit (10/day)                       | 40.3% (79/196)                        | 42.5% (17/40)                                  | 39.7% (62/156)                              | 0.8             |
| Myomectomy surgery                           | 40.8% (80/196)                        | 42.5% (17/40)                                  | 40.4% (64/156)                              | 0.8             |
| Cesarean section                             | 37.2% (73/196)                        | 35% (14/40)                                    | 37.8% (59/156)                              | 0.8             |
| Curettage history                            | 48% (94/156)                          | 52.5% (21/40)                                  | 46.8% (73/156)                              | 0.5             |
| Oral progesterone treatment                  | 27% (53/196)                          | 25% (10/40)                                    | 27.6% (43/156)                              | 0.8             |
| Levonorgestrel intrauterine device treatment | 15.3% (30/196)                        | 22.5% (9/40)                                   | 13.5% (21/156)                              | 0.2             |
| Dysmenorrhea                                 | 43.9% (86/196)                        | 35% (14/40)                                    | 46.2% (72/156)                              | 0.2             |
| Dyspareunia                                  | 28.6% (56/196)                        | 35% (14/40)                                    | 26.9% (42/156)                              | 0.3             |
| Menometrorrhagia                             | 40.8% (80/196)                        | 37.5% (15/40)                                  | 41.7% (65/156)                              | 0.7             |
| Chronic pelvic pain                          | 28.1% (55/196)                        | 37.5% (15/40)                                  | 25.6% (40/156)                              | 0.1             |

Ultrasound findings of the groups are listed in Table 2. Among the indirect features, the globular uterus and irregular junctional zone were observed to be significantly greater in the adenomyosis group (57.5 vs. 39.7%;  $p = 0.04$ , 32.5 vs. 17.3%;  $p = 0.03$ , respectively). Other indirect features did not differ between groups. Although myometrial cysts, which are direct features, were detected at a higher rate in the adenomyosis group, the difference was not significant. Hyperechogenic islands and echogenic subendometrial buds and line features were significantly higher in the adenomyosis group (17.5 vs. 5.8%;  $p = 0.01$ , 15 vs. 4.5%;  $p = 0.01$ , respectively). The median value of the total number of direct, indirect, and total features was significantly higher in the adenomyosis group ( $p = 0.04$ ;  $p = 0.04$ ;  $p < 0.01$ ). The groups were similar in terms of leiomyoma presence, site, number, and maximum diameter. The anterior location of maximum diameter myoma uteri was higher in the control group ( $p = 0.03$ ).

Interobserver agreement of ultrasound findings is shown in Table 3. Cohen's Kappa showed that there was an almost perfect agreement for asymmetrical myometrial thickening and globular uterus. Moderate agreement was found for fan-shaped shadowing and hyperechogenic islands; fair agreement was found for translesional vascularity, interrupted junctional zone, myometrial cysts, and echogenic subendometrial buds and lines; a slight agreement was found for the irregular junctional zone between observers.

The operation and pathological outcomes of the groups are listed in Table 4. The groups were similar in terms of indication and type of surgery. Although the coexistence of myoma uteri and endometrioma was detected at a higher rate in the control

group, these variables were not different between the groups. No adenomyoma coexistence was detected in the control group. This rate was reported as 15% in the adenomyosis group ( $p < 0.001$ ).

ROC analysis was conducted to calculate the cutoff score of the number of diagnostic direct, indirect, and total features for adenomyosis (Figure 1). Direct feature  $>1$  was determined as the cutoff value to predict adenomyosis ( $p = 0.1$ ). The AUC for the direct feature was 0.578 (95% CI, 0.473–0.620). Indirect feature  $>4$  was determined as the cutoff value to predict adenomyosis ( $p = 0.05$ ). The AUC for the indirect feature was 0.599 (95% CI, 0.503–0.695). Total feature  $>4$  was determined as the cutoff value to predict adenomyosis ( $p < 0.001$ ). The AUC for the total feature was 0.631 (95% CI, 0.536–0.725).

The evaluation of likelihood ratio (LR), negative predictive value (NPV), positive predictive value (PPV), specificity, sensitivity, and accuracy of ultrasonography findings are reported in Table 5. Hyperechogenic islands had 17.5% sensitivity, 94% specificity, and 78% accuracy. However, echogenic subendometrial buds and lines had more specificity (95%) and higher accuracy (79%). Among the indirect findings, the most sensitive feature was the globular uterus (57.7%) and the most specific feature was translesional vascularity (94.8%). In addition, translesional vascularity had the highest accuracy (77%). Total feature score  $>4$  had 27.5% sensitivity, 90% specificity, and 77% accuracy (Figure 2). There were four situations (“3 direct +  $\geq 2$  indirect”, “2 direct +  $\geq 3$  indirect”, “1 direct +  $\geq 4$  indirect”, and “0 direct +  $\geq 5$  indirect”) that met the total feature score  $>4$  criterion. “3 direct +  $\geq 2$  indirect” combination

TABLE 2 Ultrasound findings of the groups.

| Variables                                       | All patients<br><i>n</i> = 196 (100%) | Group 1 (adenomyosis)<br><i>n</i> = 40 (20.4%) | Group 2 (control)<br><i>n</i> = 156 (79.6%) | <i>p</i> -value |
|-------------------------------------------------|---------------------------------------|------------------------------------------------|---------------------------------------------|-----------------|
| <b>Indirect signs</b>                           |                                       |                                                |                                             |                 |
| Asymmetrical myometrial thickening              | 42.9% (84/196)                        | 55% (22/40)                                    | 39.7% (62/156)                              | 0.08            |
| Globular uterus                                 | 43.4% (85/196)                        | 57.5% (23/40)                                  | 39.7% (62/156)                              | 0.04            |
| Fan-shaped shadowing                            | 41.8% (82/196)                        | 55% (22/40)                                    | 38.5% (60/156)                              | 0.05            |
| Translesional vascularity                       | 5.6% (11/196)                         | 7.5% (3/40)                                    | 5.1% (8/156)                                | 0.5             |
| Irregular junctional zone                       | 20.4% (40/196)                        | 32.5% (13/40)                                  | 17.3% (27/156)                              | 0.03            |
| Interrupted junctional zone                     | 21.4% (42/196)                        | 30% (12/40)                                    | 19.2% (30/156)                              | 0.1             |
| <b>Direct signs</b>                             |                                       |                                                |                                             |                 |
| Myometrial cysts                                | 15.8% (31/196)                        | 17.5% (7/40)                                   | 15.4% (24/156)                              | 0.7             |
| Hyperechogenic islands                          | 8.2% (16/196)                         | 17.5% (7/40)                                   | 5.8% (9/156)                                | 0.01            |
| Echogenic subendometrial buds and lines         | 6.6% (13/196)                         | 15% (6/40)                                     | 4.5% (7/156)                                | 0.01            |
| Total number of indirect features               | 2 (0–7)                               | 3 (0–7)                                        | 2 (0–6)                                     | 0.04            |
| Total number of direct features                 | 0.3 (0–3)                             | 0 (0–3)                                        | 0 (0–2)                                     | 0.04            |
| Total number of features                        | 2 (0–9)                               | 3 (0–9)                                        | 2 (0–7)                                     | <0.01           |
| Myoma uteri                                     | 61.2% (120/196)                       | 62.5% (25/40)                                  | 60.9% (95/156)                              | 0.8             |
| <b>Location of maximum diameter myoma uteri</b> |                                       |                                                |                                             | 0.03            |
| Anterior wall                                   | 82.5% (99/120)                        | 68% (17/25)                                    | 86.3% (82/95)                               |                 |
| Posterior wall                                  | 17.5% (21/120)                        | 32% (8/25)                                     | 13.7% (13/95)                               |                 |
| <b>Site of maximum diameter myoma uteri</b>     |                                       |                                                |                                             | 0.7             |
| Type 0–2                                        | 65.8% (79/120)                        | 60% (15/25)                                    | 67.4% (64/95)                               |                 |
| Type 3–6                                        | 25% (30/120)                          | 28% (7/25)                                     | 24.2% (23/95)                               |                 |
| Type 7                                          | 9.2% (11/120)                         | 12% (3/25)                                     | 8.4% (8/95)                                 |                 |
| Number of myoma uteri (cm)                      | 3 (1–10)                              | 3 (1–10)                                       | 3 (1–10)                                    | 0.9             |
| Maximum diameter of myoma uteri (cm)            | 4 (1–10)                              | 4 (1–10)                                       | 4 (1–10)                                    | 0.9             |

TABLE 3 Interobserver agreement of ultrasound findings.

| Variables                               | Interobserver agreement rate (%) | Kappa (CI 95%)   | <i>p</i> -value |
|-----------------------------------------|----------------------------------|------------------|-----------------|
| <b>Indirect signs</b>                   |                                  |                  |                 |
| Asymmetrical myometrial thickening      | 91.8%                            | 0.83 (0.75–0.91) | <0.001          |
| Globular uterus                         | 92.3%                            | 0.84 (0.77–0.92) | <0.001          |
| Fan-shaped shadowing                    | 70.3%                            | 0.41 (0.29–0.53) | <0.001          |
| Translesional vascularity               | 85.7%                            | 0.31 (0.21–0.50) | <0.001          |
| Irregular junctional zone               | 65.8%                            | 0.2 (0.07–0.36)  | <0.01           |
| Interrupted junctional zone             | 70.9%                            | 0.33 (0.19–0.46) | <0.001          |
| <b>Direct signs</b>                     |                                  |                  |                 |
| Myometrial cysts                        | 87.7%                            | 0.58 (0.43–0.73) | <0.001          |
| Hyperechogenic islands                  | 94.3%                            | 0.65 (0.46–0.84) | <0.001          |
| Echogenic subendometrial buds and lines | 94%                              | 0.59 (0.37–0.81) | <0.001          |

had the highest specificity (100%) and accuracy (80.6%). “1 direct +  $\geq$  4 indirect” and “0 direct +  $\geq$  5 combinations” had highest sensitivity (10%).

Tables 6, 7 included logistic regression analysis for predicting adenomyosis. While hyperechogenic islands and echogenic subendometrial buds and lines were significant in univariate



TABLE 4 Operation and pathological findings of the groups.

| Variables                               | All patients<br><i>n</i> = 196 (100%) | Group 1 (adenomyosis)<br><i>n</i> = 40 (20.4%) | Group 2 (control)<br><i>n</i> = 156 (79.6%) | <i>p</i> -value |
|-----------------------------------------|---------------------------------------|------------------------------------------------|---------------------------------------------|-----------------|
| <b>Indication</b>                       |                                       |                                                |                                             | 0.1             |
| Myoma uteri                             | 40.8% (80/196)                        | 37.5% (15/40)                                  | 41.7% (65/156)                              |                 |
| Premenopausal abnormal uterine bleeding | 23% (45/196)                          | 32.5% (13/40)                                  | 20.5% (32/156)                              |                 |
| Uterine prolapse                        | 18.9% (37/196)                        | 10% (4/40)                                     | 21.2% (33/156)                              |                 |
| Chronic pelvic pain                     | 6.6% (13/196)                         | 10% (4/40)                                     | 5.8% (9/156)                                |                 |
| Benign adnexal mass                     | 4.6% (9/196)                          | 0% (0/40)                                      | 5.8% (9/156)                                |                 |
| Postmenopausal bleeding                 | 6.1% (12/196)                         | 10% (4/40)                                     | 5.1% (8/156)                                |                 |
| <b>Surgery type</b>                     |                                       |                                                |                                             | 0.3             |
| TAH + BS                                | 3.1% (6/196)                          | 5% (2/40)                                      | 2.6% (4/156)                                |                 |
| TAH + USO                               | 1% (2/196)                            | 0% (0/40)                                      | 1.3% (2/156)                                |                 |
| TAH + BSO                               | 17.5% (35/196)                        | 20% (8/40)                                     | 17.3% (27/156)                              |                 |
| TLH + BS                                | 20.4% (40/196)                        | 17.5% (7/40)                                   | 21.2% (33/156)                              |                 |
| TLH + USO                               | 1.5% (3/196)                          | 5% (2/40)                                      | 0.6% (1/156)                                |                 |
| TLH + BSO                               | 54.1% (106/196)                       | 52.5% (21/40)                                  | 54.5% (85/156)                              |                 |
| VH + BSO                                | 2% (4/196)                            | 0% (0/40)                                      | 2.6% (4/156)                                |                 |
| Coexistence of myoma uteri              | 61.2% (120/196)                       | 62.5% (25/40)                                  | 60.9% (95/156)                              | 0.8             |
| Coexistence of endometrioma             | 7.9% (12/151)                         | 15.6% (5/32)                                   | 5.9% (7/119)                                | 0.07            |
| Coexistence of adenomyoma               | 3.1% (6/196)                          | 15% (6/40)                                     | 0% (0/156)                                  | <0.001          |

TAH, total abdominal hysterectomy; TLH, total laparoscopic hysterectomy; VH, vaginal hysterectomy; BS, bilateral salpingectomy; USO, unilateral bilateral salpingo-oophorectomy; BSO, bilateral salpingo-oophorectomy.

logistic regression analysis ( $p = 0.02$ ), they were not in multivariable analysis ( $p > 0.5$ ). In univariate logistic regression analysis, the globular uterus and irregular junctional zone were significant ( $p = 0.04$ ;  $p = 0.03$ , respectively). In multivariate logistic regression analysis, the globular uterus was the only feature that showed a significant difference ( $p = 0.02$ ).

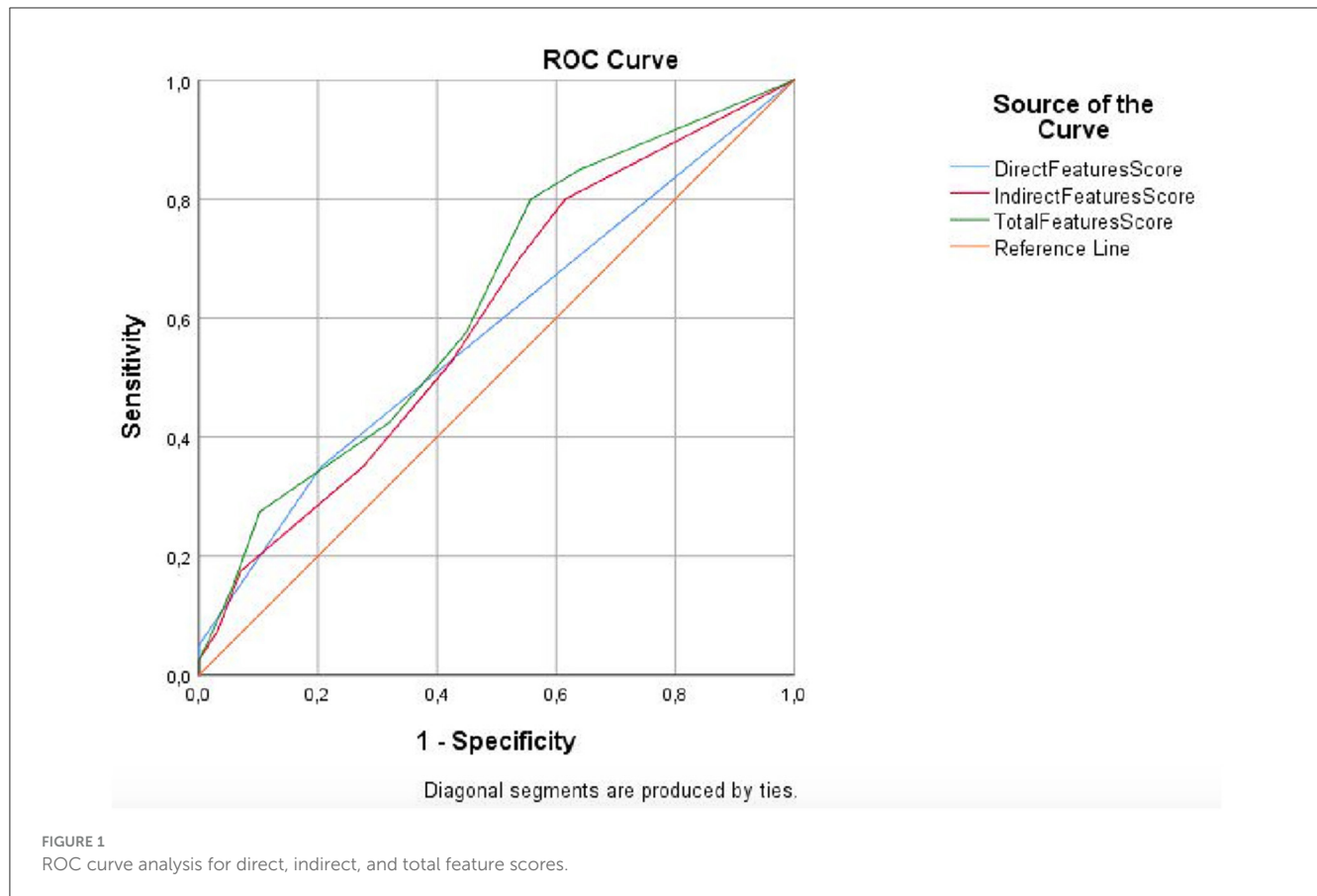
## 4 Discussion

In this current study, we aimed to predict the diagnosis of histopathologically confirmed adenomyosis in patients undergoing hysterectomy using the revised definitions of MUSA features. The overall prevalence of adenomyosis was 20.4%. Hyperechogenic islands and echogenic subendometrial buds and lines were the most predictive direct features. The globular uterus and irregular junctional zone were the most predictive indirect features. Among all indirect features, the globular uterus was the most predictive. Total feature  $>4$  was determined as the statistically significant cutoff value to predict adenomyosis.

The accuracy of TVS criteria in the adenomyosis diagnosis was investigated by Kepkep et al. (8). The sensitivity, specificity, and accuracy of TVS in the diagnosis of adenomyosis were 80.8, 61.4, and 68.6%, respectively (8). In another study, Bazot et al. reported the sensitivity (80.9%), specificity (100%), and accuracy (82.6%) of TVS for the diagnosis of adenomyosis in individuals with menometrorrhagia (4). Unfortunately, the sensitivity of TVS

was found to be poor (38.4%) in an unselected patient population scheduled for hysterectomy (4). Unlike these studies, our criteria were defined according to the revised definitions of MUSA features of adenomyosis (5).

Naftalin et al. reported the histopathological coexistence of leiomyoma and adenomyosis as 21%, and the presence of leiomyoma without adenomyosis as 20% (3). In our study, the coexistence of adenomyosis and leiomyoma was three times higher than that reported in the literature. This rate was greater than the control group, but it was not statistically significant. Although it was thought that including patients with various site, number, and maximum diameter leiomyomas in our study group would affect sonographic sensitivity and specificity, there was no difference between the groups. Only, the rate of anterior location of the maximum diameter myoma uteri was statistically higher in the control group. Exacoustos et al. reported that the accuracy of the overall two-dimensional (2D) and three-dimensional (3D TVS) diagnoses, depending on whether two or more of the particular ultrasonographic characteristics were present, was 83 and 89%, respectively (7). There was no significant change in the specificity and accuracy of 3D sonography parameters compared to 2D sonography parameters, although there was a significantly increased sensitivity and NPV in the diagnosis of adenomyosis (7). Despite the presence of leiomyomas with various characteristics in our study, the fact that sonography evaluation was performed with 3D TVS enables better determination of the sonographic features as stated in the literature (5, 7).



A three-round modified Delphi procedure was designed among gynecologists with expertise in the ultrasonographic diagnosis of adenomyosis to reach a consensus. The Delphi procedure is a qualitative research method aimed at determining the collective opinions of experts on a specific subject. Two rounds of surveys were conducted. The surveys included ultrasound images and video clips of the uteri of women suspected to have adenomyosis. The purposes of presenting the images and video clips were: (1) to investigate the agreement among experts regarding the presence of MUSA features that may necessitate a revised definition due to poor agreement; (2) to gather suggestions regarding revised definitions; and (3) to reach a consensus on the proposed revised definitions. In the revised definitions of MUSA features of adenomyosis, consensus was achieved regarding the categorization of MUSA features into direct and indirect ultrasound indicators of adenomyosis (5). Direct features signify the existence of ectopic endometrial tissue within the myometrium (5). The consensus was attained at rates of 80, 93.3, and 60% for hyperechogenic islands, myometrial cysts, and echogenic subendometrial buds and lines, respectively (5). In our study, the interobserver agreement rate for hyperechogenic islands, myometrial cysts, and echogenic subendometrial buds and lines was found to be 94.3, 87.7, and 94%, respectively. There was a fair agreement for myometrial cysts and echogenic subendometrial buds and lines, while a moderate agreement was found for hyperechogenic islands. Indirect features encompass those that arise as secondary effects of the existence of endometrial tissue in the myometrium,

including muscular hypertrophy (resulting in a globular uterus) or artifacts (e.g., shadowing). Consensus was attained at rates of 86.7, 86.7, 100, 80, 66.7, and 60% for the globular uterus, asymmetrical myometrial thickening, fan-shaped shadowing, translesional vascularity, irregular junctional zone, and interrupted junctional zone, respectively (5). In our study, total agreement for globular uterus, asymmetrical myometrial thickening, fan-shaped shadowing, translesional vascularity, irregular junctional zone, and interrupted junctional zone was found to be 92.3, 91.8, 70.3, 85.7, 65.8, and 85.7%, respectively. Asymmetric thickening was defined as the thickness difference between the anterior and posterior myometrial walls exceeding 5 mm or the ratio between the anterior and posterior wall thickness being well-above 1 or well-below 1 (5). A globular uterus was defined as one in which the myometrial serosa deviates from the cervix in at least two directions, rather than following a path parallel to the endometrium, and the measured diameters of the uterine corpus are approximately equal. We based our study on the suggested criteria (5). In our study, compared with the Delphi study, the total interobserver agreement for asymmetric myometrial thickening and globular uterus was higher, with almost perfect interobserver agreement.

Our results revealed higher total agreement for echogenic subendometrial buds and lines as well as the interrupted junctional zone in comparison to the modified Delphi study. Conversely, fan-shaped shadowing exhibited a lower total agreement in our study. We posit that these discrepancies may be attributed to factors such as the presence of myoma uteri, the number of myoma uteri, the site

TABLE 5 Evaluation of sensitivity, specificity, negative predictive value, positive predictive value, likelihood ratio, and accuracy of ultrasound findings.

| Variables                               | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | LR + | LR – | Accuracy (%) |
|-----------------------------------------|-----------------|-----------------|---------|---------|------|------|--------------|
| <b>Direct features</b>                  |                 |                 |         |         |      |      |              |
| Myometrial cysts                        | 17.5            | 79              | 22.5    | 80      | 0.8  | 1    | 70           |
| Hyperechogenic islands                  | 17.5            | 94              | 43      | 94      | 2.9  | 0.8  | 78           |
| Echogenic subendometrial buds and lines | 15              | 95.5            | 46      | 81      | 3.3  | 0.8  | 79           |
| <b>Indirect features</b>                |                 |                 |         |         |      |      |              |
| Asymmetrical myometrial thickening      | 55              | 60              | 26.1    | 83.9    | 1.3  | 0.7  | 59           |
| Globular uterus                         | 57.7            | 60.2            | 27      | 84.6    | 1.4  | 0.7  | 59           |
| Fan-shaped shadowing                    | 55              | 61              | 26.8    | 84.2    | 1.4  | 0.7  | 60           |
| Translesional vascularity               | 7.5             | 94.8            | 27.2    | 80      | 0.3  | 0.9  | 77           |
| Irregular junctional zone               | 32.5            | 82.6            | 32.5    | 82.6    | 1.8  | 0.8  | 72           |
| Interrupted junctional zone             | 30              | 80              | 28.5    | 81.8    | 1.5  | 0.8  | 70           |
| Total feature score > 4                 | 27.5            | 90              | 40      | 82      | 2.6  | 0.8  | 77           |
| Direct feature score > 1                | 12.5            | 95              | 38.4    | 80.8    | 2.4  | 0.9  | 78           |
| Indirect feature score > 4              | 17.5            | 93              | 38.8    | 81.4    | 2.4  | 0.8  | 77.5         |
| 0 direct + $\geq 5$ indirect            | 10              | 95.5            | 36.3    | 80.5    | 2.2  | 1    | 78           |
| 1 direct + $\geq 4$ indirect            | 10              | 95.5            | 22      | 79.6    | 2.2  | 1    | 77           |
| 2 direct + 0 indirect                   | NA              | 98.7            | NA      | 79.3    | NA   | 0.9  | 78.5         |
| 2 direct + 1 indirect                   | NA              | 98              | NA      | 79.3    | NA   | 0.9  | 78           |
| 2 direct + 2 indirect                   | NA              | 99.3            | NA      | 79.4    | NA   | 0.9  | 79           |
| 2 direct + $\geq 3$ indirect            | NA              | 98.7            | 60      | 80      | 5.7  | 1    | 80.1         |
| 3 direct + 0 indirect                   | NA              | 100             | NA      | 79.5    | NA   | 1    | 79.5         |
| 3 direct + 1 indirect                   | NA              | 100             | NA      | 79.5    | NA   | 1    | 79.5         |
| 3 direct + $\geq 2$ indirect            | 5               | 100             | 100     | 95.1    | NA   | 1    | 80.6         |

PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; NA, not acceptable.

of the maximum diameter of myoma uteri, the location of myoma uteri, and the overall dimensions of myoma uteri.

In our study, myometrial cysts of all sizes were included based on consensus among experts in the revised definitions of MUSA features of adenomyosis. Myometrial cysts were detected in 15.4% of the patients in the adenomyosis group. The sensitivity, specificity, and accuracy of myometrial cysts were 17.5, 79, and 70%, respectively. Bazot et al. reported that the presence of a myometrial cyst on TVS had low sensitivity (65.3%) but high specificity (97.5%) for adenomyosis, regardless of the patient group (4). According to Exacoustos et al., the existence of a myometrial cyst as the sole diagnostic feature for adenomyosis was detected in 53% of patients, with a high specificity (98%) and the highest accuracy (78%) (7). In contrast, myometrial heterogeneity alone emerged as the most sensitive feature (88%) (7). Kepkep et al. demonstrated that myometrial heterogeneity was the most sensitive (80.8%), echogenic subendometrial lines and buds were the most specific (95.5%), and the globular uterus was the most accurate (80%) criteria (8). Similar to Kepkep et al.'s result, echogenic subendometrial lines and buds were analyzed as the most specific (95.5%) feature. According to our study, the sensitivity (57.7%) and accuracy (59%) of the globular uterus were found to be low.

Although the groups in the study population were found to be similar in terms of leiomyoma, the predictivity of the globular uterus was found to be statistically significant. It was concluded that the globular uterus feature has an important place in the diagnosis of adenomyosis, even in the presence of leiomyoma. The results of our study are partially similar to those in the literature. The use of different sonography techniques and criteria in studies creates differences in the results.

In revised definitions of MUSA features of adenomyosis, researchers suggested that the echogenic subendometrial buds and lines feature may lead to diagnostic confusion between adenomyosis and malignancies in older and postmenopausal patients (5). In our study, gynecological malignancies were excluded from the study. Hyperechogenic islands and echogenic subendometrial buds and lines features were significant differences in the adenomyosis group. Echogenic subendometrial buds and lines and hyperechogenic islands had low sensitivity (15 and 17%, respectively). However, they had high specificity (95.5 and 94%, respectively), high NPV (81 and 94%, respectively), high accuracy (79 and 78%, respectively), and high positive LR (3.3 and 2.9, respectively). The predictivity of both criteria was found to be statistically significant in univariate regression analysis.

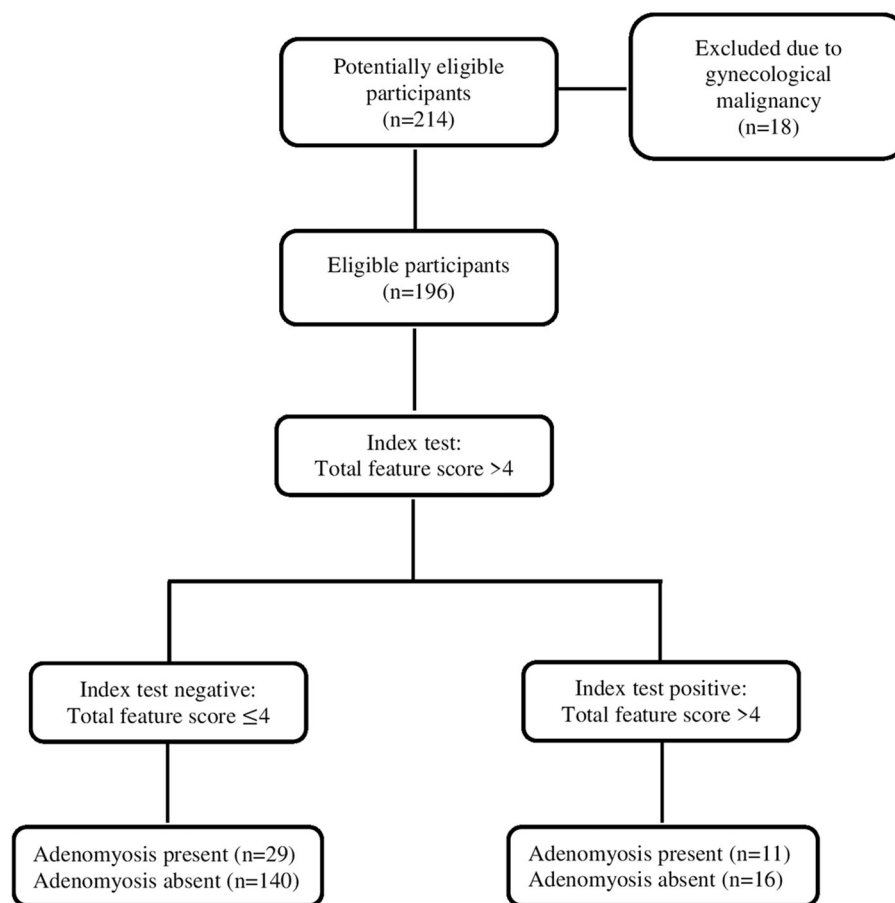


FIGURE 2  
STARD diagram to report the flow of participants throughout the study.

TABLE 6 Results of univariate logistic regression analysis for predicting adenomyosis.

| Variables                               | B      | S.E.  | Wald  | OR (95% CI)         | p-value |
|-----------------------------------------|--------|-------|-------|---------------------|---------|
| Myometrial cysts                        | 0.154  | 0.472 | 0.107 | 1.167 (0.463–2.940) | 0.7     |
| Hyperechogenic islands                  | −1.243 | 0.540 | 5.305 | 0.289 (0.100–0.831) | 0.02    |
| Echogenic subendometrial buds and lines | −1.323 | 0.588 | 5.067 | 0.266 (0.084–0.843) | 0.02    |
| Asymmetrical myometrial thickening      | −0.617 | 0.357 | 2.978 | 0.540 (0.268–1.087) | 0.08    |
| Globular uterus                         | −0.718 | 0.359 | 3.999 | 0.488 (0.241–0.986) | 0.04    |
| Fan-shaped shadowing                    | −0.671 | 0.358 | 3.512 | 0.511 (0.254–1.031) | 0.06    |
| Translesional vascularity               | −0.405 | 0.702 | 0.334 | 0.667 (0.169–2.637) | 0.5     |
| Irregular junctional zone               | −0.833 | 0.389 | 4.372 | 0.435 (0.199–0.949) | 0.03    |
| Interrupted junctional zone             | −0.588 | 0.400 | 2.155 | 0.556 (0.253–1.218) | 0.1     |

CI, confidence interval; OR, odds ratio.

The specificity, NPV, PPV, and accuracy of these direct features were remarkable.

It has been stated that the irregular junctional zone was weaker than other criteria in the revised definitions of MUSA features of adenomyosis (5). Contrary to this view, Tellum et al. reported that this feature reflects good discrimination ability (6). The common opinion in the literature is that junctional zone

evaluation should be performed by expert gynecologists in 3D TVS (5, 6, 12). According to our analysis, the irregular junctional zone feature was a significant difference in the adenomyosis group. The irregular junctional zone had 32.5% sensitivity, 82.6% specificity, and 82.6% NPV. Its predictivity was observed to be statistically significant in univariate regression analysis. In our study, junctional zone evaluation was performed by

TABLE 7 Results of multivariable logistic regression analysis for predicting adenomyosis.

| Variables                               | B*    | S.E.* | B <sup>†</sup> | T <sup>†</sup> | (95% CI)        | R    | R <sup>2</sup> | p-value |
|-----------------------------------------|-------|-------|----------------|----------------|-----------------|------|----------------|---------|
| Constant                                | 0.599 | 0.296 | –              | 2.025          | 0.015–1.182     | 0.08 | 0.08           | 0.04    |
| Hyperechogenic islands                  | 0.189 | 0.106 | 0.126          | 1.789          | –0.019 to 0.397 | –    | –              | 0.07    |
| Echogenic subendometrial buds and lines | 0.226 | 0.116 | 0.139          | 1.954          | –0.002 to 0.454 | –    | –              | 0.05    |
| Globular uterus                         | 0.127 | 0.056 | 0.157          | 2.255          | 0.16 to 0.239   | –    | –              | 0.02    |
| Irregular junctional zone               | 0.110 | 0.071 | 0.110          | 1.566          | –0.029 to 0.250 | –    | –              | 0.1     |

\*Non-standard coefficients.

†Standard coefficients.

CI, confidence interval.

an expert gynecologist on 3D TVS, as recommended in the current literature.

As stated in the revised definitions of MUSA features of the adenomyosis study, it is unclear which feature or features are required to diagnose adenomyosis (5). In our study, we determined the cutoff values for direct features >1 and indirect features >4. These values were not statistically significant. However, the total number of features was 1.5 times more significantly different in the adenomyosis group. Moreover, the cutoff value of total features >4 was statistically significant. Four combinations that provide this cutoff value were identified. However, the sensitivities of the combinations were found to be quite low. “0 direct + 5 indirect” and “1 direct +  $\geq 4$  indirect” were the weakest combinations among the others, with an accuracy of 78 and 77%, respectively. The combination with the highest specificity, PPV, NPV, and accuracy was “3 direct +  $\geq 2$  indirect” (100, 100, 95.1, and 80.6%, respectively). As mentioned in the revised definitions of MUSA features of the adenomyosis study, all three direct traits might not be present in the same uterus, and direct features are frequently modest and difficult to see. It could be simpler to identify indirect traits than direct ones (5). In this regard, the above combinations can be included in adenomyosis sonography practice.

Zannoni et al. aimed to determine the diagnostic accuracy of ultrasound features related to adenomyosis according to the MUSA statement and two additional markers (question mark sign and TVS uterine tenderness) (13). In the adenomyosis group, compared to the control group, the question mark sign was approximately 10 times higher and the uterine tenderness was  $\sim 2$  times higher. It has been reported in the literature that the question mark sign may be a marker of adenomyosis, which is strongly associated with posterior deep infiltrating endometriosis (14, 15). In that study, the question mark was proven to be an independent marker of adenomyosis. The question mark sign also showed great specificity (96%) and PPV (83%) for 2D TVS features. The authors reported that these results suggest that the question mark sign may have a broader application in diagnosing adenomyosis than previously thought. It is known that there is a relationship between adenomyosis and pelvic pain, especially in patients with adenomyosis accompanied by endometriosis. The use of TVS as a dynamic examination can indicate whether the pain is due to gentle pressure and mobilization of the uterus. The sensitivity of uterine tenderness was found to be 67.3%, and the NPV was 81%.

Several studies have described the relationship between ultrasound features of adenomyosis and clinical outcomes (16–18), but MUSA descriptions of ultrasound features have been addressed in only one of them (18). It was reported that women with TVS features of adenomyosis had more severe menstrual pain than women without these features, and a positive correlation was reported between the number of ultrasound features and the severity of menstrual pain (18). The relationship between the presence of one or more direct or indirect MUSA features and clinical symptoms, as well as the relationship between the number and size of features and their location and symptoms, also needs to be further investigated. Since the reference standard is hysterectomy, it is difficult to perform clinically useful diagnostic accuracy studies in women with suspected adenomyosis who are not planned for surgery. Additionally, there is no common guideline regarding histopathological diagnostic criteria for adenomyosis. For this reason, there is no standard approach among pathologists (5).

Raimondo et al. evaluated the diagnostic performance of the deep learning (DL) machine for the detection of adenomyosis on uterine ultrasonographic images and compared it to intermediate ultrasound skilled trainees (19). The DL model achieved a low diagnostic performance for the detection of adenomyosis with an accuracy of 51%, lower than that of intermediate-skilled trainees. The sensitivity of the intermediate-skilled trainees was higher than that of DL as well. However, the DL model showed potential for excluding adenomyotic uteri, with higher specificity and NPV than those of intermediate-skilled trainees (19).

The robustness of our study was underscored by several key strengths. First, the utilization of an updated classification system, optimized and standardized through the revised definitions of MUSA features of adenomyosis, ensures a contemporary and consistent framework for analysis. The incorporation of 3D TVS for the examination of features adds a layer of sophistication to our methodology, enabling a more nuanced and detailed assessment. Conducting the study within a single tertiary center contributes to result homogeneity, minimizing potential external influences. The limitations of our study were its retrospective design, the heterogeneity of hysterectomy indications, and the inclusion of patients with multiple and large leiomyomas. Additionally, while interobserver agreement assessment was conducted, intraobserver agreement assessment was not performed.

In conclusion, this study shows that combinations with a total number of features >4 can be practically used in the



evaluation of adenomyosis using the revised definitions of MUSA features. Prospective studies correlating ultrasound findings with standardized histopathological criteria and clinical findings will yield more accurate and precise results. Moreover, in the future, DL will be used more effectively in the diagnosis of adenomyosis.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by Dokuz Eylül University Institutional Ethics Committee (File number: 7737-GOA, Registration number: 2023/02-13). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

## Author contributions

OY: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original

draft, Writing – review & editing, Funding acquisition. AA: Formal analysis, Writing – original draft, Data curation. MÖ: Data curation, Software, Writing – original draft. BE: Data curation, Writing – original draft. SK: Supervision, Validation, Writing – review & editing. EU: Data curation, Writing – original draft. MG: Project administration, Supervision, Visualization, Writing – review & editing.

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## References

1. Ferenczy A. Pathophysiology of adenomyosis. *Hum Reprod Update*. (1998) 4:312–22. doi: 10.1093/humupd/4.4.312
2. Tahlan A, Nanda A, Mohan H. Uterine adenomyoma: a clinicopathologic review of 26 cases and a review of the literature. *Int J Gynecol Pathol*. (2006) 25:361–5. doi: 10.1097/01.pgp.0000209570.08716.b3
3. Naftalin J, Hoo W, Pateman K, Mavrellos D, Holland T, Jurkovic D. How common is adenomyosis? A prospective study of prevalence using transvaginal ultrasound in a gynaecology clinic. *Hum Reprod*. (2012) 27:3432–9. doi: 10.1093/humrep/des332
4. Bazot M, Daraï E, Rouger J, Detchev R, Cortez A, Uzan S. Limitations of transvaginal sonography for the diagnosis of adenomyosis, with histopathological correlation. *Ultrasound Obstet Gynecol*. (2002) 20:605–11. doi: 10.1046/j.1469-0705.2002.00852.x
5. Harmsen MJ, Van den Bosch T, de Leeuw RA, Dueholm M, Exacoustos C, Valentin L, et al. Consensus on revised definitions of Morphological Uterus Sonographic Assessment (MUSA) features of adenomyosis: results of modified Delphi procedure. *Ultrasound Obstet Gynecol*. (2022) 60:118–31. doi: 10.1002/uog.24786
6. Tellum T, Nygaard S, Lieng M. Noninvasive diagnosis of adenomyosis: a structured review and meta-analysis of diagnostic accuracy in imaging. *J Minim Invasive Gynecol*. (2020) 27:408–18.e3. doi: 10.1016/j.jmig.2019.11.001
7. Exacoustos C, Brienza L, Di Giovanni A, et al. Adenomyosis: three-dimensional sonographic findings of the junctional zone and correlation with histology. *Ultrasound Obstet Gynecol*. (2011) 37:471–9. doi: 10.1002/uog.8900
8. Kepkep K, Tuncay YA, Göynümer G, Tatal E. Transvaginal sonography in the diagnosis of adenomyosis: which findings are most accurate? *Ultrasound Obstet Gynecol*. (2007) 30:341–5. doi: 10.1002/uog.3985
9. Van Den Bosch T, Dueholm M, Leone FPG, Valentin L, Rasmussen CK, Votino A, et al. Terms, definitions and measurements to describe sonographic features of myometrium and uterine masses: a consensus opinion from the Morphological Uterus Sonographic Assessment (MUSA) group. *Ultrasound Obstet Gynecol*. (2015) 46:284–98. doi: 10.1002/uog.14806
10. Munro MG, Critchley HOD, Broder MS, Broder MS, Fraser IS, FIGO Working Group on Menstrual Disorders. FIGO classification system (PALM-COEIN) for causes of abnormal uterine bleeding in nongravid women of reproductive age. *Int J Gynaecol Obstet*. (2011) 113:3–13. doi: 10.1016/j.ijgo.2010.11.011
11. Liu Z, Guo Y, Pan X, Liu G, Yang X. Histopathological characteristics of adenomyosis: structure and microstructure. *Histol Histopathol*. (2023) 38:1099–107. doi: 10.14670/HH-18-594
12. Rasmussen CK, Van den Bosch T, Exacoustos C, Manegold-Brauer G, Benacerraf BR, Froyman W, et al. Intra- and inter-rater agreement describing myometrial lesions using morphologic uterus sonographic assessment: a pilot study. *J Ultrasound Med*. (2019) 38:2673–83. doi: 10.1002/jum.14971
13. Zannoni L, Ambrosio M, Raimondo D, Arena A, Forno SD, Borghese G, et al. Question mark sign and transvaginal ultrasound uterine tenderness for the diagnosis of adenomyosis: a prospective validation. *J Ultrasound Med*. (2020) 39:1405–12. doi: 10.1002/jum.15237
14. Di Donato N, Bertoldo V, Montanari G, Zannoni L, Caprara G, Seracchioli R. Question mark form of uterus: a simple sonographic sign associated with the presence of adenomyosis. *Ultrasound Obstet Gynecol*. (2015) 46:126–7. doi: 10.1002/uog.14750
15. Di Donato N, Seracchioli R. How to evaluate adenomyosis in patients affected by endometriosis? *Minim Invasive Surg*. (2014) 2014:507230. doi: 10.1155/2014/507230
16. Pinzauti S, Lazzari L, Tosti C, Centini G, Orlandini C, Luisi S, et al. Transvaginal sonographic features of diffuse adenomyosis in 18–30-year-old nulligravid women without endometriosis: association with symptoms. *Ultrasound Obstet Gynecol*. (2015) 46:730–6. doi: 10.1002/uog.14834
17. Naftalin J, Hoo W, Pateman K, Mavrellos D, Foo X, Jurkovic D. Is adenomyosis associated with menorrhagia? *Hum Reprod*. (2014) 29:473–9. doi: 10.1093/humrep/det451
18. Naftalin J, Hoo W, Nunes N, Holland T, Mavrellos D, Jurkovic D, et al. Association between ultrasound features of adenomyosis and severity of menstrual pain. *Ultrasound Obstet Gynecol*. (2016) 47:779–83. doi: 10.1002/uog.15798
19. Raimondo D, Raffone A, Aru AC, Giorgi M, Giaquinto I, Spagnolo E, et al. Application of deep learning model in the sonographic diagnosis of uterine adenomyosis. *Int J Environ Res Public Health*. (2023) 20:1724. doi: 10.3390/ijerph20031724



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# The vicious cycle of chronic endometriosis and depression—an immunological and physiological perspective

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Endometriosis is a chronic, estrogen-dependent, proinflammatory disease that can cause various dysfunctions. The main clinical manifestations of endometriosis include chronic pelvic pain and impaired fertility. The disease is characterized by a spectrum of dysfunctions spanning hormonal signaling, inflammation, immune dysregulation, angiogenesis, neurogenic inflammation, epigenetic alterations, and tissue remodeling. Dysregulated hormonal signaling, particularly involving estrogen and progesterone, drives abnormal growth and survival of endometrial-like tissue outside the uterus. Chronic inflammation, marked by immune cell infiltration and inflammatory mediator secretion, perpetuates tissue damage and pain. Altered immune function, impaired ectopic tissue clearance, and dysregulated cytokine production contribute to immune dysregulation. Enhanced angiogenesis promotes lesion growth and survival. Epigenetic modifications influence gene expression patterns, e.g., HSD11B1 gene, affecting disease pathogenesis. Endometriosis related changes and infertility lead to depression in diagnosed women. Depression changes lifestyle and induces physiological and immunological changes. A higher rate of depression and anxiety has been reported in women diagnosed with endometriosis, unleashing physiological, clinical and immune imbalances which further accelerate chronic endometriosis or vice versa. Thus, both endometriosis and depression are concomitantly part of a vicious cycle that enhance disease complications. A multidimensional treatment strategy is needed which can cater for both endometrial disease and depression and anxiety disorders.

## KEYWORDS

estrogen, endometriosis, estrogen receptor, inflammation, depression, immune imbalance

## 1 Introduction

Endometriosis stands as one of the commonly encountered benign gynecological conditions in women, where endometrial glands and stroma exhibit extrauterine location, with a prevalence ranging from 6 to 10% among those of reproductive age (1, 2). Aberrant endometrial cells, characterized by genetic polymorphisms and proliferation rather than

apoptosis, in response to local signals, lead to disease progression. Additionally, these cells when anomalously displaced into the peritoneal cavity, not only evade peritoneal destruction but also exploit the immediate environment to sustain proliferation in a clonal manner, while normal cells of the individual are systematically removed. Despite its non-malignant character, the inflammatory and erosive nature of the disease contributes to enduring alterations in a woman's life, manifesting as persistent pelvic pain, dysmenorrhea, dyspareunia, and infertility (3). The disease can lead to additional symptoms such as painful bowel movements or urination, excessive bleeding, fatigue, diarrhea, constipation, bloating and nausea (4, 5). The challenge is exacerbated by the recurrent delay in diagnosis following the manifestation of symptoms and the restricted scope of available intervention strategies. Despite the potential existence of endometriotic lesions in asymptomatic women, a conclusive diagnosis of endometriosis is typically established when the presence of endometrial tissue or lesions is established beyond the confines of the uterus, frequently through surgical means (6). Endometriosis exhibits diverse classifications based on its anatomical location, including superficial peritoneal lesions which is the most common, ovarian endometrioma, deep sub-peritoneal infiltrating endometriosis and adenomyoma, which represents internal endometriosis within the myometrium (7). Endometriotic lesions have been identified in extra-pelvic locations, such as upper abdominal visceral organs, abdominal wall, diaphragm, and pleura, as well as within the nervous system (8). Patients may exhibit various forms concurrently.

The predominant classification method in use is an updated scoring system established by the American Society for Reproductive Medicine. This system is employed to ascertain the stage of endometriosis, denoted by Roman numerals I to IV, which represent the spectrum from 'minimal' to 'severe'. It involves an assessment of type, location, appearance, depth of lesions, and an evaluation of overall extent of disease as well as presence of adhesions (9). However, grading using the ASRM criteria often demonstrates weak correlations of the abundance and location of lesions with the type of lesions, and symptoms of pain reported by patients, when compared to the disease stage. The occurrence of endometriosis in asymptomatic women, along with ambiguous reasons for its manifestation, contributes to varying perspectives on considering endometriosis as a 'syndrome' (10). Diagnosis is typically established only when a patient presents with both observable lesions and symptomatic manifestations.

An in-depth understanding of immune imbalance in endometriosis related depression and vice versa may include enhanced immune cell function, altered cytokine and chemokine levels and malfunctioning of regulatory proteins such as growth factors. Major immune cells such as macrophages, neutrophils, dendritic cells (DCs), natural killer cells (NK cells), T cells, and B cells exhibit great importance in the pathogenesis of endometriosis and depression. Increased levels of macrophages were observed in the peritoneal fluid of endometriotic patients (11). Neutrophil to lymphocyte ratio (NLR) was shown to be a clinically relevant indicator of endometriosis and associated outcomes. Increased NLR was also observed in a recent study showing higher numbers of neutrophils in endometriosis subjects (12, 13). Dendritic cells are important antigen presenting cells and in endometriotic patients, peritoneal DCs are found to increase. Furthermore, numbers of immature DCs are found to be greater as compared to mature DCs (14).

Endometriosis is associated with dysfunction in NK cell cytotoxicity and immunomodulation, by tolerating or inhibiting implantation, proliferation, and survival of endometrial cells, impairing their ability to eliminate these cells at ectopic sites (15). This review also sheds light on the role of the adaptive immune response in endometriosis, including helper T and B cells, whose roles remain incompletely understood. Several serum cytokines such as interleukins (IL) IL1 $\beta$ , IL-5, IL-6, IL-7 and IL-12 are involved, and their levels were found to be altered in endometriosis as compared to in healthy women (16). Cytokines IL-6, IL-8, IL-10, TNF- $\alpha$  also have important roles in the development of VEGF, which is involved in the pathogenesis of the disease (17–23).

Chronic stress or chronic depression events can modulate innate and adaptive immune responses with the involvement of enhanced inflammation and lowering the activity of immune protective cells (24). Inflammatory responses can be increased in stress (25). Furthermore, animal studies, showed that administration of proinflammatory cytokines (TNF or IL-1 $\beta$ ) affect the central nervous system through decreased motor activity as well as increased social alienation, disturbed sleep patterns, altered appetite, reduced water intake and greater sensitivity to pain (26–28). Immune dysregulation and associated outcomes are the hallmarks of endometriosis. Immune dysregulation has also been shown to cause depression in susceptible individuals and hence may be the primary cause of depression in women with endometriosis.

Women diagnosed with endometriosis exhibit imbalanced immunological states often because of which major lifestyle changes are inevitable (29–31). Endometriosis patients may undergo mental health issues such as depression, physiological stress and anxiety (32). These women may bear day-to-day abdominal pain, painful bowel movements or urination, excessive bleeding, fatigue, diarrhea, constipation, bloating, nausea, fatigue and painful intercourse (4, 5), leading to a stressful life. In chronic cases infertility is very common (3, 33). This review aims to explore the potential links between depression, immunological factors and endometriosis.

## 1.1 Literature search for the review article

An electronic literature search was meticulously carried out by the authors S.S., M.W.A.K, S.R., K.M., Q.H., and W.A.K., as published by Centini et al. (34). The search team evaluated the existing literature on endometriosis, which included disease identification, symptoms, diagnosis, pathogenesis and immune dysregulations. The search was performed using the online medical MEDLINE database (accessed via PubMed). Terminologies included endometriosis, biomarkers, endometriotic symptoms and diagnosis, gynecological issues in endometriosis, pathogenesis in endometriosis, endometriotic depression. This review includes the most updated published articles as well as original articles which include randomized and non-randomized clinical trials, prospective observational studies, retrospective cohort studies, and case-control studies, review articles, and case reports. The selected articles were further checked for relevance with the aim and objective of the review. The bibliography of the selected articles was thoroughly checked for additional relevant articles. This procedure effectively helped in compiling more relevant, updated and high-quality peer-reviewed articles, providing a nuanced understanding

of the specified topics “endometriosis, depression, and their associated immune imbalances.”

## 1.2 Etiology and incidence

Various physiological factors, including hormonal, metabolic, neurological, and immunological elements, play a role in the processes leading to the manifestation of symptoms. Epidemiological investigations reveal an increased susceptibility to various cancers (ovarian, breast and melanoma), rheumatoid arthritis, asthma and cardiovascular disease among women with endometriosis lesions (10). Endometriosis has familial incidence with heritability of up to 50% (35). It has been reported that having a first degree relative with a severe form of endometriosis raises the risk by up to seven times (36). A study focusing solely on relatives of individuals with endometriosis revealed that 16% of mothers and 22% of sisters of reproductive age had received a surgical diagnosis of endometriosis (36). Genome-wide association studies have found overrepresented single nucleotide polymorphisms (SNPs) in cases of severe disease. Gynecological disorders such as infertility, fibroids, and cancer were found to have overlaps with common SNPs associated with endometriosis, the etiology of which all involve steroid hormones (34, 37–40).

Additionally, five loci significantly associated with endometriosis risk were identified through a meta-analysis of 11 GWAS datasets, which genetically involved sex steroid hormone pathways (41). Irregularities in the role of extracellular matrix protein signaling such as fibronectin (42), laminin (43) and collagen (44) are implicated in abnormal cell migration and adhesion, contributing to fibrosis. Genomic studies have revealed associations between endometriosis and various biological pathways and cellular regulators. Notably, vezatin, a transmembrane adherens junctions’ protein, has been implicated (45), along with vascular endothelial growth factor receptor 2 (VEGFR-2) (46), the mitogen activated protein (MAP) kinase signaling cascade (47), IL1A (48), wingless related integration site (WNT) signaling (49), and steroid metabolism (50). Meta-analysis has highlighted common genetic signatures between migraine and depression in endometriosis, of which depression underscores an association with changes in gut mucosa (51). Additional determinants for endometriosis are low BMI, low birth weight, lower parity, Mullerian abnormalities, early menarche, short menstrual cycles or heavy and prolonged menstrual flow. Scientific evidence indicates variations in prevalence of endometriosis diagnosis across racial and ethnic groups. A systematic review revealed that Asian women exhibited an elevated risk, while Black women demonstrated a reduced risk compared to White women. However, it is plausible that these estimates may be influenced by biases linked to diagnosis and healthcare accessibility (52). The prevalence of endometriosis amongst Asian women of reproductive age is reported to range from 6.8% to as high as 16% (53) (see Table 1).

## 2 Clinical symptoms and diagnosis

Medical diagnosis of endometriosis is often difficult and delayed due to a lack of awareness and knowledge of the condition among healthcare professionals and limited understanding of its pathogenesis (35). Further, the complex nature of the disease as well as its

**TABLE 1** Meta-analysis of genome wide association studies on endometriosis.

| No. | Article                    | Reference | Gene/ pathway                                                                               |
|-----|----------------------------|-----------|---------------------------------------------------------------------------------------------|
| 1   | Gallagher et al., 2019     | (37)      | WNT4, CDC42, GREB1, ESR1, FSHB                                                              |
| 2   | Masuda et al., 2020        | (38)      | GREB1, LOC730100, PDE1C, TNRC6B                                                             |
| 3   | Sapkota et al., 2017, 2015 | (41, 50)  | WNT4, GREB1, ETAA1, ILIA, KDR, ID4, 7p15.2, CDKN2B, VEZT, FN1, CCDC170, SYNE1, 7p12.3, FSHB |

manifestations, varying from asymptomatic to its evident phenotypes, add to a complicated diagnosis (3). Pelvic pain stands out as the primary indicator of endometriosis, manifesting in various forms such as dysmenorrhea, dyspareunia, or chronic pelvic pain (54). The intensity of pelvic pain is correlated with type of lesion classification and disease progression (55). Additional symptoms which are commonly found in individuals with the disease include abdominal discomfort, bloating, menometrorrhagia, lower back pain, and fatigue (3). Surgery remains the main method of obtaining a conclusive histopathological diagnosis, with Laparoscopy considered the gold standard diagnostic test. However, prevailing guidelines advocate for a non-surgical diagnostic approach reliant upon symptomatology, physical examination outcomes, and imaging findings. This strategy aims to mitigate delays in commencing treatment. In female patients undergoing surgical interventions, more than 50% will necessitate subsequent surgical interventions within a five-year timeframe (1). Numerous hormonal medical interventions are associated with adverse effects (56).

Research indicates that the greatest prevalence of endometriosis is observed between 25 and 29 years of age (57). However, there is often a significant diagnostic delay, with the average time from the onset of first symptoms to final diagnosis ranging from 4.4 years in the United States to 10.4 years in Germany (58, 59). The primary reasons for this delay may include intermittent use of contraceptives, misdiagnosis, and self-treatment of pain with over-the-counter painkillers. These findings align with the presented study’s results, which report a mean age of 26.9 years at the time of disease recognition and symptom onset ranging from 18.8 years for dysmenorrhea to 24.0 years for dyspareunia. This underscores the importance of early and accurate diagnosis to mitigate prolonged suffering and improve patient outcomes.

Central sensitization (CS) is a type of nociplastic pain characterized by a central nervous system response to peripheral nociceptive or neuropathic triggers, often seen in patients with chronic pains (60). Symptoms of CS include chronic pain, allodynia (pain from stimuli that do not usually provoke pain), hypersensitivity, hyperalgesia (increased sensitivity to painful stimuli), and mood changes (anxiety, panic attacks, and depression) (61–63). A Central Sensitization Inventory (CSI) score of 40 or higher has been effective



in identifying CS in women with chronic pelvic pain, including those with endometriosis (62, 64). In a recent study, it has been showed that in endometriosis patients, CS can significantly worsen pain symptoms and is prevalent particularly among those with moderate to severe chronic pelvic pain, involvement of the posterolateral parametrium, high tone pelvic floor (HTF), and comorbid with central sensitivity syndromes like irritable bowel syndrome, anxiety, migraines or severe headaches (65). Therefore, recognizing and addressing CS is crucial for early and accurate diagnosis to mitigate prolonged suffering and improve endometriosis patient outcomes.

The need for reliable noninvasive biomarkers for the diagnosis, potential treatment response and disease prognosis persists as a significant unaddressed requirement. While certain types of endometriosis diagnosis can be expedited through imaging modalities, progress towards validating a dependable noninvasive blood test has been sluggish thus far (66). Other non-surgical diagnostic methods such as transvaginal ultrasonography and magnetic resonance imaging (MRI) have enabled identification of deep endometriosis types (67).

## 3 Hormones

Female sex hormones, estrogen and progesterone, play critical roles in the pathogenesis of endometriosis. Increased levels of estrogen with decreased progesterone receptor pathway signaling are implicated in disease pathogenesis (Figure 1).

### 3.1 Enhanced estrogen production

Elevated estrogen production consistently emerges as a dysregulated endocrine characteristic in eutopic endometrium and ectopic endometriotic lesions. The predominant estrogen, estradiol (E2), has a pivotal role in the post-menstrual endometrial regeneration (7). Both proliferation of endothelial cells and the re-establishment of microvasculature in this layer are orchestrated by E2, through interactions with its estrogen receptors (ERs), ER $\alpha$  and ER $\beta$  (68). Distinct intracellular localizations of the ERs lead to intricately coordinated and precisely regulated estrogen (E2) signaling pathways, which govern cellular proliferation, differentiation, and apoptosis. Endometrial E2 predominantly originates from the ovaries and, to a lesser extent, from adipocytes and the adrenal gland, transported to tissues through the circulatory system (69). Aromatase P450 (aromP450) is a rate limiting hormone in estrogen biosynthesis that catalyzes the conversion of androgens to estrogen, with subsequent transformation into E2 facilitated by 17 $\beta$ -hydroxysteroid dehydrogenase type 1 (17 $\beta$ HSDT1) (70). Prostaglandin E2 (PGE2) synthesis step is initiated by the rate-limiting cyclooxygenase-2 (COX-2) enzyme, acting on arachidonic acid, inducing dose-dependent aromatase P450 synthesis in endometriotic lesions (69). In healthy women's endometrium, aromatase P450 activity is negligible (71). Cell-specific and menstrual cycle phase-dependent expression of receptors that bind to estrogens (ER $\alpha$ , ER $\beta$  and GPER1), androgens, progestins and glucocorticoids are observed in the healthy endometrium (72). However, both the endometrium and ectopic endometriotic lesions in women with endometriosis exhibit significantly elevated levels of aromatase P450, facilitating local E2

production. The capacity of the lesion to independently generate E2, coupled with the synthesis of the necessary enzymes, may enhance intraperitoneal endometriotic tissue implantation (56). It has been observed that the expression of ER $\beta$  is extraordinarily higher in stromal cells of women with endometriosis as compared to ER $\alpha$ . It is suggested that rather than just estrogen dependent, endometriosis should be considered steroid-dependent. Thus, the abnormal functioning of estrogen, its receptors, and estradiol synthesis-related enzymes is closely associated with endometriosis.

### 3.2 Progesterone resistance

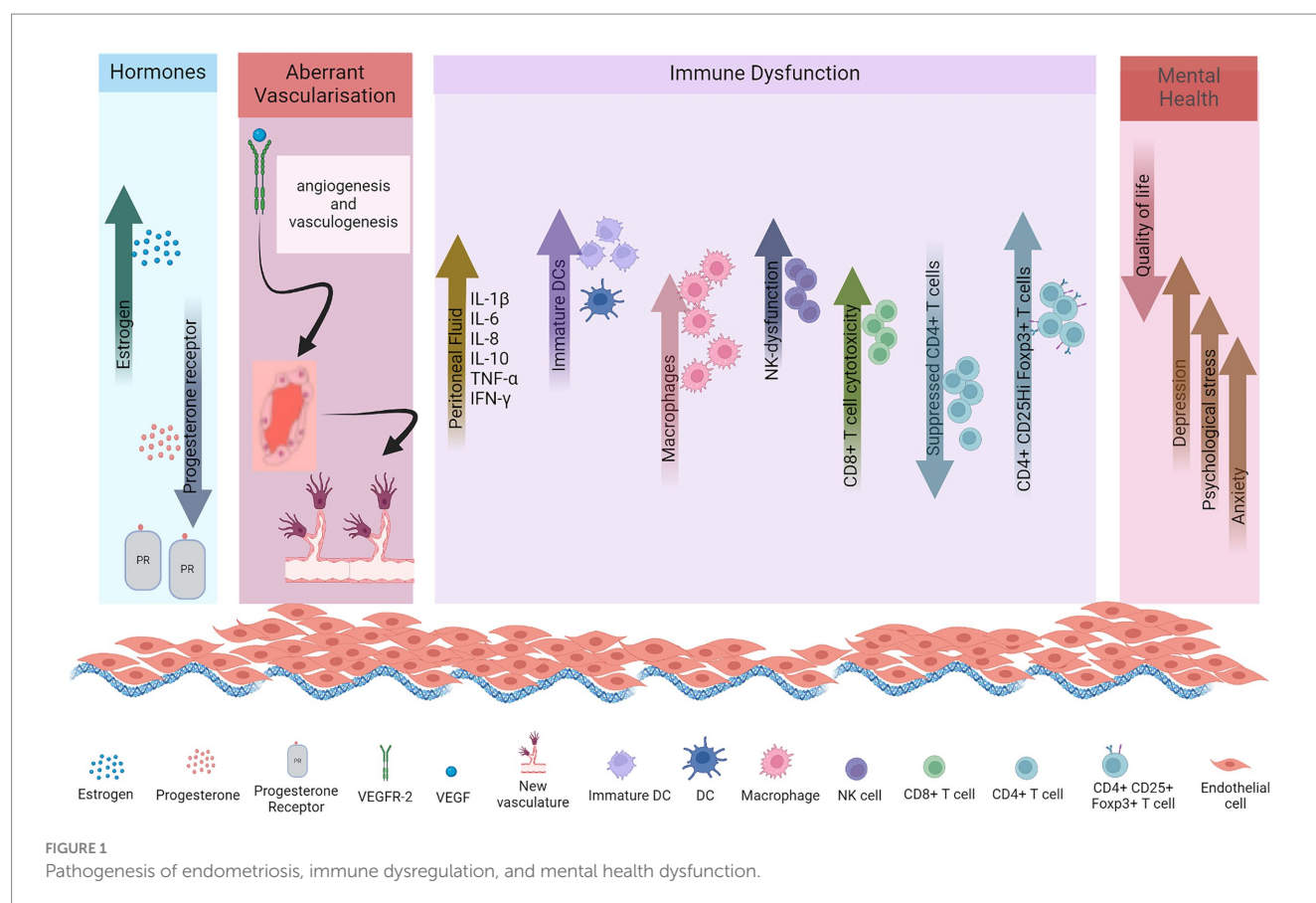
Progesterone is the dominant hormone in the secretory phase of the menstrual cycle, where it counteracts effects of estrogen and prepares the uterus for supporting an embryo. It plays a decisive role in facilitating the differentiation of endometrial epithelial and stromal cells. Suppressed progesterone receptor (PR) expression, a characteristic feature of endometriosis, leads to resistance to progesterone and contributes to the development of severe endometriosis conditions (Figure 1). Endometriotic stromal cells demonstrate resistance to progesterone with reduced responsiveness to hormone (73). This diminished communication between stromal and epithelial cells leads to a subsequent elevation in the expression of ER $\beta$  within endometriotic lesions and stromal cells (1). PR-A and PR-B are the two functionally distinct receptor isoforms which interact with progesterone. In mice, the absence of PR-A results in abnormalities in the ovary and uterus, while the lack of PR-B has negligible impact on their function (74). Notably, the transcript for both receptor isoforms originate from the same gene, with PR-A having a shorter transcript than PR-B. This structure allows transrepression of PR-B and other nuclear receptors (75). Lesions in endometriosis exhibit a deficiency in PR-B expression, with minimal expression of the transrepressor PR-A, offering molecular substantiation for progesterone resistance. Subsequently, this leads to elevated local levels of estrogen (E2) as progesterone fails to stimulate 17 $\beta$ -hydroxysteroid dehydrogenase type 2 (17 $\beta$ -HSDT2) (69).

## 4 Aberrant vascularisation

The normal endometrium constitutes a steroid responsive tissue comprising richly vascularized epithelial and stromal cells as well as a diverse range of immune cells. Cells released from this tissue during menstruation encompass epithelial cells, stromal fibroblasts, vascular cells, and immune cells (neutrophils, monocytes, macrophages and uterine natural killer cells) (76). In retrograde menstruation, these cell types can potentially lead to lesions provided they maintain viability and evade the innate immune response and clearance within the intraperitoneal space. The three most implicated cells in peritoneal lesions are stem/progenitor cells, stromal fibroblasts, and immune cells, particularly stromal and immune cells, which play pivotal roles.

Endometriosis is postulated to originate due to endometrial fragment implantation within the peritoneal space. It potentially employs angiogenesis and vasculogenesis mechanisms to develop vascularization, essential for its sustenance (77, 78). The viability of endometriotic implants within the peritoneal cavity relies on





establishing a blood supply to deliver oxygen and nutrients to the developing lesions. Concurrent with endometrial growth, the endometrial vasculature undergoes cyclical proliferation and regeneration orchestrated by ovarian steroids, particularly E2. Vascular endothelial growth factor (VEGF) serves a pivotal function in initiating angiogenesis in endometriosis, particularly in ectopic lesions (69, 79). As a vasoactive agent, it participates in numerous physiological functions, such as reestablishment of a vascular network and subsequent healing of the uterus, by modulating proliferation and migration of endothelial cells. Heightened expression of VEGF mRNA in the superficial endometrial layer was reported during both the two phases of the uterine cycle, i.e., proliferative and secretory, suggesting ongoing angiogenesis (80). Furthermore, it was also demonstrated that estradiol was responsible for stimulating expression of VEGF in endometrial cells. Administration of E2 resulted in elevated levels of VEGF mRNA expression compared to endometrial cells not exposed to E2 stimulation. Given the intrinsic angiogenic capacity of healthy endometrium regulated by estradiol, it becomes apparent that dysregulated VEGF expression and E2 levels promote neovascularization in lesions, facilitating their establishment in ectopic sites. Studies indicate that peritoneal fluid (PF) from subjects with advanced endometriosis harbors elevated VEGF concentrations versus those with mild disease or healthy individuals (81). Various immune cells participate in angiogenesis by generating and subsequently increasing levels of proinflammatory and angiogenic cytokines, as well as cellular adhesion factors within the PF, surrounding endometriotic lesions. Secretion of VEGF by neutrophils and macrophages within intraperitoneal lesions facilitates

angiogenesis (82). Disruptions in peritoneal homeostasis, coupled with the induction of proinflammatory and proangiogenic cytokine production in endometriosis, collectively contribute to modified innervation and the modulation of pain pathways in affected individuals (54). DCs have also been linked to angiogenesis (83). This was evidenced by a study revealing heightened perivascular localization of VEGFR-2 secreting immature dendritic cells within such lesions. These DCs exhibited the ability to stimulate endothelial cell migration *in vitro*. Intraperitoneal DCs in the peritoneal cavity led to the development of endometriotic lesions in the murine model (84). An investigation employing a transgenic murine model featuring diphtheria toxin mediated conditional depletion of DCs, scientists observed that endometriotic lesions in DC-depleted mice exhibited notable increased size versus control counterparts, along with reduced CD69 expression, indicative of antigen stimulated T and natural killer cell activation. These results underscore the direct involvement of DCs in regulating the angiogenic process and modulating immune activation subsets during the development of lesions (85). Endometrial cells exhibit enhanced resistance to cell mediated immunity, alongside enhanced proliferation and heightened aromatase expression, culminating in elevated estrogen levels (69, 70, 86).

Comparative studies investigating stromal fibroblast phenotypes in women with endometriosis have revealed behavioral disparities, notably epigenetic alterations leading to aberrant responses to estrogen (87). It is plausible that cell plasticity evolved to expedite endometrial repair post-menstruation, leading to multicellular lesion formation in extrauterine locations. Mechanistic similarities between

menstrual regulation and lesion formation encompass transient hypoxia (88), iron release, and platelet activation (89, 90).

## 5 Immune dysfunction

Endometrial lesions adhere to the peritoneum or are closely associated with the ovaries, exposing them to an altered peritoneal environment comprising immune cells, cytokines, and regulatory proteins such as growth factors, with a high potential for anomalous behavior of these entities. Endometriosis animal model studies are suggestive of the fact that immune cells within lesions consist of a combination of cells from endometrial shedding as well as cells from peritoneal microenvironment (91). Fragments of endometrial tissue elicit intraperitoneal inflammation, which results in activation and recruitment of neutrophils and macrophages to the area. Hence, women with the disease often exhibit elevated concentrations of activated macrophages secreting proinflammatory and chemotactic cytokines in the peritoneal fluid (92). Given that various estrogen receptors are expressed on both macrophages and nerve fibers, estrogen is postulated to modulate macrophage and nerve fibers behavior. Thus, estrogen regulation encompasses macrophage recruitment, atypical neurogenesis atypical inflammation observed in endometriosis (93).

### 5.1 Cytokines

Several studies were conducted for the involvement of cytokines in the pathogenesis of endometriosis (Figure 1) (16–23). Multan et al., found that serum cytokines IL1 $\beta$ , IL-5, IL-6, IL-7 and IL-12 levels were elevated in serum samples of endometriosis patients compared to normal women (16). Cytokines (IL-6, IL-8, IL-10, TNF- $\alpha$ ) and chemokines (CCL-2) as well as growth factor VEGF increased in peritoneal fluid of patients (18–23).

Nerve fibers demonstrate an exceptional capacity to recruit macrophages to the injury site. Numerous mediators identified in this process including leukemia inhibitory factor, IL1 $\alpha$ , IL1 $\beta$  (94) and pancreatitis-associated protein 3 (PAP3) (95). Estrogen has also been shown to promote colony-stimulating factor 1 and C-C motif ligand 2 (CCL2) secretions from PNS, thereby amplifying macrophage movement towards lesions (96). Additionally, macrophages contribute to the proliferation of peritoneal implants and act as significant sources of angiogenic factors like TNF- $\alpha$  and IL-8. They also contribute to hypoxia-induced angiogenesis (92).

Endometriosis, like cancer, can be categorized as a metabolic disorder. Under the influence of transforming TGF- $\beta$ 1, tumor cells adopt aerobic glycolytic phenotype, leading to enhanced lactate secretion and accumulation (97). Elevated levels of TGF- $\beta$ 1 and lactate are observed in endometriotic PF. Concurrently, there is a shift from typical mitochondrial phosphorylation to glycolysis in the mesothelial cells lining the peritoneum to support cell survival in a tumor like microenvironment (98). Like in tumorigenesis, endometrial cells also exhibit the Warburg effect, where cells adjacent to tumors exhibit a programmed utilization of aerobic glycolysis induced by TGF- $\beta$ 1, leading to lactate production. This lactate serves as a nutrient source for neighboring tumor cells, thereby establishing a cohesive metabolic microenvironment conducive to tumor progression (99). Lactate

induces lactylation or the covalent modification of lysine residues on histones and other proteins. Research findings indicate that elevated levels of lactate and lactate dehydrogenase-A, contribute to enhanced lactylation of histone H3 lysine 18 in ectopic endometrial tissues and ectopic endometrial stromal cells, compared to normal cells (100). Furthermore, lactate promotes cell proliferation, migration, and invasion in endometriosis progression, further linked to immune suppression and possible transformation to a malignant form.

### 5.2 Macrophages

Macrophages represent the predominant immune cell population in the peritoneum. Alterations in macrophage phenotype, or polarization, are linked to significant metabolic shifts. The peritoneal fluid of patients with endometriosis exhibits increased levels of macrophages (11), as shown in Figure 1. These macrophages do not effectively clear endometrial tissue; instead, they significantly contribute to high levels of cytokines (95). Proinflammatory macrophages primarily rely on glycolysis, whereas anti-inflammatory M2 macrophages exhibit a greater dependence on oxidative phosphorylation (101). Moreover, macrophages produce angiogenic mediators, such as TNF- $\alpha$  and IL-8, thereby promoting the growth of lesions (102). While macrophages appear to play a role in the growth and development of endometriotic tissue, depletion of macrophages does not prevent the implantation of endometrial cells in the peritoneum.

### 5.3 Neutrophils

Neutrophils are postulated to essay a pivotal role in endometriosis pathogenesis. Neutrophils significantly contribute to the resolution of inflammatory responses. A study found that when neutrophils from healthy women were exposed to endometrial plasma or PF, reduced neutrophil apoptosis was observed versus controls, elucidating the presence of antiapoptotic factors in the plasma and PF (12). Interleukin-8 stood out in the study due to its proinflammatory nature and its involvement in neutrophil chemotaxis during inflammation (12).

### 5.4 Dendritic cells

Dendritic cells are antigen-presenting cells which initiate and modulate adaptive immune responses. DCs additionally serve a crucial function in the prevention of autoimmunity by functioning as mobile sentinels. They transport self-antigens to naïve T cells residing in lymphoid organs, thereby facilitating the induction of self-tolerance (103). In healthy women, immature dendritic cells are absent from the peritoneal membrane. In endometriosis they are present within endometriotic lesions and adjacent to peritoneum. Additionally, the numbers of mature DCs are significantly reduced in the endometrium throughout the menstrual cycle in women with endometriosis compared to those with healthy endometrium (Figure 1). Endometriotic conditions may impede the maturation of immature DCs and prompt their transition into a macrophage phenotype. Moreover, the progression and

vascularization of lesions necessitate the presence of endogenous DCs, which infiltrate these lesions and augment endothelial cell migration through the secretion of proangiogenic factors (104). In murine models, the cell density of peritoneal dendritic cells increased promptly following the injection of endometrial tissues, peaking at 14 days. The proportion of mature DCs within peritoneal DCs initially decreased post-injection, then gradually rose over time, although remaining lower than the control group at 42 days. Conversely, the proportion of immature DCs exhibited contrasting changes (14). The administration of lipopolysaccharide resulted in a significant increase in mature DCs proportion, consequently leading to reduced volume and weight of endometriosis lesions. While DC maturation suppresses the angiogenic response, immature DCs actively promote angiogenesis and lesion growth, thus undergoing a shift in their immunological function from antigen presentation to supporting angiogenesis and the progression of the disease.

## 5.5 Natural killer

NK cells are cytotoxic effector lymphocytes of the innate immune response characterized by their capacity to induce lysis of target cells independent of prior antigen exposure. Endometriosis is associated with a dysfunction in NK cell cytotoxicity and immunomodulation, by tolerating or inhibiting implantation, proliferation, and survival of endometrial cells, impairing their ability to eliminate these cells at ectopic sites (15). A study identified soluble immunosuppressive factors present in the media of both normal endometrial cells and endometriotic stromal cells. Healthy endometrium possesses immunosuppressive capabilities against NK cell cytotoxicity, potentially facilitating embryo implantation (Figure 1). However, in endometriosis, the immunosuppression is more pronounced, potentially allowing retrogradely displaced endometrial tissue to develop into lesions within the peritoneal environment (105). Functional defects and dysregulation of NK cell cytotoxicity are attributed to various cytokines and inhibitory factors present in both serum and PF. The reduction in NK cytotoxicity appears to result from functional defects. The dysregulated cytotoxicity of peritoneal NK cells in endometriosis can be attributed to various cytokines (IL-6, IL-8, IL-1 $\beta$ , IFN- $\gamma$ , and TNF- $\alpha$ ) and inhibitory factors present in both serum and peritoneal fluid. Also, for such patients, there is a notable reduction in the populations of mature NK cells (CD32CD56+), while immature NK cells are elevated in the PF, leading to apoptosis (106). The observed abnormalities in NK cells among women with endometriosis may indeed be outcomes resulting from the local regulation of microenvironment due to the pathology itself.

Treatment modalities such as inhibition of receptor-ligand interactions involving KIR2DL1, NKG2A, LILRB1/2, and PD-1/PD-L1, TGF- $\beta$ ; stimulation of NK cells via IL-2; and mycobacterial therapy utilizing *Bacillus Calmette-Guérin* (BCG) (82, 107–109). Moreover, ongoing research is exploring the potential of adoptive NK cell therapy for managing endometriosis. Endometriosis holds promise as a candidate for immunotherapy aimed at blocking negative regulatory checkpoints of NK cells, such as inhibitory NK cell receptors. Attenuating the cellular cytotoxicity of NK cells could potentially mitigate the progression of pelvic pain in individuals affected by the disease. The principal inhibitory receptors on NK cells,

which are potential checkpoints for eradication of ectopic endometrial tissue, are leukocyte immunoglobulin-like receptors (LILRs).

## 5.6 T and B cells

Adaptive immune response entails helper T and B cells, in endometriosis, which remains incompletely understood. A study showed that a higher number of CD8 T cells are present in endometriotic lesions compared to eutopic endometrium (110). However, in blood circulation the CD8 T cell populations show no difference between patients and healthy women. It has been noted that CD8 T cell cytotoxicity is enhanced in menstrual effluent of patients, specifically CD8 T effector memory cells are enriched in eutopic endometrium of patients (Figure 1) (110).

Suppressed CD4 T cells have been reported in endometriosis due to the systemic and local alterations in immune responses (Figure 1). These impaired CD4 T cells potentially contribute to the pathogenesis of endometriosis disease through cytokines, which are important for implantation and proliferation of ectopic endometrial cells, inflammation and angiogenesis (111). In women with this condition, there appears to be a bias towards Th2 cell polarization, as evidenced by robust intracellular IL-4 expression and the absence of IL-2 in ectopic lesion derived lymphocytes (82). The equilibrium of CD4 cells in endometriosis remains contentious, with studies indicating reduced activation of both Th1 and Th2 cells in the peritoneal fluid of affected individuals (110).

Regulatory T (Treg) cells constitute a distinct subset within the T cell population, balancing immunological self-tolerance and homeostasis, thus modulating the immune system's response to prevent excessive reactions against the host (97). Nevertheless, the precise involvement and significance of Treg cells in the context of endometriosis remain inadequately elucidated. The Forkhead box 3 protein (Foxp3), identified as a pivotal transcriptional factor, serves as a master regulator gene governing the differentiation of CD4+ Treg cells (112). Berbic et al. (113), demonstrated heightened expression levels of Foxp3 within both eutopic and ectopic endometrial tissues during the secretory phase of the menstrual cycle in patients afflicted with endometriosis. Furthermore, elevated Foxp3 expression at the messenger RNA level within ovarian endometrioma tissue (114), along with a relatively higher ratio of CD4+ Foxp3+ cells within the CD4+ cell population (115).

Additionally, recent studies have shown a significant increase in the proportion of CD4+ CD25hiFoxp3+ cells within the PF, but not in peripheral blood, of endometriosis patients, as opposed to those without the disease (Figure 1) (116, 117). These collective findings proved the abundance of Treg cells within localized endometrial lesions, implicating their potential involvement in the pathophysiology of endometriosis.

Additionally, heightened activation of B cells has been observed in both eutopic endometrium and lesions compared to healthy endometrium. Notably, the presence of anti-endometrial antibodies in the serum of endometriosis subjects has led to its occasional classification as an autoimmune disease (118).

## 5.7 Stem cells

Traditional hypotheses concerning the development of endometriotic lesions have lacked detailed mechanistic explanations

for their proliferation and survival until recent studies revealed the involvement of mesenchymal stem cells (MSCs) and myeloid-derived suppressor cells (MDSCs) within a complex network of immune-endocrine signaling. MDSCs typically have strong immunosuppressive and angiogenic characteristics and are found in low numbers in healthy tissue. However, their accumulation is linked to interactions with inflammatory cytokines and has been implicated in several inflammatory diseases. Increased levels of these pro-inflammatory cytokines within the PF of individuals with endometriosis-associated pain may influence the differentiation of monocytes into MDSCs (119).

## 6 Immunological pathogenesis of endometriosis

Estrogen dominance fosters immune dysregulation, whereby many features observed in endometriosis mirror immune processes observed in various cancers, including heightened somatic mutations in endometrial epithelial cells. This elevated mutational burden contributes to the development of endometriosis-specific neoantigens, potentially altering the immune microenvironment of the lesions. Additionally, endometriosis often coexists with several chronic inflammatory conditions, characterized by shared dysregulation of the IL-23/IL-17 pathway, as evidenced in inflammatory bowel disease, psoriasis, and rheumatoid arthritis (120).

The crosstalk between immune cells, nerves, and central pain pathways plays a significant role in the pathophysiology of endometriosis. Endometrium is unique among mucosal tissues in the body in that it typically lacks innervation under normal physiological conditions. Nerve fibers are rare within the functional layer of the endometrium in women without any pathology (121). Sensory nerves surrounding endometriotic lesions drive the chronic pain associated with the condition and contribute to a pro-growth phenotype (122). Substantial alterations in nerve activity occur both within endometriotic lesions and the nervous system. Studies indicate that women experiencing pain symptoms associated with endometriosis exhibit notably higher nerve fiber density within the endometrium, myometrium and lesions as compared to those without the condition (123). Nerve fibers within endometriotic lesions consist of a combination of sensory, sympathetic, and parasympathetic fibers, collectively contributing to pain and inflammatory processes (124). The pain associated with endometriosis implies neuronal mechanisms that culminate in CS.

The interplay between macrophages and nerve fibers fosters inflammation and pain manifestations in endometriosis. Given their abundance within endometriotic lesions, macrophages stimulate sensory innervation and sensitization, thereby contributing to lesion proliferation and the prevalent pain experienced in endometriosis (19, 23). Moreover, immune cells release pro-nociceptive and pro-inflammatory mediators that can sensitize nerve fibers, leading to neurogenic inflammation (125). This communication between immune cells and nerves presents promising avenues for therapeutic interventions in endometriosis.

Prostaglandins, particularly prostaglandin E2 (PGE2), also play a significant role in the pathophysiology of endometriosis, contributing to pain and inflammation. Women with endometriosis produce an

excess of PGE2, which is responsible for uterine contractions, pain, and inflammation (126). PGE2 is upregulated in the peritoneal cavity in endometriosis and is produced by macrophages and ectopic endometrial cells (127). It is involved in the development and continued growth of endometriosis, as it increases estrogen synthesis, inhibits apoptosis, promotes cell proliferation, affects leukocyte populations, and promotes angiogenesis (127). The presence of endometriosis lesions can trigger inflammation, which further promotes PGE2 activity (128). The release of PGE2 is associated with the development of symptoms and the progression of endometriosis, making it a potential target for therapeutic interventions. These changes in nerve activity contribute to the complex and debilitating pain experienced by individuals with endometriosis. However, the use of painkillers or non-steroidal anti-inflammatory drugs (NSAIDs) alone is not always ideal for managing endometriosis pain, as they may have limited efficacy and potential adverse effects (1). Therefore, understanding the role of PGE2 in endometriosis is important for developing targeted treatment strategies to address the associated pain and inflammation.

## 7 Clinical consequences of depression in endometriosis

Endometriosis changes the lifestyle of women and may lead to mental health issues such as depression, physiological stress and anxiety as depicted in Figure 1. Endometriosis is linked to psychological disorders in several ways. The disease in chronic stage can cause life impacting abdominal pain during periods, painful bowel movements or urination, chronic pelvic pain, excessive bleeding, fatigue, diarrhea, constipation, bloating, nausea, fatigue and painful intercourse (Figure 2), leading to a compromised quality of life and in several cases infertility. These co-occurring conditions may cause stress, anxiety and psychological disorders (4, 5).

A study conducted by Pope et al. (129) highlighted the correlation between endometriosis and a diverse array of psychiatric symptoms, notably depression, anxiety, psychosocial stress, and diminished quality of life. Recent literature further substantiates the prevalence of depression and anxiety as the predominant psychiatric comorbidities in individuals with endometriosis (129–136). In an investigation by Low et al. (137), for the potential role of a distinct psychological profile associated with endometriosis, the author included 81 women participants in the study who were experiencing pelvic pain. Of these, 40 were diagnosed with endometriosis disease and 41 presenting with alternative gynecological issues. All the subjects underwent evaluation through six standardized psychometric assessments, including the Eysenck Personality Questionnaire (EPQ), Beck Depression Inventory (BDI), General Health Questionnaire, State-Trait Anxiety Inventory (STAI), The Golombok Rust Inventory of Marital State, and The Short-Form McGill Pain Questionnaire. In assessments using these criteria, the endometriosis patients exhibited increased level of psychoticism, introversion and anxiety scores than women with other gynecological issues (138).

In a recent study conducted by Warzecha et al. (139), 15.1% of women with endometriosis were diagnosed with depression which aligns with findings by Fried et al., who reported a 14.5% incidence of depressive symptoms. In another study the incidence of symptoms



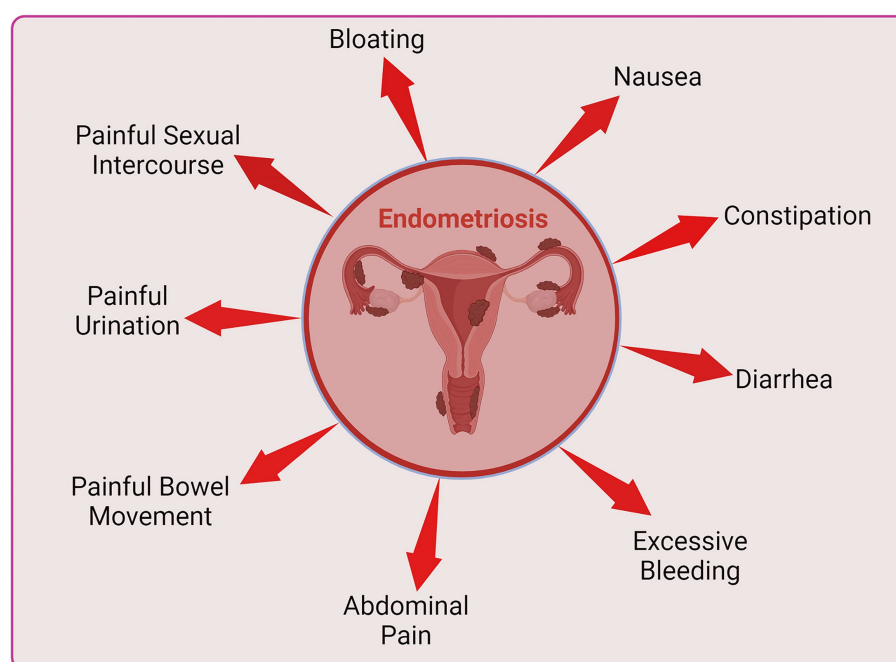


FIGURE 2  
Various health issues in individuals with chronic endometriosis.

of anxiety were estimated to be 29% among Austrian women with endometriosis (130). A meta-analysis by Gambadauro et al. (140), encompassing 24 studies and 99,614 women, confirmed higher levels of depression in such subjects. Researchers indicate that women with endometriosis accompanied by pelvic pain, the rate of depressive symptoms is significantly higher than in cases of endometriosis without pain. This evidence suggests that endometriosis associated complications such as pain may be a more critical factor in the development of depressive symptoms than the presence of endometriosis alone (140). Furthermore, Warzecha et al. (140), revealed that the mean age at the onset of depressive symptoms among women with endometriosis was 22.2 years, which is closely aligned with the age range for the onset of endometriosis symptoms between 18.8 and 24 years. Additionally, the study found that certain types of pain, specifically chronic pelvic pain and painful defecation, significantly increased the incidence of depressive symptoms (140). These findings underscore the profound impact that specific pain manifestations can have on the mental health of women suffering from endometriosis. In these studies, clinicians caring for women with chronic pelvic pain, particularly when coexisting with endometriosis, should be cognizant of the elevated risk of depressive disorders in this population. Understanding the strong correlation between chronic pain and mental health is essential for providing holistic care. Early recognition and intervention for depressive symptoms in these patients can significantly improve their overall quality of life and treatment outcomes.

Another study based on meta-analysis included 18 relevant quantitative studies (129). Out of the 18 studies, 17 included clinical patients' samples. Fourteen out of eighteen studies indicated that endometriosis or chronic pelvic pain significantly impaired at least

some aspects of psychological functioning, mental health, elevated risk for depression, hypomanic, or anxiety symptoms among affected women.

From the 18 studies, 4 studies (137, 141–143) used clinical diagnostic criteria to assess psychiatric diagnosis. Out of these 4 studies, 3 were used as comparator group (137, 141, 142). From the clinical samples of women (age from late teens to mid-40s), 37% of participants showed endometriosis and 50% exhibited pelvic pain with a reported family history of mood disorders. From the 3 comparator group studies, 2 showed higher risk of psychiatric disorders in women with endometriosis (137, 141). Data from these three studies exhibited that 44 (56%) of the 79 women with endometriosis met the criteria for at least one psychiatric disorder.

Another study was conducted on a Brazilian population including 103 women with an age range of 15 to 49 years (average age 33.4 years) (144). Out of 103 patients, 53 (51.5%) were diagnosed with endometriosis and 50 (48.5%) without endometriosis (control). Subjects were evaluated using a questionnaire (Beck Depression Inventory) providing different levels of depression (mild, moderate, moderate to severe, and severe). Based on the questionnaire, symptoms for depression were observed in 35 (66%) women with endometriosis. Out of these, 20 (37.7%) women showed mild depression, 4 women (7.5%) exhibited mild to moderate, 6 women (11.3%) were found to have moderate to severe depression, and 5 (9.4%) women had severe depression. However, according to the Fisher's exact test, there was no relationship between endometriosis and depressive symptoms ( $p = 0.423$ ) (144).

Traumatic stress is very likely in endometriosis diagnosed women compared with the women without endometriosis (145, 146). Harris et al. concluded in a study that children who experienced physical or sexual abuse were likely to develop endometriosis in later stages of life



(147). Post-traumatic stress disorder and childhood trauma can impact individuals and may contribute to the development of depression at an early stage (148). Furthermore, a study conducted by Reis et al., showed that depression or stress in the early stages of life may be considered an important factor for the development of endometriosis (149). The persistence of such conditions over a long period of time may lead to hormonal imbalance, neuroendocrine dysfunction, chronic inflammation which are leading factors in the development of depression and endometriosis (146, 150).

## 8 Immunological aspects of depression in endometriosis

Depression is very much associated with the secretion or formation of proinflammatory molecules such as IL-6, IFN- $\gamma$ , TNF- $\alpha$ , and IL1 $\beta$  (151–153). Additionally, depression also enhances oxidative stress and increases oxidative molecules such as protein bound carbonyl content and methylglyoxal (151, 152, 154). A study revealed that methylglyoxal, which is a well-known reactive metabolite, plays a vital role in various central nervous system associated cognitive functions and can be linked to stress, depression, anxiety, and neurodegenerative diseases (154, 155). Numerous studies conducted in this area have proven that endometriosis is strongly linked with the increased risk of psychological depression, anxiety, and eating disorders (156).

Studies indicated that endometriosis patients have an increased incidence of autoimmune diseases and cancer (157–159). Women diagnosed with endometriosis are more prone to several autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, and Sjogren's Syndrome (159, 160). Higher estrogen levels in women during endometriosis lead to the modification of macromolecules like insulin, serum albumin etc. (161–163). These modifications not only compromise the functioning of these macromolecules but also lead to the formation of neo-antigens on these molecules that activate a cascade of the reactions causing production of autoantibodies (161–163). Higher levels of autoantibodies were detected in patients with depression (161–163). These elevated levels of autoantibodies, together with several pathological complications in endometriosis as discussed above, further aggravate the disease to extremely severe levels.

T cell involvement in depression has not been investigated in detail. Some studies conducted in this area revealed that T cell responses decrease in depression (164–166). T cell responses were found to decrease against antigens encountered in the skin of depressed individuals (164, 167). In a meta-analysis conducted by Zorrilla et al. (166), depression was associated with a decreased percentage of T cells. CD4+ T cells in depressed individuals exhibited increased expression of Fas (CD95) which is known as death receptor as it triggers apoptosis when it interacts with its ligand (168, 169).

T cell function can be inhibited by glucocorticoid pathways in major depression. Increased levels of glucocorticoids in circulatory blood are hallmark of depression (170). Glucocorticoids mediate cell migration and induce apoptosis of immune cells including T cells (166, 171). Endometriosis is characterized by elevated expression of the *HSD11B1* gene, which converts inactive cortisone to cortisol, a biologically potent glucocorticoid in peripheral tissues. Receptor for

glucocorticoid expression increases up to 3.5-fold in endometriosis. The interaction of higher levels of glucocorticoids with increased level of receptors in endometriosis may increase the proinflammatory environment surrounding the endometriotic lesion and enhance the activity that supports endometriotic cell survival (172). Higher levels of glucocorticoids also induce infertility in women. Infertility treatment, which are often long and painful processes, as well as the condition itself induce depression and compromises in quality of life.

Finally, it has been suggested that chronic endometriosis arises due to various dysfunctions imbalances and may lead to infertility which may cause women to develop depression and subsequently unleash further physiological, clinical and immune imbalances which further accelerate chronic endometriosis or vice versa (Figure 3). Thus, both endometriosis and depression concomitantly develop a vicious cycle which enhance and exacerbate disease complications.

## 9 Links between depression and immunological factors for potential malignant transformation of endometriosis

Although endometriosis is classified as a benign disease, it has the potential to transform into malignancy, which occurs in about 1% of endometriosis patients (173, 174). This malignant transformation most frequently affects the ovaries, with ovarian endometrioid carcinoma and ovarian clear cell carcinoma being the most common types. These two malignancies account for 76% of all endometriosis-related ovarian cancers (174, 175).

Recently, several carcinogenic pathways have been identified for endometriosis-related malignant transformation. Uncontrolled cell division, tissue infiltration, neoangiogenesis, and apoptosis evasion may result from oncogene demethylation and tumor suppressor gene hypermethylation (173, 174). Key events include hypermethylation of the hMLH1 gene promoter, reducing DNA mismatch repair gene expression, and hypomethylation of LINE-1. Tumor suppressor genes *RUNX3* and *RASSF2* are inactivated by promoter hypermethylation (173). In endometrioid cancer, *KRAS* oncogene activation and *PTEN* tumor suppressor gene inactivation is significant (175, 176). Loss of *PTEN* activity, an early event in malignant transformation, is linked to *PTEN* gene mutations (177). Additionally, somatic mutations in cancer driver genes *ARID1A*, *PIK3CA*, *KRAS*, and *PPP2R1A* are found in deep infiltrating endometriosis (178).

A recent meta-analysis study conducted by Centini et al. (34), focusses on atypical endometriosis, which is present in 12–35% of ovarian endometriosis cases and 60–80% of endometriosis associated ovarian cancers. The *SWItch/Sucose Non-Fermentable* (*SWI/SNF*) complex and *ARID1A* gene alterations offer valuable insights into the pathogenesis of endometriosis and endometriosis-associated ovarian cancer. Also, the use of potential therapeutics based on inhibitors and suggested the use of PARP inhibitors in treating ovarian cancer which may potentially improve outcomes for these conditions.

Retrograde menstruation, where menstrual blood containing erythrocytes, macrophages, and endometrial tissue travels through the fallopian tubes to the peritoneal cavity, is crucial for understanding endometriosis pathogenesis (179, 180). Periodic hemorrhage from ectopic endometriotic lesions causes iron overload, with

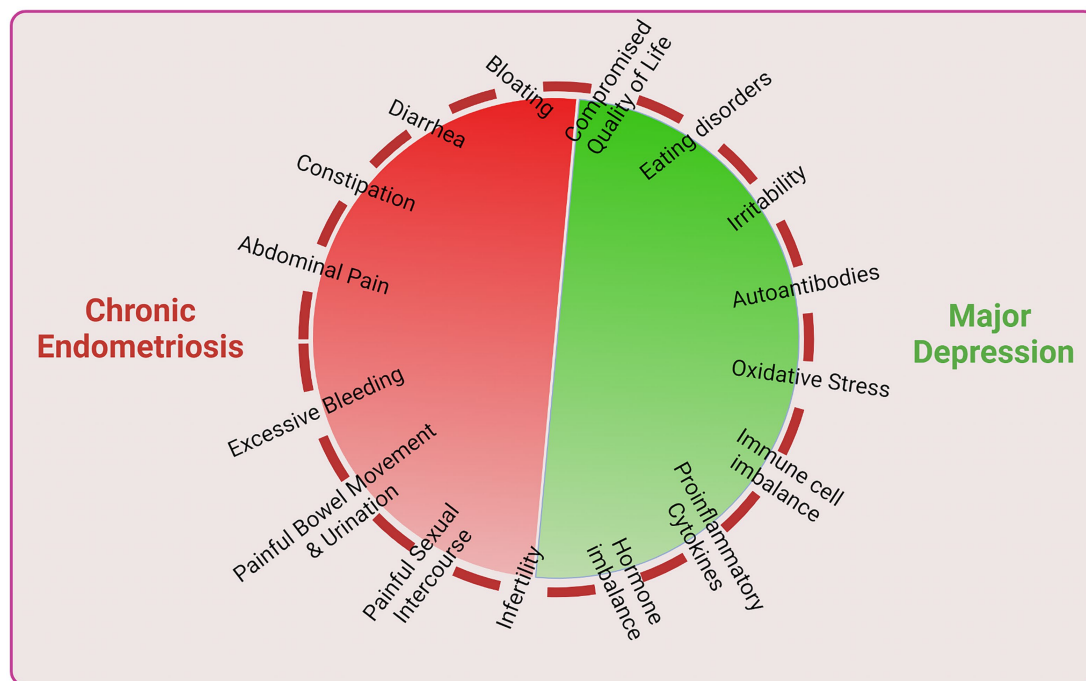


FIGURE 3  
Interlink between chronic endometriosis and major depression.

erythrocyte-derived iron being a well-known inducer of oxidative stress (180). This altered iron metabolism can contribute to endometriosis development and progression (181). At moderate levels, iron-induced reactive oxygen species (ROS) stimulate ectopic endometrial cell proliferation, angiogenesis, and adhesion. Animal models show that iron treatment increases the number and size of endometriotic lesions compared to controls, suggesting that imbalances in iron homeostasis regulate endometriotic cell proliferation (182). These findings suggest that alterations in iron hemostasis may promote endometriotic cell proliferation. Iron overload intensifies intracellular oxidative stress through the Fenton reaction ( $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^\bullet$ ), leading to DNA, lipid, and protein damage, and resulting in cytotoxic effects on cells (183). This reaction generates reactive hydroxyl radicals that contribute to cellular injury and dysfunction. Furthermore, excess iron can decrease transferrin concentration in follicular fluid due to increased transferrin saturation. This iron overload and transferrin insufficiency lead to elevated ROS levels, compromising mitotic spindle integrity and promoting chromosome instability (184, 185). Consequently, this may affect the number and maturation of oocytes retrieved from women with endometriosis (184, 185). High content of iron in ovarian endometriomas exert negative effect on granulosa cells via increased level of ROS cause decrease in the number and quality of oocytes leading to impaired fertility (186–188). The increased levels of free radical generation in physiological stress concomitant with impaired fertility in endometriosis may be due to an imbalance in ROS homeostasis.

Furthermore, there is a persistent production of antioxidants, where endometriotic cells adapt to oxidative stress with the support of macrophages. This adaptation enhances antioxidative defenses and influences redox signaling, energy metabolism, and the tumor immune microenvironment, potentially leading to malignant

transformation. Moreover, specific molecular alterations, including mutations in *ARID1A/BAF250a*, *PIK3CA*, *CTNNB1*, and *PTEN*, as well as microsatellite instability and loss of heterozygosity, have been reported (189–193).

## 10 Conclusion

Endometriosis is a chronic, estrogen-dependent, proinflammatory disease that can cause various dysfunctions. Hormonal imbalance, inflammation, immune dysregulation, angiogenesis, neurogenic inflammation, epigenetic alterations, and tissue remodeling are common in the pathogenesis of endometriosis. Higher numbers of women diagnosed with endometriosis showed increased levels of depression which can potentially further aggravate the disease. According to published literature strong synergisms were observed in endometriosis patients with depression or vice versa. This review article focuses on the immunological aspects of depression in endometriosis patients by looking at the links between depression and immunological factors responsible for potential malignant transformation of endometriosis. There is a huge gap in the awareness of endometriosis and proper counselling and treatment, especially in underdeveloped and developing countries due to continued reluctance of open discussion of female gynecological issues. Clinicians, academicians, and scientists should reach out to these communities and provide vital information promoting regular screening, early detection of the disease and counselling to prevent further complications. Importantly, increased funding is critical for investigation and identification of factors against which multifactorial drug development is critical to alleviate the pain and suffering of women diagnosed with endometriosis and depression.

## Author contributions

SS: Conceptualization, Data curation, Supervision, Writing – original draft, Writing – review & editing. MK: Data curation, Writing – original draft, Writing – review & editing. SR: Data curation, Writing – original draft. KA-M: Data curation, Writing – review & editing. QH: Data curation, Writing – review & editing. WK: Data curation, Writing – review & editing.

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## References

1. Saunders PTK, Horne AW. Endometriosis: etiology, pathobiology, and therapeutic prospects. *Cell*. (2021) 184:2807–24. doi: 10.1016/j.cell.2021.04.041
2. Zondervan KT, Becker CM, Missmer SA. Endometriosis. *N Engl J Med*. (2020) 382:1244–56. doi: 10.1056/NEJMr1810764
3. Allaire C, Bedaiwy MA, Yong PJ. Diagnosis and management of endometriosis. *CMAJ*. (2023) 195:E363–71. doi: 10.1503/cmaj.220637
4. Maroun P, Cooper MJ, Reid GD, Keirse MJ. Relevance of gastrointestinal symptoms in endometriosis. *Aust N Z J Obstet Gynaecol*. (2009) 49:411–4. doi: 10.1111/j.1479-828X.2009.01030.x
5. Velho RV, Werner F, Mechsner S. Endo belly: what is it and why does it happen?—a narrative review. *J Clin Med*. (2023) 12:7176. doi: 10.3390/jcm12227176
6. Parasar P, Ozcan P, Terry KL. Endometriosis: epidemiology, diagnosis and clinical management. *Curr Obstet Gynecol Rep*. (2017) 6:34–41. doi: 10.1007/s13669-017-0187-1
7. Chantalat E, Valera MC, Vaysse C, Noirrit E, Rusidze M, Weyl A, et al. Estrogen receptors and endometriosis. *Int J Mol Sci*. (2020) 21:2815. doi: 10.3390/ijms21082815
8. Andres MP, Arcoverde FVL, Souza CCC, Fernandes LFC, Abrão MS, Kho RM. Extrapelvic endometriosis: a systematic review. *J Minim Invasive Gynecol*. (2020) 27:373–89. doi: 10.1016/j.jmig.2019.10.004
9. Lee SY, Koo YJ, Lee DH. Classification of endometriosis. *Yeungnam Univ J Med*. (2021) 38:10–8. doi: 10.12701/yujm.2020.00444
10. Kvaskoff M, Mu F, Terry KL, Harris HR, Poole EM, Farland L, et al. Endometriosis: a high-risk population for major chronic diseases? *Hum Reprod Update*. (2015) 21:500–16. doi: 10.1093/humupd/dmv013
11. Hogg C, Horne AW, Greaves E. Endometriosis-associated macrophages: origin, phenotype, and function. *Front Endocrinol*. (2020) 11:7. doi: 10.3389/fendo.2020.00007
12. Kwak JY, Park SW, Kim KH, Na YJ, Lee KS. Modulation of neutrophil apoptosis by plasma and peritoneal fluid from patients with advanced endometriosis. *Hum Reprod*. (2002) 17:595–600. doi: 10.1093/humrep/17.3.595
13. Tabatabaei F, Tahernia H, Ghaedi A, Bazrgar A, Khanzadeh S. Diagnostic significance of neutrophil to lymphocyte ratio in endometriosis: a systematic review and meta-analysis. *BMC Womens Health*. (2023) 23:576. doi: 10.1186/s12905-023-02692-7
14. Qiaomei Z, Ping W, Yanjing Z, Jinhua W, Shaozhan C, Lihong C. Features of peritoneal dendritic cells in the development of endometriosis. *Reprod Biol Endocrinol*. (2023) 21:4. doi: 10.1186/s12958-023-01058-w
15. Sikora J, Mielczarek-Palacz A, Kondra-Anasz Z. Role of natural killer cell activity in the pathogenesis of endometriosis. *Curr Med Chem*. (2011) 18:200–8. doi: 10.2174/092986711794088416
16. Malutan AM, Drugan T, Costin N, Ciortea R, Bucuri C, Rada MP, et al. Pro-inflammatory cytokines for evaluation of inflammatory status in endometriosis. *Cent Eur J Immunol*. (2015) 40:96–102. doi: 10.5114/ceji.2015.50840
17. Luckow Invitti A, Schor E, Martins Parreira R, Kopelman A, Kamergorodsky G, Goncalves G, et al. Inflammatory cytokine profile of co-cultivated primary cells from the endometrium of women with and without endometriosis. *Mol Med Reports*. (2018) 18:1287–96. doi: 10.3892/mmr.2018.9137
18. Lie I, Ilie R. Cytokines and endometriosis—the role of immunological alterations. *Biotechnol Mol Biol Nanomed*. (2013) 1:8–19.

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19. Sikora J, Smycz-Kubańska M, Mielczarek-Palacz A, Kondra-Anasz Z. Abnormal peritoneal regulation of chemokine activation—the role of IL-8 in pathogenesis of endometriosis. *Am J Reprod Immunol*. (2017) 77:e12622. doi: 10.1111/aji.12622
20. Sikora J, Mielczarek-Palacz A, Kondra-Anasz Z. Association of the precursor of interleukin-1 $\beta$  and peritoneal inflammation—role in pathogenesis of endometriosis. *J Clin Lab Anal*. (2016) 30:831–7. doi: 10.1002/jcla.21944
21. May CD, Sphyris N, Evans KW, Werden SJ, Guo W, Mani SA. Epithelial-mesenchymal transition and cancer stem cells: a dangerously dynamic duo in breast cancer progression. *Breast Cancer Res*. (2011) 13:1–10. doi: 10.1186/bcr2789
22. Fan YY, Chen HY, Chen W, Liu YN, Fu Y, Wang LN. Expression of inflammatory cytokines in serum and peritoneal fluid from patients with different stages of endometriosis. *Gynecol Endocrinol*. (2018) 34:507–12. doi: 10.1080/09513590.2017.1409717
23. Jørgensen H, Hill AS, Beste MT, Kumar MP, Chiswick E, Fedorcsak P, et al. Peritoneal fluid cytokines related to endometriosis in patients evaluated for infertility. *Fertil Steril*. (2017) 107:1191–1199.e2. doi: 10.1016/j.fertnstert.2017.03.013
24. Dhabhar FS. Effects of stress on immune function: the good, the bad, and the beautiful. *Immunol Res*. (2014) 58:193–210. doi: 10.1007/s12026-014-8517-0
25. Bartolomucci A, Palanza P, Parmigiani S, Pederzani T, Merlot E, Neveu PJ, et al. Chronic psychosocial stress down-regulates central cytokines mRNA. *Brain Res Bull*. (2003) 62:173–8. doi: 10.1016/j.brainresbull.2003.09.009
26. Bluthé RM, Laye S, Michaud B, Combe C, Dantzer R, Parnet P. Role of interleukin-1 $\beta$  and tumour necrosis factor- $\alpha$  in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice. *Eur J Neurosci*. (2000) 12:4447–56. doi: 10.1046/j.1460-9568.2000.01348.x
27. Dantzer R. Cytokine, sickness behavior, and depression. *Immunol Allergy Clin N Am*. (2009) 29:247–64. doi: 10.1016/j.jiac.2009.02.002
28. Seiler A, Fagundes CP, Christian LM. The impact of everyday stressors on the immune system and health In: A Chouker, editor. Stress challenges and immunity in space. Cham: Springer (2020). 71–92.
29. Culley L, Law C, Hudson N, Denny E, Mitchell H, Baumgarten M, et al. The social and psychological impact of endometriosis on women's lives: a critical narrative review. *Hum Reprod Update*. (2013) 19:625–39. doi: 10.1093/humupd/dmt027
30. De Graaff AA, D'hooghe TM, Dunselman GA, Dirksen CD, Hummelshoj L, WERF EndoCost Consortium et al. The significant effect of endometriosis on physical, mental and social wellbeing: results from an international cross-sectional survey. *Hum Reprod*. (2013) 28:2677–85. doi: 10.1093/humrep/det284
31. Della Corte L, Di Filippo C, Gabrielli O, Reppucci S, La Rosa VL, Ragusa R, et al. The burden of endometriosis on women's lifespan: a narrative overview on quality of life and psychosocial wellbeing. *Int J Environ Res Public Health*. (2020) 17:4683. doi: 10.3390/ijerph17134683
32. Szyplowska M, Tarkowski R, Kulak K. The impact of endometriosis on depressive and anxiety symptoms and quality of life: a systematic review. *Front Public Health*. (2023) 11:1230303. doi: 10.3389/fpubh.2023.1230303
33. Bonavina G, Taylor HS. Endometriosis-associated infertility: from pathophysiology to tailored treatment. *Front Endocrinol*. (2022) 13:1020827. doi: 10.3389/fendo.2022.1020827



34. Centini G, Schettini G, Pieri E, Giorgi M, Lazzeri L, Martire FG, et al. Endometriosis-related ovarian Cancer: where are we now? A narrative review towards a pragmatic approach. *J Clin Med*. (2024) 13:1933. doi: 10.3390/jcm13071933
35. Lalami I, Abo C, Borghese B, Chapron C, Vaiman D. Genomics of endometriosis: from genome wide association studies to exome sequencing. *Int J Mol Sci*. (2021) 22:7297. doi: 10.3390/ijms22147297
36. Nouri K, Ott J, Krupitz B, Huber JC, Wenzl R. Family incidence of endometriosis in first, second-, and third-degree relatives: case-control study. *Reprod Biol Endocrinol*. (2010) 8:85. doi: 10.1186/1477-7827-8-85
37. Gallagher CS, Mäkinen N, Harris HR, Rahmioglu N, Uimari O, Cook JP, et al. Genome-wide association and epidemiological analyses reveal common genetic origins between uterine leiomyomata and endometriosis. *Nat Commun*. (2019) 10:4857. doi: 10.1038/s41467-019-12536-4
38. Masuda T, Low SK, Akiyama M, Harita M, Ueda Y, Matsuda K, et al. GWAS of five gynecologic diseases and cross-trait analysis in Japanese. *Eur J Hum Genet*. (2020) 28:95–107. doi: 10.1038/s41431-019-0495-1
39. Saunders PTK. Insights from genomic studies on the role of sex steroids in the aetiology of endometriosis. *Reprod Fertil*. (2022) 3:R51–65. doi: 10.1530/RAF-21-0078
40. Zhang Z, Suo L, Chen Y, Zhu L, Wan G, Han X. Endometriotic peritoneal fluid promotes Myofibroblast differentiation of endometrial mesenchymal stem cells. *Stem Cells Int*. (2019) 2019:1–13. doi: 10.1155/2019/6183796
41. Sapkota Y, Steinthorsdottir V, Morris AP, Fassbender A, Rahmioglu N, De Vivo I, et al. Meta-analysis identifies five novel loci associated with endometriosis highlighting key genes involved in hormone metabolism. *Nat Commun*. (2017) 8:15539. doi: 10.1038/ncomms15539
42. Warzecha D, Zalecka J, Mańka G, Kieka M, Lipa M, Spaczynski R, et al. Plasma and peritoneal fluid fibronectin and collagen IV levels as potential biomarkers of endometriosis. *Int J Mol Sci*. (2022) 23:15669. doi: 10.3390/ijms232415669
43. Béliard A, Donnez J, Nisolle M, Foidart JM. Localization of laminin, fibronectin, E-cadherin, and integrins in endometrium and endometriosis. *Fertil Steril*. (1997) 67:266–72. doi: 10.1016/S0015-0282(97)81909-7
44. Yuge A, Nasu K, Matsumoto H, Nishida M, Narahara H. Collagen gel contractility is enhanced in human endometriotic stromal cells: a possible mechanism underlying the pathogenesis of endometriosis-associated fibrosis. *Hum Reprod*. (2007) 22:938–44. doi: 10.1093/humrep/del485
45. Holdsworth-Carson SJ, Fung JN, Luong HT, Sapkota Y, Bowdler LM, Wallace L, et al. Endometrial vezatin and its association with endometriosis risk. *Hum Reprod*. (2016) 31:999–1013. doi: 10.1093/humrep/dew047
46. Machado DE, Berardo PT, Palmero CY, Nasciutti LE. Higher expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Flk-1) and metalloproteinase-9 (MMP-9) in a rat model of peritoneal endometriosis is similar to cancer diseases. *J Exp Clin Cancer Res*. (2010) 29:4. doi: 10.1186/1756-9966-29-4
47. Bora G, Yaba A. The role of mitogen-activated protein kinase signaling pathway in endometriosis. *J Obstet Gynaecol Res*. (2021) 47:1610–23. doi: 10.1111/jog.14710
48. Sikora J, Ferrero S, Mielczarek-Palacz A, Kondera-Anasz Z. The delicate balance between the good and the bad IL-1 Proinflammatory effects in endometriosis. *Curr Med Chem*. (2018) 25:2105–21. doi: 10.2174/0929867325666180111093547
49. Matsuzaki S, Darcha C. Involvement of the Wnt/ $\beta$ -catenin signaling pathway in the cellular and molecular mechanisms of fibrosis in endometriosis. *PLoS One*. (2013) 8:e76808. doi: 10.1371/journal.pone.0076808
50. Sapkota Y, Fassbender A, Bowdler L, Fung JN, Peterse D, Dorien O, et al. Independent replication and Meta-analysis for endometriosis risk loci. *Twin Res Hum Genet*. (2015) 18:518–25. doi: 10.1017/thg.2015.61
51. Adewuyi EO, Mehta D, Sapkota Y, Auta A, Yoshihara K, Nyegaard M, et al. Genetic analysis of endometriosis and depression identifies shared loci and implicates causal links with gastric mucosa abnormality. *Hum Genet*. (2021) 140:529–52. doi: 10.1007/s00439-020-02223-6
52. Bougie O, Yap MI, Sikora L, Flaxman T, Singh S. Influence of race/ethnicity on prevalence and presentation of endometriosis: a systematic review and meta-analysis. *BJOG*. (2019) 126:1104–15. doi: 10.1111/1471-0528.15692
53. Yen CF, Kim MR, Lee CL. Epidemiologic factors associated with endometriosis in East Asia. *Gynecol Minim Invasive Ther*. (2019) 8:4–11. doi: 10.4103/GMIT.GMIT\_83\_18
54. Triolo O, Laganà AS, Sturlese E. Chronic pelvic pain in endometriosis: an overview. *J Clin Med Res*. (2013) 5:153–63. doi: 10.4021/jocmr1288w
55. Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani PG. Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: a multivariate analysis of over 1000 patients. *Hum Reprod*. (2007) 22:266–71. doi: 10.1093/humrep/del339
56. Vannuccini S, Clemenza S, Rossi M, Petraglia F. Hormonal treatments for endometriosis: the endocrine background. *Rev Endocr Metab Disord*. (2022) 23:333–55. doi: 10.1007/s11154-021-09666-w
57. Missmer SA, Hankinson SE, Spiegelman D, Barbieri RL, Marshall LM, Hunter DJ. Incidence of laparoscopically confirmed endometriosis by demographic, anthropometric, and lifestyle factors. *Am J Epidemiol*. (2004) 160:784–96. doi: 10.1093/aje/kwh275
58. Hudelist G, Fritzer N, Thomas A, Niehues C, Oppelt P, Haas D, et al. Diagnostic delay for endometriosis in Austria and Germany: causes and possible consequences. *Hum Reprod*. (2012) 27:3412–6. doi: 10.1093/humrep/des316
59. Soliman AM, Fuldeore M, Snabes MC. Factors associated with time to endometriosis diagnosis in the United States. *J Women's Health*. (2017) 26:788–97. doi: 10.1089/jwh.2016.6003
60. Littlejohn G. Neuroinflammation in fibromyalgia and CRPS: top-down or bottom-up? *Nat Rev Rheumatol*. (2016) 12:242–2. doi: 10.1038/nrrheum.2016.26
61. Orr NL, Wahl KJ, Noga H, Allaire C, Williams C, Bedaiwy MA, et al. Phenotyping sexual pain in endometriosis using the central sensitization inventory. *J Sex Med*. (2020) 17:761–70. doi: 10.1016/j.jsxm.2019.12.019
62. Orr NL, Wahl KJ, Lisonek M, Joannou A, Noga H, Albert A, et al. Central sensitization inventory in endometriosis. *Pain*. (2022) 163:e234–45. doi: 10.1097/j.pain.0000000000002351
63. Chiarotto A, Viti C, Sulli A, Cutolo M, Testa M, Piscitelli D. Cross-cultural adaptation and validity of the Italian version of the central sensitization inventory. *Musculoskeletal Sci Pract*. (2018) 37:20–8. doi: 10.1016/j.msksp.2018.06.005
64. Mayer TG, Neblett R, Cohen H, Howard KJ, Choi YH, Williams MJ, et al. The development and psychometric validation of the central sensitization inventory. *Pain Pract*. (2012) 12:276–85. doi: 10.1111/j.1533-2500.2011.00493.x
65. Raimondo D, Raffone A, Renzulli F, Sanna G, Raspollini A, Bertoldo L, et al. Prevalence and risk factors of central sensitization in women with endometriosis. *J Min Invas Gynecol*. (2023) 30:73–80.e1. doi: 10.1016/j.jmig.2022.10.007
66. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, et al. Blood biomarkers for the non-invasive diagnosis of endometriosis. *Cochrane Database Syst Rev*. (2016) 2016:CD012179. doi: 10.1002/14651858.CD012179
67. Horne AW, Saunders PTK. Snapshot: endometriosis. *Cell*. (2019) 179:1677–7. doi: 10.1016/j.cell.2019.11.033
68. Yaşar P, Ayaz G, User SD, Güpür G, Muyan M. Molecular mechanism of estrogen-estrogen receptor signaling. *Reprod Med Biol*. (2016) 16:4–20. doi: 10.1002/rmb.2.12006
69. Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and immune dysfunction in endometriosis. *Biomed Res Int*. (2015) 2015:795976:1–12. doi: 10.1155/2015/795976
70. Zhao H, Zhou L, Shangguan AJ, Bulun SE. Aromatase expression and regulation in breast and endometrial cancer. *J Mol Endocrinol*. (2016) 57:R19–33. doi: 10.1530/JME-15-0310
71. Mettler L, Schmidt D, Maher P. The impact of endometriosis on the health of women 2016. *Biomed Res Int*. (2016) 2016:1747280. doi: 10.1155/2016/1747280
72. Yilmaz BD, Bulun SE. Endometriosis and nuclear receptors. *Hum Reprod Update*. (2019) 25:473–85. doi: 10.1093/humupd/dmz005
73. Zhang P, Wang G. Progesterone resistance in endometriosis: current evidence and putative mechanisms. *Int J Mol Sci*. (2023) 24:6992. doi: 10.3390/ijms24086992
74. Azeez JM, Susmi TR, Remadevi V, Ravindran V, Sujatha AS, Sreeja S. New insights into the functions of progesterone receptor (PR) isoforms and progesterone signaling. *Am J Cancer Res*. (2021) 11:5214–32.
75. Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR, Bulun SE. Progesterone receptor isoform A but not B is expressed in endometriosis. *J Clin Endocrinol Metab*. (2000) 85:2897–902. doi: 10.1210/jcem.85.8.6739
76. Armstrong GM, Maybin JA, Murray AA, Nicol M, Walker C, Saunders PTK, et al. Endometrial apoptosis and neutrophil infiltration during menstruation exhibits spatial and temporal dynamics that are recapitulated in a mouse model. *Sci Rep*. (2017) 7:17416. doi: 10.1038/s41598-017-17565-x
77. Rocha AL, Reis FM, Taylor RN. Angiogenesis and endometriosis. *Obstet Gynecol Int*. (2013) 2013:859619:1–8. doi: 10.1155/2013/859619
78. Laschke MW, Giebels C, Menger MD. Vasculogenesis: a new piece of the endometriosis puzzle. *Hum Reprod Update*. (2011) 17:628–36. doi: 10.1093/humupd/dmr023
79. Donnez J, Smoes P, Gillerot S, Casanas-Roux F, Nisolle M. Vascular endothelial growth factor (VEGF) in endometriosis. *Hum Reprod*. (1998) 13:1686–90. doi: 10.1093/humrep/13.6.1686
80. Shifren JL, Tseng JF, Zaloudek CJ, Ryan IP, Meng YG, Ferrara N, et al. Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. *J Clin Endocrinol Metab*. (1996) 81:3112–8. doi: 10.1210/jcem.81.8.8768883
81. Wang H, Gorpudolo N, Li Y, Feng D, Wang Z, Zhang Y. Elevated vascular endothelial growth factor-a in the serum and peritoneal fluid of patients with endometriosis. *J Huazhong Univ Sci Technol Med Sci*. (2009) 29:637–41. doi: 10.1007/s11596-009-0520-7
82. Abramciuk M, Grywalska E, Małkowska P, Sierawska O, Hryniewicz R, Niedzwiedzka-Rystwej P. The role of the immune system in the development of endometriosis. *Cells*. (2022) 11:2028. doi: 10.3390/cells11132028
83. Sozzani S, Rusnati M, Riboldi E, Mitola S, Presta M. Dendritic cell-endothelial cell cross-talk in angiogenesis. *Trends Immunol*. (2007) 28:385–92. doi: 10.1016/j.it.2007.07.006

84. Fainaru O, Adini A, Benny O, Adini I, Short S, Bazinet L, et al. Dendritic cells support angiogenesis and promote lesion growth in a murine model of endometriosis. *FASEB J.* (2008) 22:522–9. doi: 10.1096/fj.07-9034com
85. Stanic AK, Kim M, Styer AK, Rueda BR. Dendritic cells attenuate the early establishment of endometriosis-like lesions in a murine model. *Reprod Sci.* (2014) 21:1228–36. doi: 10.1177/1933719114525267
86. Liu H, Lang JH. Is abnormal eutopic endometrium the cause of endometriosis? The role of eutopic endometrium in pathogenesis of endometriosis. *Med Sci Monit.* (2011) 17:RA92–99:CR542–6.
87. Houshdaran S, Oke AB, Fung JC, Vo KC, Nezhat C, Giudice LC. Steroid hormones regulate genome-wide epigenetic programming and gene transcription in human endometrial cells with marked aberrancies in endometriosis. *PLoS Genet.* (2020) 16:e1008601. doi: 10.1371/journal.pgen.1008601
88. Li Y, Zhao L, Li XF. Hypoxia and the tumor microenvironment. *Technol Cancer Res Treat.* (2021) 20:153303382110363. doi: 10.1177/15330338211036304
89. Zhang Y, Liu X, Deng M, Xu C, Zhang Y, Wu D, et al. Ferroptosis induced by iron overload promotes fibrosis in ovarian endometriosis and is related to subpopulations of endometrial stromal cells. *Front Pharmacol.* (2022) 13:930614. doi: 10.3389/fphar.2022.930614
90. Ding D, Liu X, Duan J, Guo SW. Platelets are an undicted culprit in the development of endometriosis: clinical and experimental evidence. *Hum Reprod.* (2015) 30:812–32. doi: 10.1093/humrep/dev025
91. Bruner-Tran KL, Mokshagundam S, Herington JL, Ding T, Osteen KG. Rodent models of experimental endometriosis: identifying mechanisms of disease and therapeutic targets. *Curr Womens Health Rev.* (2018) 14:173–88. doi: 10.2174/1573404813666170921162041
92. Chen S, Liu Y, Zhong Z, Wei C, Liu Y, Zhu X. Peritoneal immune microenvironment of endometriosis: role and therapeutic perspectives. *Front Immunol.* (2023) 14:1134663. doi: 10.3389/fimmu.2023.1134663
93. Liang Y, Xie H, Wu J, Liu D, Yao S. Villainous role of estrogen in macrophage-nerve interaction in endometriosis. *Reprod Biol Endocrinol.* (2018) 16:122. doi: 10.1186/s12958-018-0441-z
94. Zigmund RE, Echevarria FD. Macrophage biology in the peripheral nervous system after injury. *Prog Neurobiol.* (2019) 173:102–21. doi: 10.1016/j.pneurobio.2018.12.001
95. Wu J, Xie H, Yao S, Liang Y. Macrophage and nerve interaction in endometriosis. *J Neuroinflammation.* (2017) 14:53. doi: 10.1186/s12974-017-0828-3
96. Greaves E, Temp J, Esnal-Zufurre A, Mechsner S, Horne AW, Saunders PT. Estradiol is a critical mediator of macrophage-nerve cross talk in peritoneal endometriosis. *Am J Pathol.* (2015) 185:2286–97. doi: 10.1016/j.ajpath.2015.04.012
97. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell.* (2008) 133:775–87. doi: 10.1016/j.cell.2008.05.009
98. Young VJ, Brown JK, Maybin J, Saunders PT, Duncan WC, Horne AW. Transforming growth factor- $\beta$  induced Warburg-like metabolic reprogramming may underpin the development of peritoneal endometriosis. *J Clin Endocrinol Metab.* (2014) 99:3450–9. doi: 10.1210/jc.2014-1026
99. Hirschhaeuser F, Sattler UG, Mueller-Klieser W. Lactate: a metabolic key player in cancer. *Cancer Res.* (2011) 71:6921–5. doi: 10.1158/0008-5472.CAN-11-1457
100. Chen J, Qin P, Sun Y, Han S. Histone lactylation promotes cell proliferation, migration and invasion through targeting HMGB1 in endometriosis. *J Biomed Res.* (2023) 37:470–8. doi: 10.7555/JBR.37.20230095
101. Viola A, Munari F, Sánchez-Rodríguez R, Scolaro T, Castegna A. The metabolic signature of macrophage responses. *Front Immunol.* (2019) 10:1462. doi: 10.3389/fimmu.2019.01462
102. Ramírez-Pavez TN, Martínez-Esparza M, Ruiz-Alcaraz AJ, Marín-Sánchez P, Machado-Linde F, García-Peñarrubia P. The role of peritoneal macrophages in endometriosis. *Int J Mol Sci.* (2021) 22:10792. doi: 10.3390/ijms221910792
103. Zanna MY, Yasmin AR, Omar AR, Arshad SS, Mariatulqabiah AR, Nur-Fazila SH, et al. Review of dendritic cells, their role in clinical immunology, and distribution in various animal species. *Int J Mol Sci.* (2021) 22:8044. doi: 10.3390/ijms22158044
104. Laginha PA, Arcoverde FVL, Riccio LGC, Andres MP, Abrão MS. The role of dendritic cells in endometriosis: a systematic review. *J Reprod Immunol.* (2022) 149:103462. doi: 10.1016/j.jri.2021.103462
105. Somigliana E, Viganò P, Gaffuri B, Candiani M, Busacca M, Blasio AM, et al. Modulation of NK cell lytic function by endometrial secretory factors: potential role in endometriosis. *Am J Reprod Immunol.* (1996) 36:295–300. doi: 10.1111/j.1600-0897.1996.tb00179.x
106. Jeung I, Cheon K, Kim MR. Decreased cytotoxicity of peripheral and peritoneal natural killer cell in endometriosis. *Biomed Res Int.* (2016) 2016:1–6. doi: 10.1155/2016/2916070
107. Hoogstad-van Evert J, Paap R, Nap A, van der Molen R. The promises of natural killer cell therapy in endometriosis. *Int J Mol Sci.* (2022) 23:5539. doi: 10.3390/ijms23105539
108. Thiruchelvam U, Wingfield M, O'Farrelly C. Natural killer cells: key players in endometriosis. *Am J Reprod Immunol.* (2015) 74:291–301. doi: 10.1111/aji.12408
109. Maruyama T, Yoshimura Y. Molecular and cellular mechanisms for differentiation and regeneration of the uterine endometrium. *Endocr J.* (2008) 55:795–810. doi: 10.1507/endocrj.K08E-067
110. Maksym RB, Hoffmann-Młodzianowska M, Skibińska M, Rąbajewski M, Mackiewicz A, Kieda C. Immunology and immunotherapy of endometriosis. *J Clin Med.* (2021) 10:5879. doi: 10.3390/jcm10245879
111. Pashizeh F, Mansouri R, Davari-Tanha F, Hosseini R, Asgari Z, Aghaei H, et al. Alterations of CD4+ T cell subsets in blood and peritoneal fluid in different stages of endometriosis. *Int J Fertil Steril.* (2020) 14:201. doi: 10.22074/ijfs.2020.6127
112. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* (2003) 299:1057–61. doi: 10.1126/science.1079490
113. Berbic M, Hey-Cunningham AJ, Ng C, Tokushige N, Ganewatta S, Markham R, et al. The role of Foxp3+ regulatory T-cells in endometriosis: a potential controlling mechanism for a complex, chronic immunological condition. *Hum Reprod.* (2010) 25:900–7. doi: 10.1093/humrep/deq020
114. Budiu RA, Diaconu I, Chrissluis R, Dricu A, Edwards RP, Vlad AM. A conditional mouse model for human MUC1-positive endometriosis shows the presence of anti-MUC1 antibodies and Foxp3+ regulatory T cells. *Dis Model Mec.* (2009) 2:593–603. doi: 10.1242/dmm.002535
115. Basta P, Majka M, Jozwicki W, Lukaszewska E, Knafel A, Grabiec M, et al. The frequency of CD25+ CD4+ and FOXP3+ regulatory T cells in ectopic endometrium and ectopic decidua. *Reprod Biol Endocrinol.* (2010) 8:1–7. doi: 10.1186/1477-7827-8-116
116. Olkowska-Truchanowicz J, Bocian K, Maksym RB, Białoszewska A, Włodarczyk D, Baranowski W, et al. CD4+ CD25+ FOXP3+ regulatory T cells in peripheral blood and peritoneal fluid of patients with endometriosis. *Hum Reprod.* (2013) 28:119–24. doi: 10.1093/humrep/des346
117. Tanaka Y, Mori T, Ito F, Koshiba A, Takaoka O, Kataoka H, et al. Exacerbation of endometriosis due to regulatory T-cell dysfunction. *J Clin Endocrinol Metab.* (2017) 102:3206–17. doi: 10.1210/jc.2017-00052
118. Vanni VS, Villanacci R, Salmeri N, Papaleo E, Delprato D, Ottolina J, et al. Publisher correction: concomitant autoimmunity may be a predictor of more severe stages of endometriosis. *Sci Rep.* (2021) 11:17715. doi: 10.1038/s41598-021-97506-x
119. Kapur S, Santibanez JF. Myeloid-derived suppressor cells and mesenchymal stem/stromal cells in myeloid malignancies. *J Clin Med.* (2021) 10:2788. doi: 10.3390/jcm10132788
120. McCallion A, Sisnett DJ, Zutautes KB, Hayati D, Spiess KG, Aleksieva S, et al. Endometriosis through an immunological lens: a pathophysiology based in immune dysregulation. *Explor Immunol.* (2022) 2:454–83. doi: 10.37349/ei.2022.00062
121. Tokushige N, Markham R, Russell P, Fraser IS. Nerve fibres in peritoneal endometriosis. *Hum Reprod.* (2006) 21:3001–7. doi: 10.1093/humrep/del260
122. Velho RV, Taube E, Sehoul J, Mechsner S. Neurogenic inflammation in the context of endometriosis-what do we know? *Int J Mol Sci.* (2021) 22:13102. doi: 10.3390/ijms222313102
123. Morotti M, Vincent K, Brawn J, Zondervan KT, Becker CM. Peripheral changes in endometriosis-associated pain. *Hum Reprod Update.* (2014) 20:717–36. doi: 10.1093/humupd/dmu021
124. Miller EJ, Fraser IS. The importance of pelvic nerve fibers in endometriosis. *Womens Health.* (2015) 11:611–8. doi: 10.2217/whe.15.47
125. Domoto R, Sekiguchi F, Tsubota M, Kawabata A. Macrophage as a peripheral pain regulator. *Cells.* (2021) 10:1881. doi: 10.3390/cells10081881
126. García-Gómez E, Vázquez-Martínez ER, Reyes-Mayoral C, Cruz-Orozco OP, Camacho-Arroyo I, Cerbón M. Regulation of inflammation pathways and Inflammasome by sex steroid hormones in endometriosis. *Front Endocrinol.* (2020) 10:935. doi: 10.3389/fendo.2019.00935
127. Sacco K, Portelli M, Pollacco J, Schembri-Wismayer P, Calleja-Aguis J. The role of prostaglandin E2 in endometriosis. *Gynecol Endocrinol.* (2012) 28:134–8. doi: 10.3109/09513590.2011.588753
128. Lai ZZ, Yang HL, Ha SY, Chang KK, Mei J, Zhou WJ, et al. Cyclooxygenase-2 in endometriosis. *Int J Biol Sci.* (2019) 15:2783–97. doi: 10.7150/ijbs.35128
129. Pope CJ, Sharma V, Sharma S, Mazmanian D. A systematic review of the association between psychiatric disturbances and endometriosis. *J Obstet Gynaecol Can.* (2015) 37:1006–15. doi: 10.1016/S1701-2163(16)30050-0
130. Friedl F, Riedl D, Fessler S, Wildt L, Richter R, Schubler G, et al. Impact of endometriosis on quality of life, anxiety, and depression: an Austrian perspective. *Arch Gynecol Obstet.* (2015) 292:1393–9. doi: 10.1007/s00404-015-3789-8
131. Vitale SG, La Rosa VL, Rapisarda AM, Laganà AS. Impact of endometriosis on quality of life and psychological well-being. *J Psychosom Obstet Gynaecol.* (2017) 38:317–9. doi: 10.1080/0167482X.2016.1244185
132. De Graaff AA, Van Lankveld J, Smits LJ, Van Beek JJ, Dunselman GA. Dyspareunia and depressive symptoms are associated with impaired sexual functioning in women with endometriosis, whereas sexual functioning in their male partners is not affected. *Hum Reprod.* (2016) 31:2577–86. doi: 10.1093/humrep/dew215
133. Chen LC, Hsu JW, Huang KL, Bai YM, Su TP, Li CT, et al. Risk of developing major depression and anxiety disorders among women with endometriosis: a longitudinal follow-up study. *J Affect Disord.* (2016) 190:282–5. doi: 10.1016/j.jad.2015.10.030



134. Laganà AS, La Rosa V, Petrosino B, Vitale SG. Comment on “risk of developing major depression and anxiety disorders among women with endometriosis: a longitudinal follow-up study”. *J Affect Disord.* (2017) 208:672–3. doi: 10.1016/j.jad.2016.07.016
135. Laganà AS, Condemni I, Retto G, Muscatello MR, Bruno A, Zoccali RA, et al. Analysis of psychopathological comorbidity behind the common symptoms and signs of endometriosis. *Eur J Obstet Gynecol Reprod Biol.* (2015) 194:30–3. doi: 10.1016/j.ejogrb.2015.08.015
136. Cavaggioni G, Lia C, Resta S, Antonielli T, Benedetti Panici P, Megiorni F, et al. Are mood and anxiety disorders and alexithymia associated with endometriosis? A preliminary study. *Biomed Res Int.* (2014) 2014:1–5. doi: 10.1155/2014/786830
137. Low WY, Edelmann RJ, Sutton C. A psychological profile of endometriosis patients in comparison to patients with pelvic pain of other origins. *J Psychosom Res.* (1993) 37:111–6. doi: 10.1016/0022-3999(93)90077-S
138. Laganà AS, La Rosa VL, Rapisarda AM, Valenti G, Sapia F, Chiofalo B, et al. Anxiety and depression in patients with endometriosis: impact and management challenges. *Int J Women's Health.* (2017) 9:323–30. doi: 10.2147/IJWH.S119729
139. Warzecha D, Szymusik I, Wielgos M, Pietrzak B. The impact of endometriosis on the quality of life and the incidence of depression—a cohort study. *Int J Environ Res Public Health.* (2020) 17:3641. doi: 10.3390/ijerph17103641
140. Gambadauro P, Carli V, Hadlaczyk G. Depressive symptoms among women with endometriosis: a systematic review and meta-analysis. *Am J Obstet Gynecol.* (2019) 220:230–41. doi: 10.1016/j.ajog.2018.11.123
141. Kumar V, Khan M, Vilos GA, Sharma V. Revisiting the association between endometriosis and bipolar disorder. *J Obstet Gynaecol Can.* (2011) 33:1141–5. doi: 10.1016/S1701-2163(16)35082-4
142. Walker E, Katon W, Jones LM, Russo J. Relationship between endometriosis and affective disorder. *Am J Psychiatry.* (1989) 146:380–1. doi: 10.1176/ajp.146.3.380
143. Lewis DO, Comite F, Mallouh C, Zadunaisky L, Hutchinson-Williams K, Cherksey BD, et al. Bipolar mood disorder and endometriosis: preliminary findings. *Am J Psychiatry.* (1987) 144:1588–91. doi: 10.1176/ajp.144.12.1588
144. Novais RF, da Câmara-França BE, Lasmar RB, Lasmar BP. Endometriosis and its relationship with depression. *Int J Clin Med.* (2018) 9:71–8. doi: 10.4236/ijcm.2018.92008
145. Carbone MG, Campo G, Papaleo E, Marazziti D, Maremmani I. The importance of a multi-disciplinary approach to the Endometriotic patients: the relationship between endometriosis and psychic vulnerability. *J Clin Med.* (2021) 10:1616. doi: 10.3390/jcm10081616
146. Van Stein K, Schubert K, Ditzgen B, Weise C. Understanding psychological symptoms of endometriosis from a research domain criteria perspective. *J Clin Med.* (2023) 12:4056. doi: 10.3390/jcm12124056
147. Harris HR, Wieser F, Vitonis AF, Rich-Edwards J, Boynton-Jarrett R, Bertone-Johnson ER, et al. Early life abuse and risk of endometriosis. *Hum Reprod.* (2018) 33:1657–68. doi: 10.1093/humrep/dey248
148. Harricharan S, McKinnon MC, Lanius RA. How processing of sensory information from the internal and external worlds shape the perception and engagement with the world in the aftermath of trauma: implications for PTSD. *Front Neurosci.* (2021) 15:360. doi: 10.3389/fnins.2021.625490
149. Reis FM, Coutinho LM, Vannuccini S, Luisi S, Petraglia F. Is stress a cause or a consequence of endometriosis? *Reprod Sci.* (2020) 27:39–45. doi: 10.1007/s43032-019-00053-0
150. Liebermann C, Schwartz ASK, Charpidou T, Geraedts K, Rauchfuss M, Wölfler M, et al. Maltreatment during childhood: a risk factor for the development of endometriosis? *Hum Reprod.* (2018) 33:1449–58. doi: 10.1093/humrep/dey111
151. Sherwani S, Khan MW. Autoantibodies against ROS-human serum albumin-a potent immunological marker in depressed individuals with smoking history. *Rev Romana Med Lab.* (2022) 30:399–411. doi: 10.2478/rrlm-2022-0039
152. Sherwani S, Raafat M, Rajendrasozhan S, Khan M, Saleem M, Husain Q, et al. Increased levels of autoantibodies against ROS-modified proteins in depressed individuals with decrease in antibodies against SARS-CoV-2 antigen (S1-RBD). *Curr Issues Mol Biol.* (2022) 44:5260–76. doi: 10.3390/cimb44110358
153. Farooq RK, Asghar K, Kanwal S, Zulqernain A. Role of inflammatory cytokines in depression: focus on interleukin-1 $\beta$ . *Biomed Rep.* (2017) 6:15–20. doi: 10.3892/br.2016.807
154. Patil M, Kulsange S, Kazi R, Chirmade T, Kale V, Mote C, et al. Behavioral and proteomic studies reveal methylglyoxal activate pathways associated with Alzheimer's disease. *ACS Pharmacol Transl Sci.* (2022) 6:65–75. doi: 10.1021/acspsci.2c00143
155. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt A. Advanced glycation end-products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. *Glycobiology.* (2005) 15:16R–28R. doi: 10.1093/glycob/cwi053
156. Koller D, Pathak GA, Wendt FR, Tylee DS, Levey DF, Overstreet C, et al. Epidemiologic and genetic associations of endometriosis with depression, anxiety, and eating disorders. *JAMA Netw Open.* (2023) 6:e2251214. doi: 10.1001/jamanetworkopen.2022.51214
157. Kajiyama H, Suzuki S, Yoshihara M, Tamauchi S, Yoshikawa N, Niimi K, et al. Endometriosis and cancer. *Free Rad Biol Med.* (2019) 133:186–92. doi: 10.1016/j.freeradbiomed.2018.12.015
158. Bulun SE, Yilmaz BD, Sison C, Miyazaki K, Bernardi L, Liu S, et al. Endometriosis. *Endocr Rev.* (2019) 40:1048–79. doi: 10.1210/er.2018-00242
159. Szukiewicz D. Epigenetic regulation and T-cell responses in endometriosis—something other than autoimmunity. *Front Immunol.* (2022) 13:943839. doi: 10.3389/fimmu.2022.943839
160. Shigesu N, Kvaskoff M, Kirtley S, Feng Q, Fang H, Knight JC, et al. The association between endometriosis and autoimmune diseases: a systematic review and meta-analysis. *Hum Reprod Update.* (2019) 25:486–503. doi: 10.1093/humupd/dmz014
161. Khan WA, Malik A, Khan MW. Depression linked to higher antibodies production against estrogenized insulin in type 1 diabetes. *Int Immunopharmacol.* (2020) 86:106712. doi: 10.1016/j.intimp.2020.106712
162. Khan WA, Malik A, Khan MW. Estrogenization of insulin by catecholesterogen produced high affinity autoantibodies and altered the normal function of insulin in type 1 diabetes. *Life Sci.* (2020) 256:117910. doi: 10.1016/j.lfs.2020.117910
163. Khan WA, Alsamghan AS, Khan MW. Endometrial cancer patients have high affinity antibodies for estrogen metabolite–receptor aggregate: a potential biomarker for EC. *J Obstet Gynaecol Res.* (2020) 46:2115–25. doi: 10.1111/jog.14413
164. Miller AH. Depression and immunity: a role for T cells? *Brain Behav Immun.* (2010) 24:1–8. doi: 10.1016/j.bbi.2009.09.009
165. Irwin MR, Miller AH. Depressive disorders and immunity: 20 years of progress and discovery. *Brain Behav Immun.* (2007) 21:374–83. doi: 10.1016/j.bbi.2007.01.010
166. Zorrilla EP, Luborsky L, McKay JR, Rosenthal R, Houldin A, Tax A, et al. The relationship of depression and stressors to immunological assays: a meta-analytic review. *Brain Behav Immun.* (2001) 15:199–226. doi: 10.1006/brbi.2000.0597
167. Sephton SE, Dhabhar FS, Keuroghlian AS, Giese-Davis J, McEwen BS, Ison AC, et al. Depression, cortisol, and suppressed cell-mediated immunity in metastatic breast cancer. *Brain Behav Immun.* (2009) 23:1148–55. doi: 10.1016/j.bbi.2009.07.007
168. Ivanova SA, Semke VY, Vetlugina TP, Rakitina NM, Kudryakova TA, Simutkin GG. Signs of apoptosis of immunocompetent cells in patients with depression. *Neurosci Behav Physiol.* (2007) 37:527–30. doi: 10.1007/s11055-007-0047-y
169. Szuster-Ciesielska A, Słowińska M, Stachura A, Marmurowska-Michałowska H, Dubas-Słemp H, Bojarska-Junak A, et al. Accelerated apoptosis of blood leukocytes and oxidative stress in blood of patients with major depression. *Prog Neuro-Psychopharmacol Biol Psychiatry.* (2008) 32:686–94. doi: 10.1016/j.pnpbp.2007.11.012
170. Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry.* (2001) 49:391–404. doi: 10.1016/S0006-3223(00)01088-X
171. McEwen BS, Biron CA, Brunson KW, Bulloch K, Chambers WH, Dhabhar FS, et al. The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. *Brain Res Brain Res Rev.* (1997) 23:79–133. doi: 10.1016/S0165-0173(96)00012-4
172. Monsivais D, Bray JD, Su E, Pavone ME, Dyson MT, Navarro A, et al. Activated glucocorticoid and eicosanoid pathways in endometriosis. *Fertil Steril.* (2012) 98:117–25. doi: 10.1016/j.fertnstert.2012.03.030
173. Koukoura O, Sifakis S, Spandidos DA. DNA methylation in endometriosis. *Mol Med Rep.* (2016) 13:2939–48. doi: 10.3892/mmr.2016.4925
174. Lamceva J, Uljanovs R, Strumfa I. The main theories on the pathogenesis of endometriosis. *Int J Mol Sci.* (2023) 24:4254. doi: 10.3390/ijms24054254
175. Králíčková M, Losan P, Vetricka V. Endometriosis and cancer. *Womens Health.* (2014) 10:591–7. doi: 10.2217/WHE.14.43
176. Esfandiari F, Favaedi R, Heidari-Khoei H, Chitsazian F, Yari S, Piryaee A, et al. Insight into epigenetics of human endometriosis organoids: DNA methylation analysis of HOX genes and their cofactors. *Fertil Steril.* (2021) 115:125–37. doi: 10.1016/j.fertnstert.2020.08.1398
177. Teague EM, Print CG, Hull ML. The role of microRNAs in endometriosis and associated reproductive conditions. *Hum Reprod Update.* (2010) 16:142–65. doi: 10.1093/humupd/dmp034
178. Anglesio MS, Papadopoulos N, Ayhan A, Nazeran TM, Noë M, Horlings HM, et al. Cancer-associated mutations in endometriosis without cancer. *N Engl J Med.* (2017) 376:1835–48. doi: 10.1056/NEJMoa1614814
179. Van Langendonck A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. *Fertil Steril.* (2002) 77:861–70. doi: 10.1016/S0015-0282(02)02959-X
180. Scutiero G, Iannone P, Bernardi G, Bonaccorsi G, Spadaro S, Volta CA, et al. Oxidative stress and endometriosis: a systematic review of the literature. *Oxidative Med Cell Longev.* (2017) 2017:7265238. doi: 10.1155/2017/7265238
181. Kobayashi H, Yamada Y, Kanayama S, Furukawa N, Noguchi T, Haruta S, et al. The role of iron in the pathogenesis of endometriosis. *Gynecol Endocrinol.* (2009) 25:39–52. doi: 10.1080/09513590802366204

182. Defrère S, Van Langendonck A, Vaesen S, Jouret M, González Ramos R, Gonzalez D, et al. Iron overload enhances epithelial cell proliferation in endometriotic lesions induced in a murine model. *Hum Reprod.* (2006) 21:2810–6. doi: 10.1093/humrep/del261
183. Iwabuchi T, Yoshimoto C, Shigetomi H, Kobayashi H. Cyst fluid hemoglobin species in endometriosis and its malignant transformation: the role of metallobiology. *Oncol Lett.* (2016) 11:3384–8. doi: 10.3892/ol.2016.4383
184. Ni Z, Li Y, Song D, Ding J, Mei S, Sun S, et al. Iron-overloaded follicular fluid increases the risk of endometriosis-related infertility by triggering granulosa cell ferroptosis and oocyte dysmaturity. *Cell Death Dis.* (2022) 13:579. doi: 10.1038/s41419-022-05037-8
185. Li A, Ni Z, Zhang J, Cai Z, Kuang Y, Yu C. Transferrin insufficiency and iron overload in follicular fluid contribute to oocyte dysmaturity in infertile women with advanced endometriosis. *Front Endocrinol.* (2020) 11:391. doi: 10.3389/fendo.2020.00391
186. Xu B, Guo N, Zhang XM, Shi W, Tong XH, Iqbal F, et al. Oocyte quality is decreased in women with minimal or mild endometriosis. *Sci Rep.* (2015) 5:10779. doi: 10.1038/srep10779
187. Sanchez AM, Papaleo E, Corti L, Santambrogio P, Levi S, Viganò P, et al. Iron availability is increased in individual human ovarian follicles in close proximity to an endometrioma compared with distal ones. *Hum Reprod.* (2014) 29:577–83. doi: 10.1093/humrep/det466
188. Giacomini E, Sanchez AM, Sarais V, Al Beitawi S, Candiani M, Viganò P. Characteristics of follicular fluid in ovaries with endometriomas. *Eur J Obstet Gynecol Reprod Biol.* (2017) 209:34–8. doi: 10.1016/j.ejogrb.2016.01.032
189. Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med.* (2010) 363:1532–43. doi: 10.1056/NEJMoa1008433
190. McConechy MK, Ding J, Senz J, Yang W, Melnyk N, Tone AA, et al. Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles. *Mod Pathol.* (2014) 27:128–34. doi: 10.1038/modpathol.2013.107
191. Matsumoto T, Yamazaki M, Takahashi H, Kajita S, Suzuki E, Tsuruta T, et al. Distinct  $\beta$ -catenin and PIK3CA mutation profiles in endometriosis-associated ovarian endometrioid and clear cell carcinomas. *Am J Clin Pathol.* (2015) 144:452–63. doi: 10.1309/AJCPZ5T2POOFMQVN
192. Catasús L, Bussaglia E, Rodríguez I, Gallardo A, Pons C, Irving JA, et al. Molecular genetic alterations in endometrioid carcinomas of the ovary: similar frequency of beta-catenin abnormalities but lower rate of microsatellite instability and PTEN alterations than in uterine endometrioid carcinomas. *Hum Pathol.* (2004) 35:1360–8. doi: 10.1016/j.humpath.2004.07.019
193. Sato N, Tsunoda H, Nishida M, Morishita Y, Takimoto Y, Kubo T, et al. Loss of heterozygosity on 10q23. 3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. *Cancer Res.* (2000) 60:7052–6.



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# Incidental findings on MRI for the evaluation of endometriosis: prevalence and clinical significance

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**Objectives:** This study aimed to analyze the prevalence and clinical significance of incidental findings on MRI for endometriosis. Differences between patients with and without evidence of deep infiltrating endometriosis on MRI were to be examined.

**Methods:** This was a retrospective, descriptive cross-sectional single-center study. All patients who received a pelvic MRI for endometriosis between April 2021 and February 2023 were included. The presence and frequency of incidental findings were noted after review of all MR images and radiology reports. The potential clinical significance of the findings was analyzed. Differences in the frequency of incidental findings between patients with and without evidence of deep infiltrating endometriosis on MRI were evaluated, utilizing the Chi-square test, Fisher's exact test and Mann-Whitney *U*-test.

**Results:** 303 consecutive patients (mean age, 33.4 years  $\pm$  8.3) were evaluated. Incidental findings were noted in 299/303 (98.7%) patients. Most frequently, ossification of the hip acetabular rim and degenerative changes of the lumbar spine were noted. In 25/303 (8.3%) patients, incidental findings had high clinical significance. For specific incidental findings, significantly higher prevalences were found in patients with than in patients without evidence of deep infiltrating endometriosis on MRI (hip acetabular rim ossification,  $p = 0.041$ ; annulus fibrosus fissures,  $p = 0.006$ ; gallstones,  $p = 0.042$ ).

**Conclusions:** Incidental findings are very common on pelvic MRI for endometriosis. The detection of incidental findings can lead to the diagnosis of relevant diseases and thus enable early treatment. On the other hand, many incidental findings have no, only minor, or uncertain consequences.

## KEYWORDS

pelvis, endometriosis, incidental findings, diagnostic imaging, magnetic resonance imaging

## 1 Introduction

Endometriosis is a disease characterized by the presence of endometrium-like tissue outside the endometrium and myometrium, usually accompanied by inflammatory changes (1). Endometriosis can affect various structures: The peritoneum, the ovaries, the intestinal wall, the urinary bladder, or extra-abdominal structures. Deep infiltrating

endometriosis (DIE) is a subtype of endometriosis, characterized by the presence of endometrial-like tissue in the abdominal cavity, which spreads on or under the peritoneal surface and can infiltrate adjacent organs (1).

Endometriosis can cause various symptoms, including chronic pelvic pain, dyspareunia, fatigue, and infertility (2). About 10%–15% of women of childbearing age and 35%–50% of women with pelvic pain and/or infertility are affected by endometriosis (3). Laparoscopy has traditionally been the method of choice for diagnosing endometriosis. Recently, however, there has been a growing body of research highlighting the value of imaging (MRI and transvaginal ultrasound) in the diagnosis of endometriosis (4–6). The recommendation of imaging in current guidelines (2) and the advantages of MRI (large examination field, non-invasiveness, standardization, little operator dependency) give reason to expect an increasingly broad application (4).

As with many other radiological examinations, the description and interpretation of incidental findings (IFs) on MRI for endometriosis can present a challenge. No data on IFs in endometriosis MRI are currently available (level of evidence: n/a) (7). IFs are defined as findings beyond the primary clinical indication of a study and may be clinically relevant but do not necessarily have to be (8–10). IFs include both insignificant marginal findings and false positive findings. IFs can lead to uncertainty among radiologists, referring physicians, and patients. The main reasons for this are a lack of information about the frequency and relevance of IFs and difficulties in differentiating relevant findings from physiological changes and normal variants. The radiological reporting of IFs may lead to further diagnostic examinations and medical interventions. These additional measures can be helpful and potentially life-saving but can also be unnecessary, costly, and risky. Therefore, an adequate strategy for the disclosure of IFs must be chosen. In order to develop such a strategy, however, data on the types and the frequency of findings are required in the first instance. As there is currently a lack of data on IFs in endometriosis MRIs, potential negative effects of this knowledge gap on patient treatment and outcomes are possible.

Challenges in the scientific analysis of IFs are that the patient's history and radiological reports are often available only in non-standardized form and that the interpretation of the images is subject to variability. In addition, a very specific imaging question (e.g., endometriosis) leads to a higher rate of IFs than a broader question (e.g., pelvic pain). A retrospective study of 1,040 abdominal CT scans for different indications revealed relevant IFs (leading to further imaging, clinical evaluation, or follow-up) in 18.8% of patients (11). As the rate of IFs in this study was based on a review of the radiology reports without a review of the images, it can be assumed that the rate of IFs was underestimated (9).

An association between endometriosis and various other diseases has been suspected, including gynecologic diseases, gastrointestinal diseases, immunological-related/autoimmune diseases, cardiovascular diseases, and cerebrovascular diseases (12, 13). This association would give reason to expect an increased rate of IFs in MRI examinations positive for endometriosis. However, no data on IFs in endometriosis MRI are currently available (7).

The objective of the present study was therefore to analyze the prevalence and distribution of IFs identified on pelvic MRI for endometriosis, including overview sequences from the kidneys to the pubic bone. In addition, differences in the frequency of IFs between patients with and without evidence of DIE on MRI and between patients with and without administration of gadolinium based contrast agents (GBCAs) were to be examined.

## 2 Materials and methods

This study was approved by the institutional review board. Due to the retrospective design of the study, informed consent was waived.

### 2.1 Study population and design

This study was conducted retrospectively on a cohort of patients from a descriptive cross-sectional single-center study. In this study all patients have been included who have received a pelvic MRI for evaluation of endometriosis between April 2021 and February 2023 after clinical gynecological examination and transvaginal sonography (14). All patients aged at least 18 years with clinically suspected pelvic endometriosis were included consecutively. The clinical gynecological suspicion of endometriosis was based on typical symptoms (e.g., chronic pelvic pain, dyspareunia, infertility) and/or findings of transvaginal sonography. No exclusion criteria were applied. The rate of patients in the study cohort with prior abdominal surgery was 187/303 (61.7%) with the following distribution (several procedures in one patient possible): Laparoscopy for endometriosis,  $n = 118$ ; appendectomy,  $n = 48$ ; cesarean section,  $n = 44$ ; total laparoscopic hysterectomy,  $n = 21$ ; laparoscopy for ovarian mass,  $n = 21$ ; laparoscopy for adhesions,  $n = 17$ ; diagnostic laparoscopy,  $n = 8$ ; rectum resection with anastomosis due to endometriosis,  $n = 7$ ; laparoscopic myomectomy,  $n = 6$ ; laparoscopic supracervical hysterectomy,  $n = 6$ ; laparoscopy for ectopic pregnancy,  $n = 5$ ; inguinal hernia repair,  $n = 5$ ; other surgical procedures,  $n = 25$ . 43 patients had at least one prior vaginal delivery. The MRI scans were positive for DIE in 106/303 (35.0%) patients and for endometriomas in 89/303 (29.4%) patients.

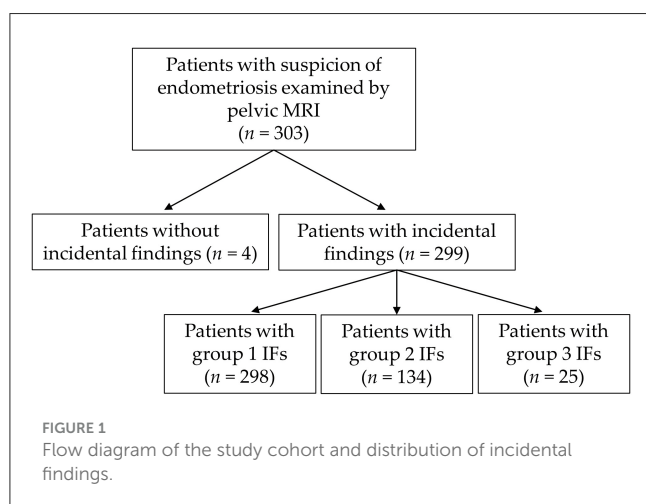
MRI scans were conducted at two 1.5 Tesla scanners (Avanto, Siemens Healthcare,  $n = 144$ ; Espree, Siemens Healthcare,  $n = 155$ ) and one 3 Tesla scanner (Skyra fit, Siemens Healthcare,  $n = 4$ ). Both field strengths are currently considered valuable for endometriosis imaging (15, 16). The scans included the key sequences recommended in recent guidelines (15, 16): T2-weighted FSE (fast spin echo) sequences (axial, sagittal, and coronal with small field of view; coronal single shot fat suppressed with large field of view for an overview of the kidneys and urinary system), and T1-weighted FSE sequences with and without fat suppression (axial with small field of view). Contrast-enhanced sequences were included optionally in 84/303 (27.7%) of patients, depending on the findings of the non-contrast sequences and the presence of additional questions (Gadoteridol, ProHance, 0.1 mmol/kg, Bracco Imaging) (14). Contrast-enhanced examinations encompassed axial and sagittal T1-weighted FSE sequences with

fat suppression (small field of view), and in 72/84 cases an additional short T1-weighted gradient-echo sequence (urography) and optional time-resolved MR angiography.

## 2.2 Image analysis and classification of incidental findings

The presence of incidental findings was noted after a second review of the images of all patients and of the original radiology reports of all patients by a radiologist with 8 years' experience in pelvic MRI (S.H.). An incidental finding was defined as an unrelated imaging abnormality on pelvic MRI for endometriosis. The clinical significance of IFs was classified following previous studies (17):

- Group 1: Not significant; no further evaluation or treatment required.
- Group 2: Moderately/potentially significant; further diagnostic studies, follow-up, or treatment possibly necessary.
- Group 3: Significant; relevant impact on the patient's prognosis or immediate treatment required.



Adnexal lesions were assessed following the Ovarian-Adnexal Reporting and Data System (O-RADS) (18) and the work by Sahin et al. (19) for non-contrast examinations. Diagnosis of polycystic ovary morphology (PCOM) was made according to Teede et al. (20). The diagnosis of leiomyomas was made in accordance with the guidelines of the European Society of Urogenital Radiology (ESUR) (21). The assessment for pelvic venous anomalies was performed in accordance with the criteria by Bookwalter et al. (22). Acetabular rim ossifications (ARO) were diagnosed following the work of Valente et al. (23). Degenerations of the lumbar spine were categorized utilizing the Modic grades (24) and the recommendations by Fardon et al. (25). Lumbar foraminal stenoses were classified using a simplified adaption of the Lee system (26). Changes in the sacroiliac joints (SIJ) were described in simplified form (27) as abnormalities with or without edema. Hydronephrosis was graded in orientation to the system by the Society of Fetal Urology (SFU) (28). The common upper limit of  $\geq 10$  mm in short axis was applied for the definition of enlarged lymph nodes (29, 30).

## 2.3 Statistical analysis

Statistical analyses were performed by S.H. using IBM SPSS Statistics 29.0. The final study population was stratified in patients with and without DIE on MRI to evaluate for possible differences in the prevalence of IFs within these two groups. In addition, differences in the number of IFs between non-contrast and contrast examinations were investigated, and differences in the frequencies of the individual IFs depending on patient age. Continuous variables are presented as mean and standard deviation, and categorical variables are presented as counts and percentages. 95% confidence intervals are Clopper-Pearson intervals. Chi-square test and Fisher's exact test were used to compare differences in the frequencies of categorical variables. The Mann-Whitney *U*-test was performed to assess the differences

TABLE 1 Prevalence of IFs and mean number of IFs per examination and by DIE diagnosis on MRI with percentages and 95% confidence intervals in brackets.

|                                    | Total ( <i>n</i> = 303) | MRI positive for DIE ( <i>n</i> = 106) | MRI negative for DIE ( <i>n</i> = 197) | <i>p</i> -values   |
|------------------------------------|-------------------------|----------------------------------------|----------------------------------------|--------------------|
| Prevalence of all IFs (%)          | 299 (98.7; 96.7–99.6)   | 106 (100.0; 96.6–100.0)                | 193 (98.0; 94.9–99.4)                  | 0.302 <sup>a</sup> |
| Mean number of IFs per MRI         | 5.84 ± 3.14             | 6.22 ± 2.81                            | 5.64 ± 3.29                            | 0.075 <sup>c</sup> |
| Prevalence of group 1 IFs (%)      | 298 (98.3; 96.2–99.5)   | 106 (100.0; 96.6–100.0)                | 192 (97.5; 94.2–99.2)                  | 0.167 <sup>a</sup> |
| Mean number of group 1 IFs per MRI | 5.17 ± 2.85             | 5.54 ± 2.62                            | 4.97 ± 2.95                            | 0.064 <sup>c</sup> |
| Prevalence of group 2 IFs (%)      | 134 (44.2; 38.5–50.0)   | 53 (50.0; 40.1–59.9)                   | 81 (41.1; 34.2–48.3)                   | 0.138 <sup>b</sup> |
| Mean number of group 2 IFs per MRI | 0.59 ± 0.78             | 0.59 ± 0.67                            | 0.59 ± 0.83                            | 0.404 <sup>c</sup> |
| Prevalence of group 3 IFs (%)      | 25 (8.3; 5.4–11.9)      | 9 (8.5; 4.0–15.5)                      | 16 (8.1; 4.7–12.9)                     | 0.911 <sup>b</sup> |
| Mean number of group 3 IFs per MRI | 0.09 ± 0.29             | 0.08 ± 0.28                            | 0.09 ± 0.30                            | 0.922 <sup>c</sup> |

IF, incidental finding; DIE, deep infiltrating endometriosis.

<sup>a</sup>Fisher's exact test.

<sup>b</sup>Chi-square test.

<sup>c</sup>Mann-Whitney *U*-test.



**TABLE 2** Prevalence of IFs and mean number of IFs per examination and by GBCA application with percentages and 95% confidence intervals in brackets.

|                                    | Total ( <i>n</i> = 303) | Application of GBCA ( <i>n</i> = 84) | No application of GBCA ( <i>n</i> = 219) | <i>p</i> -values         |
|------------------------------------|-------------------------|--------------------------------------|------------------------------------------|--------------------------|
| Prevalence of all IFs (%)          | 299 (98.7; 96.7–99.6)   | 84 (100.0; 95.7–100.0)               | 215 (98.2; 95.4–99.5)                    | 0.579 <sup>a</sup>       |
| Mean number of IFs per MRI         | 5.84 ± 3.14             | 6.79 ± 3.11                          | 5.48 ± 3.08                              | <b>0.002<sup>c</sup></b> |
| Prevalence of group 1 IFs (%)      | 298 (98.3; 96.2–99.5)   | 84 (100.0; 95.7–100.0)               | 214 (97.7; 94.8–99.3)                    | 0.327 <sup>a</sup>       |
| Mean number of group 1 IFs per MRI | 5.17 ± 2.85             | 5.83 ± 2.87                          | 4.91 ± 2.80                              | <b>0.014<sup>c</sup></b> |
| Prevalence of group 2 IFs (%)      | 134 (44.2; 38.5–50.0)   | 44 (52.4; 41.2–63.4)                 | 90 (41.1; 34.5–47.9)                     | 0.077 <sup>b</sup>       |
| Mean number of group 2 IFs per MRI | 0.59 ± 0.78             | 0.79 ± 0.92                          | 0.52 ± 0.71                              | <b>0.023<sup>c</sup></b> |
| Prevalence of group 3 IFs (%)      | 25 (8.3; 5.4–11.9)      | 13 (15.5; 8.5–25.0)                  | 12 (5.5; 2.9–9.4)                        | <b>0.005<sup>b</sup></b> |
| Mean number of group 3 IFs per MRI | 0.09 ± 0.29             | 0.17 ± 0.41                          | 0.05 ± 0.23                              | <b>0.004<sup>c</sup></b> |

Bold values denote statistical significance at the  $p \leq 0.05$  level.

IF, incidental finding; GBCA, gadolinium based contrast agent; DIE, deep infiltrating endometriosis.

<sup>a</sup>Fisher's exact test.

<sup>b</sup>Chi-square test.

<sup>c</sup>Mann–Whitney U-test.

in the mean numbers of IFs.  $p$ -values  $\leq 0.05$  were considered statistically significant.

## 3 Results

A total of 1771 IFs were noted in the study cohort of 303 patients [mean age, 33.4 years  $\pm$  8.3 (standard deviation); median age, 33; [Figure 1](#)]. IFs were recorded in 299/303 examinations (98.7%). 11 patients had one IF, 30 patients had two IFs, 31 patients had three IFs, 41 patients had four IFs and 186 patients had five or more IFs. The mean number of IFs per patient was 5.8, and the median number of IFs per patient was 6.

### 3.1 Frequency and clinical significance of incidental findings

The prevalence of IFs and the mean number of IFs per patient are presented in [Table 1](#). No statistically significant differences were found in the prevalence of IFs (all IFs, group 1 IFs, group 2 IFs, group 3 IFs) and the mean number of IFs per patient between patients with and without DIE diagnosis on MRI. In [Table 2](#), the prevalence of IFs and the mean number of IFs per patient are presented subdivided according to GBCA administration on MRI and clinical significance. The mean number of IFs per MRI in non-contrast and contrast examinations differed significantly (all IFs,  $p = 0.002$ ; group 1 IFs,  $p = 0.014$ ; group 2 IFs,  $p = 0.023$ ; group 3 IFs,  $p = 0.004$ ). The overall prevalence of IFs in non-contrast and contrast examinations did not differ significantly ( $p = 0.579$ ) and was 215/219 (98.2%) and 84/84 (100%), respectively. The prevalence of group 1 and group 2 IFs did also not differ significantly between non-contrast and contrast examinations, but it did for group 3 IFs ( $p = 0.005$ ).

### 3.2 Incidental findings with high clinical significance

[Table 3](#) and [Supplementary Table S1](#) show the number of incidental findings with high clinical significance (group 3) on MRI. Differences between patients with and without DIE diagnosis on MRI are specified in [Table 3](#). Differences between patients aged  $<33$  and  $\geq 33$  are specified in [Supplementary Table S1](#). The most frequent IFs of high clinical significance were mature ovarian teratomas (histologically proven in 5/6 cases; [Figure 2](#)) and hydronephrosis. In patients with hydronephrosis, MRI showed no evidence of causative endometriosis in 10/11 cases (e.g., hydronephrosis due to ureteropelvic junction obstruction; [Figure 3](#)). No statistically significant differences were found in the number of the individual incidental findings with high clinical significance between patients with and without DIE diagnosis on MRI.

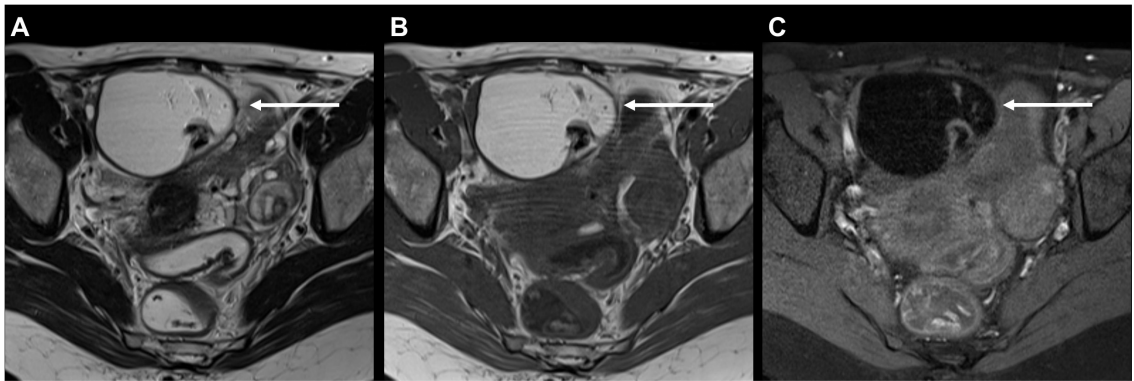
### 3.3 Incidental findings with moderate clinical significance

[Table 4](#) and [Supplementary Table S2](#) depict the number of incidental findings with moderate clinical significance (group 2) on MRI (for  $n \geq 3$ ). Differences between patients with and without DIE diagnosis on MRI are specified in [Table 4](#). Differences between patients aged  $<33$  and  $\geq 33$  are specified in [Supplementary Table S2](#). The most frequent IFs of moderate clinical significance were leiomyomas without degeneration in 44/303 (14.5%) patients ([Figure 4](#)) and degenerative changes of the lumbar spine with potential nerve root compression in 28/303 (9.2%) patients ([Figure 5A](#)). Nutcracker anatomy was detected significantly more frequently in patients without than in patients with evidence of DIE on MRI ( $p = 0.030$ ). Gallstones were detected significantly more frequently in patients with than in patients without evidence of DIE on MRI ( $p = 0.042$ ). Less frequent IFs with moderate clinical significance ( $n \leq 2$ ) were: Signs of ovarian failure, uterine polyp, degenerated leiomyoma, cyst of

**TABLE 3** Number of IFs with high clinical significance (group 3) on MRI and differences between patients with and without diagnosis of DIE on MRI with percentages and 95% confidence intervals in brackets (several findings per patient possible).

| Findings                                              | Total ( <i>n</i> = 303) | MRI DIE+ ( <i>n</i> = 106) | MRI DIE– ( <i>n</i> = 197) | <i>p</i> -values   |
|-------------------------------------------------------|-------------------------|----------------------------|----------------------------|--------------------|
| Mature ovarian teratoma                               | 6 (2.0; 0.7–4.3)        | 1 (0.9; 0.0–5.1)           | 5 (2.5; 0.8–5.8)           | 0.669 <sup>a</sup> |
| Hydronephrosis, grade 2                               | 6 (2.0; 0.7–4.3)        | 2 (1.9; 0.2–6.6)           | 4 (2.0; 0.6–5.1)           | 1.000 <sup>a</sup> |
| Hydronephrosis, grade 1                               | 3 (1.0; 0.2–2.9)        | 0 (0; 0.0–3.4)             | 3 (1.5; 0.3–4.4)           | 0.554 <sup>a</sup> |
| Hydronephrosis, grade 3                               | 2 (0.7; 0.1–2.4)        | 1 (0.9; 0.0–5.1)           | 1 (0.5; 0.0–2.8)           | 1.000 <sup>a</sup> |
| Small bowel obstruction due to postsurgical adhesions | 1 (0.3; 0.0–1.8)        | 1 (0.9; 0.0–5.1)           | 0 (0; 0.0–1.9)             | 0.350 <sup>a</sup> |
| Ovarian cyst, intermediate risk (O-RADS 4)            | 1 (0.3; 0.0–1.8)        | 0 (0; 0.0–3.4)             | 1 (0.5; 0.0–2.8)           | 1.000 <sup>a</sup> |
| Tailgut cyst                                          | 1 (0.3; 0.0–1.8)        | 1 (0.9; 0.0–5.1)           | 0 (0; 0.0–1.9)             | 0.350 <sup>a</sup> |
| Cervical cancer                                       | 1 (0.3; 0.0–1.8)        | 0 (0; 0.0–3.4)             | 1 (0.5; 0.0–2.8)           | 1.000 <sup>a</sup> |
| Pelvic inflammatory disease                           | 1 (0.3; 0.0–1.8)        | 0 (0; 0.0–3.4)             | 1 (0.5; 0.0–2.8)           | 1.000 <sup>a</sup> |
| Malpositioned intrauterine device (IUD)               | 1 (0.3; 0.0–1.8)        | 0 (0; 0.0–3.4)             | 1 (0.5; 0.0–2.8)           | 1.000 <sup>a</sup> |
| Liver cirrhosis                                       | 1 (0.3; 0.0–1.8)        | 1 (0.9; 0.0–5.1)           | 0 (0; 0.0–1.9)             | 0.350 <sup>a</sup> |
| Severe colonic wall thickening due to colitis         | 1 (0.3; 0.0–1.8)        | 1 (0.9; 0.0–5.1)           | 0 (0; 0.0–1.9)             | 0.350 <sup>a</sup> |
| Aneurysm of common femoral artery                     | 1 (0.3; 0.0–1.8)        | 1 (0.9; 0.0–5.1)           | 0 (0; 0.0–1.9)             | 0.350 <sup>a</sup> |

IF, incidental finding; MRI, magnetic resonance imaging; DIE, deep infiltrating endometriosis.  
<sup>a</sup>Fisher's exact test.



**FIGURE 2**  
41-year-old patient with typical mature ovarian teratoma on MRI for endometriosis: (A) Axial T2 FSE (fast spin echo) and (B) axial T1 FSE showing a mostly hyperintense mass with hypointense components measuring 7 cm (arrows). (C) Axial T1 FSE with fat suppression confirms the presence of macroscopic fat due to signal loss upon fat suppression. Mature ovarian teratoma was proven histologically.

the vaginal wall, bicornuate uterus, hydrosalpinx, hepatomegaly, umbilical hernia, pelvic floor prolapse (*n* = 2, 0.7%, respectively). In individual cases, the following IFs with moderate clinical significance were found: Peritoneal inclusion cyst, occlusion of common femoral vein, agenesis of common iliac vein, retroaortic left renal vein, May-Thurner syndrome, dural ectasia, enlarged inguinal lymph nodes, enlarged iliac lymph nodes, arterial elongation, osteochondroma, cartilage damage of the hip, pubic ramus fracture, bilateral kidney atrophy (unrelated to endometriosis), scar tissue of the urinary bladder after sampling, scar tissue of the urinary bladder after suturing, hematosalpinx, splenomegaly, spigelian hernia, anal fistula (*n* = 1, 0.3%, respectively).

### 3.4 Incidental findings with low clinical significance

Table 5 and Supplementary Table S3 show the number of incidental findings with low clinical significance (group 1) on MRI (for *n* ≥ 3). Differences between patients with and without DIE diagnosis on MRI are specified in Table 5. Differences between patients aged <33 and ≥33 are specified in Supplementary Table S3. The most frequently noted IFs of low clinical significance were ARO in 200/303 (66.0%) patients (Figure 6) and lumbar disc desiccation in 146/303 (48.2%) patients (Figure 5B). ARO and annular fissures of intervertebral discs were detected significantly more frequently in patients with than in

patients without evidence of DIE on MRI ( $p = 0.041$  and  $p = 0.006$ , respectively). Less frequent IFs with low clinical significance ( $n \leq 2$ ) were: Meyerding grade II spondylolisthesis, Castellvi Ib lumbosacral transitional vertebra, coxa magna, acetabular paralabral cyst, supraacetabular fossa, femoral shaft pseudolesion, focal edema of the femoral neck, sacroiliac joint ankylosis, arcuate

uterus, marked post-operative changes to the uterus not associated with cesarean section ( $n = 2$ , 0.7%, respectively). In individual cases, the following IFs with low clinical significance were found: External iliac vein ectasia, butterfly vertebra, interspinous bursitis, Castellvi Ia lumbosacral transitional vertebra, O'Driscoll type 3 morphology of first sacral intervertebral disc, muscular focus of activity, postoperative changes in the SIJ after screw fixation, benign lesion of the iliac bone, lipoma of the abdominal wall muscles, splenic cyst, transient hepatic intensity difference, minor hemoperitoneum, atrophy of the gluteal muscles, atrophy of the piriformis muscle, edema of the quadriceps femoris muscle, subcutaneous inflammatory changes ( $n = 1$ , 0.3%, respectively).

## 4 Discussion

This study analyzed the prevalence and distribution of IFs detected on pelvic MRI for endometriosis, including overview sequences from the kidneys to the pubic bone. Our findings show that IFs with high clinical relevance are common, and IFs with moderate and low clinical relevance are very common with prevalences of 25/303 (8.3%; 95% CI 5.4–11.9%), 134/303 (44.2%; 95% CI 38.5–50.0%), and 298/303 (98.3%; 95% CI 96.2–99.5%), respectively. The most frequent individual IFs were ARO (200/303 patients, 66.0%) and lumbar disc desiccation (146/303 patients, 48.2%). The overall prevalence of IFs, the prevalence of IFs grouped by clinical significance and the mean number of IFs per patient did not differ significantly between patients with and without DIE diagnosis on MRI ( $p = 0.064$  to  $p = 0.922$ ). For three individual IFs, significantly higher prevalences were found in patients with than in patients without evidence of DIE on MRI (ARO, fissures of the annulus fibrosus, gallstones).

To date, no data are available on the presence of IFs on pelvic MRI for endometriosis. Several recent studies have investigated the frequency of incidental findings in prostate MRI, although



**FIGURE 3**  
27-year-old patient with hydronephrosis of the left kidney (arrows), evident on coronal single shot fat-suppressed T2 FSE (fast spin echo) with large field of view. There is dilatation of the renal pelvis and calyces. The underlying condition was a stenosis of the ureteropelvic junction.

**TABLE 4** Number of IFs with moderate clinical significance (group 2) on MRI (for  $n \geq 3$ ) and differences between patients with and without diagnosis of DIE on MRI with percentages and 95% confidence intervals in brackets (several findings per patient possible).

| Findings                                | Total ( $n = 303$ )  | MRI DIE+ ( $n = 106$ ) | MRI DIE- ( $n = 197$ ) | $p$ -values              |
|-----------------------------------------|----------------------|------------------------|------------------------|--------------------------|
| Leiomyomas, no degeneration             | 44 (14.5; 10.8–19.0) | 14 (13.2; 7.4–21.2)    | 30 (15.2; 10.5–21.0)   | 0.634 <sup>b</sup>       |
| Potential lumbar nerve root compression | 28 (9.2; 6.2–13.1)   | 10 (9.4; 4.6–16.7)     | 18 (9.1; 5.5–14.1)     | 0.932 <sup>b</sup>       |
| Ovarian cyst, indeterminate             | 11 (3.6; 1.8–6.4)    | 5 (4.7; 1.5–10.7)      | 6 (3.0; 1.1–6.5)       | 0.525 <sup>a</sup>       |
| Lumbar nerve root compression           | 10 (3.3; 1.6–6.0)    | 2 (1.9; 0.2–6.6)       | 8 (4.1; 1.8–7.8)       | 0.503 <sup>a</sup>       |
| Nutcracker anatomy                      | 9 (3.0; 1.4–5.6)     | 0 (0; 0.0–3.4)         | 9 (4.6; 2.1–8.5)       | <b>0.030<sup>a</sup></b> |
| Pelvic venous congestion                | 9 (3.0; 1.4–5.6)     | 1 (0.9; 0.0–5.1)       | 8 (4.1; 1.8–7.8)       | 0.168 <sup>a</sup>       |
| Polycystic ovaries                      | 7 (2.3; 0.9–4.7)     | 3 (2.8; 0.6–8.0)       | 4 (2.0; 0.6–5.1)       | 0.699 <sup>a</sup>       |
| Cesarean scar diverticulum              | 7 (2.3; 0.9–4.7)     | 2 (1.9; 0.2–6.6)       | 5 (2.5; 0.8–5.8)       | 1.000 <sup>a</sup>       |
| Postsurgical bowel adhesions            | 6 (2.0; 0.7–4.3)     | 2 (1.9; 0.2–6.6)       | 4 (2.0; 0.6–5.1)       | 1.000 <sup>a</sup>       |
| Ureter duplication                      | 4 (1.3; 0.4–3.3)     | 1 (0.9; 0.0–5.1)       | 3 (1.5; 0.3–4.4)       | 1.000 <sup>a</sup>       |
| Ascites                                 | 4 (1.3; 0.4–3.3)     | 3 (2.8; 0.6–8.0)       | 1 (0.5; 0.0–2.8)       | 0.125 <sup>a</sup>       |
| Gallstones                              | 3 (1.0; 0.2–2.9)     | 3 (2.8; 0.6–8.0)       | 0 (0.0; 0.0–1.9)       | <b>0.042<sup>a</sup></b> |

Bold values denote statistical significance at the  $p \leq 0.05$  level.

IF, incidental finding; MRI, magnetic resonance imaging; DIE, deep infiltrating endometriosis.

<sup>a</sup>Fisher's exact test.

<sup>b</sup>Chi-square test.

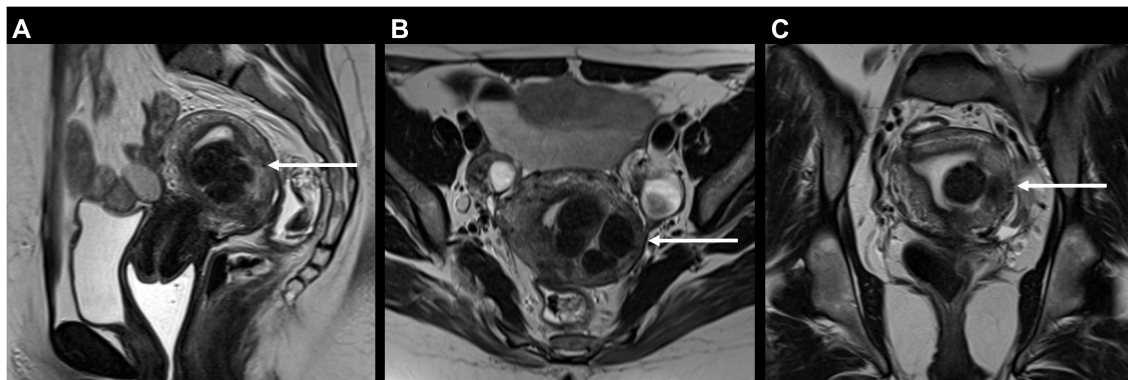


FIGURE 4

37-year-old patient with typical uterine leiomyomas (syn. fibroids) on MRI for endometriosis: (A) Sagittal, (B) axial, and (C) coronal T2 FSE (fast spin echo) showing mostly hypointense submucous and intramural masses of the uterine posterior wall measuring 3 cm in total (arrows). One submucous leiomyoma protrudes into the uterine cavity, largely surrounded by endometrium [type 1 according to the FIGO fibroid classification system (55)].

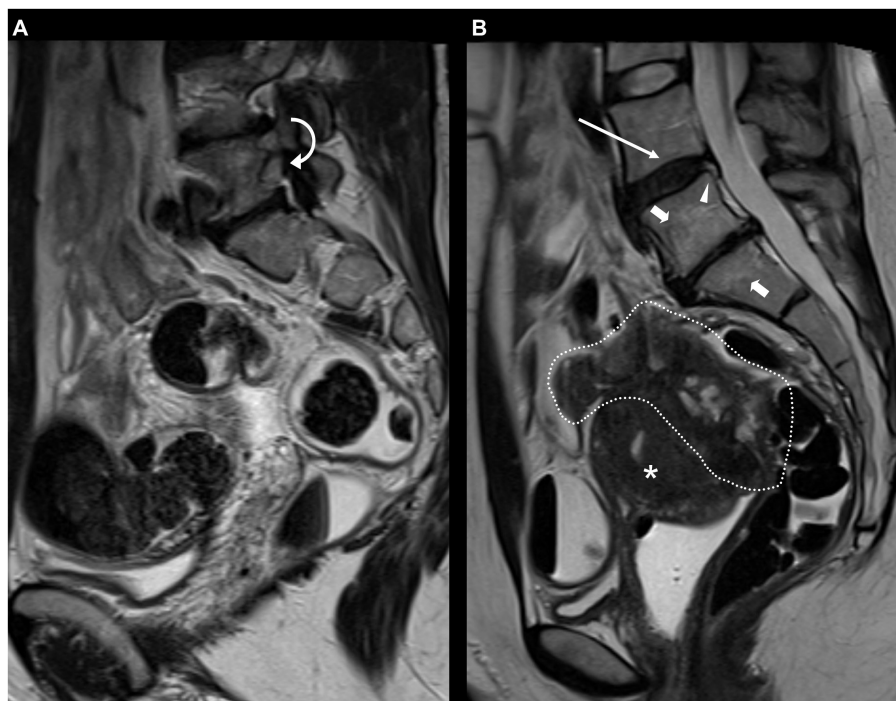


FIGURE 5

(A) 40-year-old patient with potential foraminal lumbar nerve root compression on MRI for endometriosis, depicted on sagittal T2 FSE (fast spin echo) at L5-S1 level due to decreased height of the intervertebral disc, bulging of the disc and articular process hypertrophy (curved arrow). (B) 36-year-old patient with typical deep infiltrating endometriosis (DIE) and degenerations of the lumbar spine on sagittal T2 FSE (fast spin echo). A large, inhomogeneous mass of DIE is evident in the pouch of Douglas (area encircled by dotted line) with involvement of the vaginal vault, the rectum, the sigmoid colon, and the posterior outer myometrium [A2, B3/3, C3, FA, FI according to the #Enzian classification (56)]. Also included on the MRI slice are degenerative changes of the lumbar spine with desiccation (long arrow) and annular fissure (arrowhead) of the L4-L5 disc, decreased height and extrusion of the L5-S1 disc and associated Modic type 2 signal changes (short arrows). Asterisk: Uterus.

the comparability with our results is obviously reduced due to the different patient population. Cutaia et al. found IFs in only 52.7% and Sherrer et al. in only 40.2% of patients on prostate MRI despite the older age of the patients (mean age 67.1 and 63.3 years, respectively) (17, 31). These lower prevalences of IFs are most likely attributable to the smaller field of view of prostate MRI.

Consequently, changes of the hip joints and the lumbar spine were not included in these studies. MRI for endometriosis is performed with a larger field of view, so that the entire pelvis and the lower part of the lumbar spine are included in the scans. When sequences are included for an overview of the kidneys and urinary tract, parts of the liver and other upper abdominal organs may also be visible.



**TABLE 5** Number of IFs with low clinical significance (group 1) on MRI (for  $n \geq 3$ ) and differences between patients with and without diagnosis of DIE on MRI with percentages and 95% confidence intervals in brackets (several findings per patient possible).

| Findings                                               | Total ( $n = 303$ )   | MRI DIE+ ( $n = 106$ ) | MRI DIE- ( $n = 197$ ) | $p$ -values              |
|--------------------------------------------------------|-----------------------|------------------------|------------------------|--------------------------|
| Ossification of the acetabular rim                     | 200 (66.0; 60.4–71.3) | 78 (73.6; 64.1–81.7)   | 122 (61.9; 54.8–68.7)  | <b>0.041<sup>b</sup></b> |
| Lumbar disc desiccation                                | 146 (48.2; 42.4–54.0) | 55 (51.9; 42.0–61.7)   | 91 (46.2; 39.1–53.4)   | 0.344 <sup>b</sup>       |
| T2-hyperintensity of hip labrum                        | 122 (40.3; 34.7–46.0) | 43 (40.6; 31.1–50.5)   | 79 (40.1; 33.2–47.3)   | 0.937 <sup>b</sup>       |
| Nabothian cysts of cervix uteri                        | 113 (37.3; 31.8–43.0) | 44 (41.5; 32.0–51.5)   | 69 (35.0; 28.4–42.1)   | 0.266 <sup>b</sup>       |
| Annular fissure, intervertebral disc                   | 101 (33.3; 28.0–38.9) | 46 (43.4; 33.8–53.4)   | 55 (27.9; 21.8–34.7)   | <b>0.006<sup>b</sup></b> |
| Abnormalities of SIJ w/o osseous edema                 | 72 (23.8; 19.1–29.0)  | 26 (24.5; 16.7–33.8)   | 46 (23.4; 17.6–29.9)   | 0.818 <sup>b</sup>       |
| Post-surgical pelvic scarring                          | 67 (22.1; 17.6–27.2)  | 25 (23.6; 15.9–32.8)   | 42 (21.3; 15.8–27.7)   | 0.650 <sup>b</sup>       |
| Lumbar disc protrusion                                 | 63 (20.8; 16.4–25.8)  | 25 (23.6; 15.9–32.8)   | 38 (19.3; 14.0–25.5)   | 0.380 <sup>b</sup>       |
| Lumbar disc bulge                                      | 61 (20.1; 15.8–25.1)  | 19 (17.9; 11.2–26.6)   | 42 (21.3; 15.8–27.7)   | 0.482 <sup>b</sup>       |
| Changes of symphysis pubis, no edema                   | 61 (20.1; 15.8–25.1)  | 19 (17.9; 11.2–26.6)   | 42 (21.3; 15.8–27.7)   | 0.482 <sup>b</sup>       |
| Lumbar disc extrusion                                  | 60 (19.8; 15.5–24.7)  | 23 (21.7; 14.3–30.8)   | 37 (18.8; 13.6–24.9)   | 0.543 <sup>b</sup>       |
| Simple ovarian cyst $\leq 3$ cm                        | 46 (15.2; 11.3–19.7)  | 15 (14.2; 8.1–22.3)    | 31 (15.7; 10.9–21.6)   | 0.714 <sup>b</sup>       |
| Corpus luteum $\leq 3$ cm                              | 41 (13.5; 9.9–17.9)   | 11 (10.4; 5.3–17.8)    | 30 (15.2; 10.5–21.0)   | 0.239 <sup>b</sup>       |
| Abnormalities of SIJ with osseous edema                | 31 (10.2; 7.1–14.2)   | 9 (8.5; 0.4–15.5)      | 22 (11.2; 7.1–16.4)    | 0.463 <sup>b</sup>       |
| Osseous hemangioma                                     | 27 (8.9; 6.0–12.7)    | 10 (9.4; 4.6–16.7)     | 17 (8.6; 5.1–13.5)     | 0.815 <sup>b</sup>       |
| Modic II endplate changes                              | 26 (8.6; 5.7–12.3)    | 7 (6.6; 2.7–13.1)      | 19 (9.6; 5.9–14.7)     | 0.367 <sup>b</sup>       |
| Modic I endplate changes                               | 23 (7.6; 4.9–11.2)    | 9 (8.5; 0.4–15.5)      | 14 (7.1; 3.9–11.6)     | 0.664 <sup>b</sup>       |
| Developmental dysplasia of hip                         | 19 (6.3; 3.8–9.6)     | 7 (6.6; 2.7–13.1)      | 12 (6.1; 3.2–10.4)     | 0.861 <sup>b</sup>       |
| Marked facet joint degenerations                       | 18 (5.9; 3.6–9.2)     | 9 (8.5; 0.4–15.5)      | 9 (4.6; 2.1–8.5)       | 0.168 <sup>b</sup>       |
| Simple renal cyst                                      | 17 (5.6; 3.3–8.8)     | 8 (7.5; 3.3–14.3)      | 9 (4.6; 2.1–8.5)       | 0.283 <sup>b</sup>       |
| Hip joint effusion                                     | 16 (5.3; 3.0–8.4)     | 4 (3.8; 1.0–9.4)       | 12 (6.1; 3.2–10.4)     | 0.390 <sup>b</sup>       |
| Scoliosis                                              | 14 (4.6; 2.5–7.6)     | 7 (6.6; 2.7–13.1)      | 7 (3.6; 1.4–7.2)       | 0.257 <sup>a</sup>       |
| Paralabral cyst of the hip                             | 14 (4.6; 2.5–7.6)     | 5 (4.7; 1.5–10.7)      | 9 (4.6; 2.1–8.5)       | 1.000 <sup>a</sup>       |
| Schmorl node                                           | 12 (4.0; 2.1–6.8)     | 4 (3.8; 1.0–9.4)       | 8 (4.1; 1.8–7.8)       | 1.000 <sup>a</sup>       |
| Femoral neck herniation pits                           | 12 (4.0; 2.1–6.8)     | 4 (3.8; 1.0–9.4)       | 8 (4.1; 1.8–7.8)       | 1.000 <sup>a</sup>       |
| Greater trochanteric edema                             | 11 (3.6; 1.8–6.4)     | 6 (5.7; 2.1–11.9)      | 5 (2.5; 0.8–5.8)       | 0.202 <sup>a</sup>       |
| Spondylolisthesis, grade I                             | 10 (3.3; 1.6–6.0)     | 4 (3.8; 1.0–9.4)       | 6 (3.0; 1.1–6.5)       | 0.744 <sup>a</sup>       |
| Separation of the pars interarticularis, L5            | 10 (3.3; 1.6–6.0)     | 3 (2.8; 0.6–8.0)       | 7 (3.6; 1.4–7.2)       | 1.000 <sup>a</sup>       |
| Lumbosacral transitional vertebra, type Castellvi IIa  | 10 (3.3; 1.6–6.0)     | 3 (2.8; 0.6–8.0)       | 7 (3.6; 1.4–7.2)       | 1.000 <sup>a</sup>       |
| Ovarian cyst (i.e., O-RADS 2)                          | 9 (3.0; 1.4–5.6)      | 2 (1.9; 0.2–6.6)       | 7 (3.6; 1.4–7.2)       | 0.503 <sup>a</sup>       |
| Lumbosacral transitional vertebra, type Castellvi IIb  | 9 (3.0; 1.4–5.6)      | 5 (4.7; 1.5–10.7)      | 4 (2.0; 0.6–5.1)       | 0.286 <sup>a</sup>       |
| O'Driscoll type 4 disc morphology                      | 9 (3.0; 1.4–5.6)      | 4 (3.8; 1.0–9.4)       | 5 (2.5; 0.8–5.8)       | 0.724 <sup>a</sup>       |
| Liver cysts                                            | 9 (3.0; 1.4–5.6)      | 4 (3.8; 1.0–9.4)       | 5 (2.5; 0.8–5.8)       | 0.724 <sup>a</sup>       |
| Lumbosacral transitional vertebra, type Castellvi IIIb | 7 (2.3; 0.9–4.7)      | 3 (2.8; 0.6–8.0)       | 4 (2.0; 0.6–5.1)       | 0.699 <sup>a</sup>       |
| Hamstring tendinopathy                                 | 7 (2.3; 0.9–4.7)      | 5 (4.7; 1.5–10.7)      | 2 (1.0; 0.1–3.6)       | 0.053 <sup>a</sup>       |
| Bartholin cyst                                         | 7 (2.3; 0.9–4.7)      | 2 (1.9; 0.2–6.6)       | 5 (2.5; 0.8–5.8)       | 1.000 <sup>a</sup>       |
| Pelvic floor atrophy, unilateral                       | 6 (2.0; 0.7–4.3)      | 2 (1.9; 0.2–6.6)       | 4 (2.0; 0.6–5.1)       | 1.000 <sup>a</sup>       |
| Vertebral body shiny corner                            | 5 (1.7; 0.5–3.8)      | 0 (0; 0.0–3.4)         | 5 (2.5; 0.8–5.8)       | 0.167 <sup>a</sup>       |
| Productive changes of symphysis pubis with edema       | 5 (1.7; 0.5–3.8)      | 2 (1.9; 0.2–6.6)       | 3 (1.5; 0.3–4.4)       | 1.000 <sup>a</sup>       |
| Colonic diverticulosis                                 | 4 (1.3; 0.4–3.3)      | 2 (1.9; 0.2–6.6)       | 2 (1.0; 0.1–3.6)       | 0.614 <sup>a</sup>       |
| Loss of colonic haustra                                | 4 (1.3; 0.4–3.3)      | 1 (0.9; 0.0–5.1)       | 3 (1.5; 0.3–4.4)       | 1.000 <sup>a</sup>       |

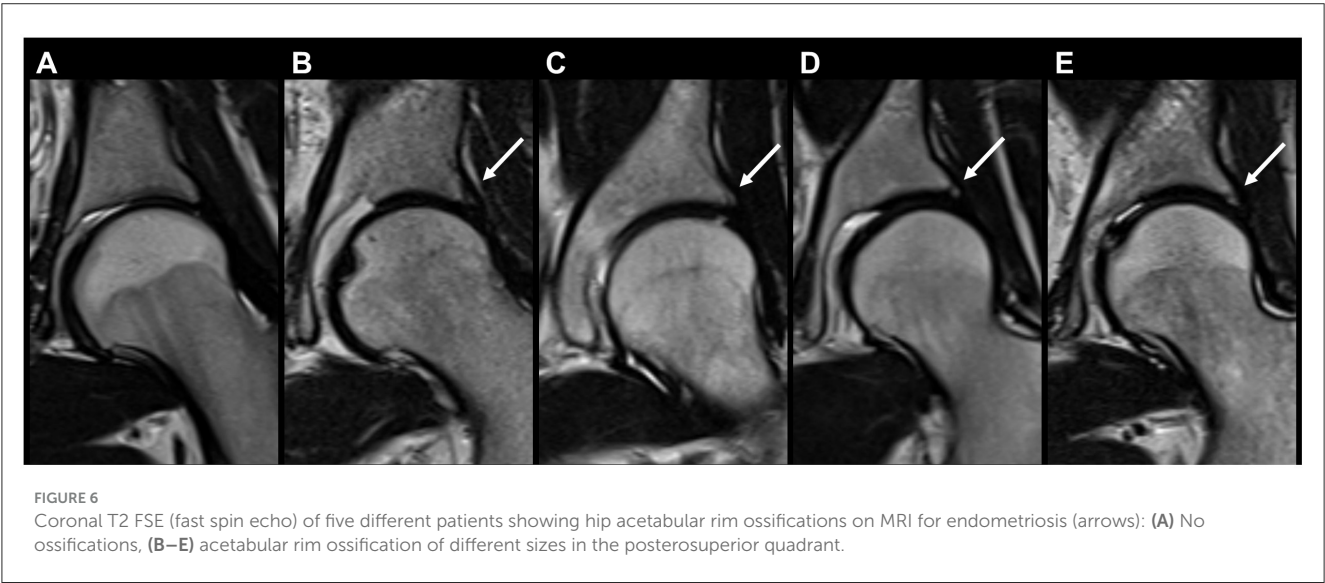
(Continued)



TABLE 5 (Continued)

| Findings                                             | Total ( <i>n</i> = 303) | MRI DIE+ ( <i>n</i> = 106) | MRI DIE− ( <i>n</i> = 197) | <i>p</i> -values   |
|------------------------------------------------------|-------------------------|----------------------------|----------------------------|--------------------|
| Lumbosacral transitional vertebra, type Castellvi IV | 4 (1.3; 0.4–3.3)        | 2 (1.9; 0.2–6.6)           | 2 (1.0; 0.1–3.6)           | 0.614 <sup>a</sup> |
| Benign lesion, proximal femur                        | 4 (1.3; 0.4–3.3)        | 3 (2.8; 0.6–8.0)           | 1 (0.5; 0.0–2.8)           | 0.125 <sup>a</sup> |
| Rectus abdominis diastasis                           | 4 (1.3; 0.4–3.3)        | 2 (1.9; 0.2–6.6)           | 2 (1.0; 0.1–3.6)           | 0.614 <sup>a</sup> |
| Tarlov/perineural cyst                               | 3 (1.0; 0.2–2.9)        | 1 (0.9; 0.0–5.1)           | 2 (1.0; 0.1–3.6)           | 1.000 <sup>a</sup> |
| Coxa valga deformity                                 | 3 (1.0; 0.2–2.9)        | 1 (0.9; 0.0–5.1)           | 2 (1.0; 0.1–3.6)           | 1.000 <sup>a</sup> |
| Parasymphyseal cyst                                  | 3 (1.0; 0.2–2.9)        | 1 (0.9; 0.0–5.1)           | 2 (1.0; 0.1–3.6)           | 1.000 <sup>a</sup> |

Bold values denote statistical significance at the  $p \leq 0.05$  level.  
IF, incidental finding; MRI, magnetic resonance imaging; DIE, deep infiltrating endometriosis; T2WI, T2 weighted image; SIJ, sacroiliac joint.  
<sup>a</sup>Fisher's exact test.  
<sup>b</sup>Chi-square test.



In the ESUR guideline for the MR imaging of endometriosis, four of the eight participating centers stated that their MRI protocol contains a T2-sequence from the kidney to the pubic bone, and a corresponding recommendation is suggested to enable a systematic visualization of kidneys and potential analysis of the right iliac fossa (16).

Even minor findings can pose difficulties for radiologists and referring physicians in everyday practice and cause uncertainty. Particularly in a young patient population, the question regularly arises as to when findings should be considered pathological, a normal variant and/or be communicated. The most common IF we found was ARO in 200/303 patients (66.0%). As this finding has received little attention to date, no reports are available on the prevalence in non-musculoskeletal pelvic MRI examinations. The importance of this very common finding lies primarily in not interpreting it as pathological, as Valente et al. (23) have pointed out: In their 2021 study, they found ARO in 96% of 75 asymptomatic patients (mean age, 47.7 years). Consequently, the diagnosis of osteoarthritis should not be made solely based on the presence of ARO.

Another very common observation in our study collective were degenerations of the lumbar spine, despite the low average age of the patients. Annular fissures of the intervertebral discs

were detected significantly more frequently in patients with than in patients without DIE diagnosis on MRI ( $p = 0.006$ ). This observation could be explained by the association between lumbar disc degeneration and comorbidities related to systemic inflammation reported by Lambrechts et al. (32), although we could not find significant differences in the prevalence of disc desiccation between patients with and without DIE on MRI ( $p = 0.344$ ). It is currently unclear whether degenerations of the spine as IFs should be reported by radiologists and communicated to patients. As Brinjikji et al. (33) stated, disc degeneration has a higher prevalence in adults with low back pain than in asymptomatic individuals. On the other hand, routine MRI reports have been found to produce a negative perception and poor functional outcomes in low back pain (34).

In our study cohort, a significantly higher prevalence of three types of IFs was found in patients with DIE diagnosis on MRI (ARO, fissures of the annulus fibrosus, gallstones). An association between endometriosis and various comorbidities has been suspected, including gynecologic diseases (fibroids, adenomyosis, ovarian cancer), gastrointestinal diseases (irritable/inflammatory bowel disease), immunological-related/autoimmune diseases (rheumatoid arthritis, psoriasis, osteoarthritis, asthma, allergy), cardiovascular and cerebrovascular diseases (12, 13). Causal

mechanisms are considered to be endometriosis-induced local and systemic inflammation, immune dysregulation, hormonal changes, and treatment sequelae. The results of our study provide a potential indication of an association of the three IFs mentioned above with endometriosis, although no statistically significant differences in the overall prevalence and mean number of IFs (with/without DIE on MRI) and no associations comparable to the age dependence of IFs could be demonstrated (Supplementary Tables S1–S3).

We found a significantly higher mean number of IFs per patient in contrast-enhanced than in non-contrast MRIs ( $p = 0.002$ ) without significant difference in the overall prevalence of IFs ( $p = 0.579$ ). However, the decision on GBCA administrations had been made depending on the findings of the non-contrast sequences and the presence of ancillary questions (14). Therefore, the higher rate of IFs must be seen as a reason for the application rather than a consequence of GBCA administrations. DIE had not been found significantly more frequently in contrast-enhanced MRIs, which is consistent with the current ESUR guidelines for endometriosis MRI that do not routinely recommend GBCA administration (16).

Various guidelines for the management of IFs in clinical imaging and research have been established recently (35–48). Due to the extensive use of imaging in modern medicine, there is an ongoing need for standardization of the management of IFs (49, 50). Further assistance for radiologists through artificial intelligence (AI) may be expected in the future (51, 52). Radiologists must nonetheless familiarize themselves with IFs to properly determine consequences and provide guidance (53, 54). Detecting an IF does not necessarily imply that it should be reported. For IFs with moderate or high clinical significance, a description in the radiologic report is warranted, if available, with reference to current guidelines on the management of the findings. Appropriate wording should be used so as not to cause unnecessary further investigations or patient distress. However, the majority of IFs has low clinical significance and a description in the radiology report is often unnecessary and not beneficial to the patient, e.g., in non-pathological findings such as ARO or in age-typical degeneration of the spine.

There are some limitations of our study. The study was conducted retrospectively at a single tertiary care center. Diagnoses were mainly based on the review of the imaging findings and radiological reports. Since not every manifestation of DIE is detectable on MRI, our results comparing the frequency of IFs between patients with and without evidence of DIE on MRI may have somewhat limited generalizability. Further studies to externally validate our results are warranted.

In conclusion, incidental findings are found very commonly on pelvic MRI for endometriosis, including overview sequences from the kidneys to the pubic bone. Many incidental findings have no, only minor, or uncertain consequences. Although less prevalent, radiologists should be aware of findings with high clinical significance.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by Institutional Review Board of the Faculty of Medicine, Justus-Liebig-University Giessen. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the retrospective design of the study.

## Author contributions

SH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft. FR: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. AB: Formal analysis, Methodology, Resources, Writing – review & editing. HK: Conceptualization, Data curation, Validation, Writing – review & editing. FZ: Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing. IM-H: Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing. GK: Conceptualization, Formal analysis, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2024.1468860/full#supplementary-material>

# References

- Tomassetti C, Johnson NP, Petrozza J, Abrao MS, Einarsson JJ, Horne AW, et al. An international terminology for endometriosis. 2021. *Hum Reprod Open*. (2021) 2021:hoab029. doi: 10.1093/hropen/hoab029
- Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, Kiesel L, et al. ESHRE guideline: endometriosis. *Hum Reprod Open*. (2022) 2022:hoac009. doi: 10.1093/hropen/hoac009
- Smolarz B, Szyłko K, Romanowicz H. Endometriosis: epidemiology, classification, pathogenesis, treatment and genetics (review of literature). *Int J Mol Sci*. (2021) 22:10554. doi: 10.3390/ijms221910554
- Avery JC, Knox S, Deslandes A, Leonardi M, Lo G, Wang H, et al. Non-invasive Diagnostic imaging for endometriosis part 2: a systematic review of recent developments in magnetic resonance imaging, nuclear medicine and computed tomography. *Fertil Steril*. (2023) 121(2):189–211. doi: 10.1016/j.fertnstert.2023.12.017
- Harth S, Roller FC, Zeppernick F, Meinhold-Heerlein I, Krombach GA. Deep infiltrating endometriosis: diagnostic accuracy of preoperative magnetic resonance imaging with respect to morphological criteria. *Diagnostics*. (2023) 13:1794. doi: 10.3390/diagnostics13101794
- Harth S, Kaya HE, Zeppernick F, Meinhold-Heerlein I, Keckstein J, Yildiz SM, et al. Application of the #Enzin classification for endometriosis on MRI: prospective evaluation of inter- and intraobserver agreement. *Front Med*. (2023) 10:1303593. doi: 10.3389/fmed.2023.1303593
- Marti-Bonmati L. Evidence levels in radiology: the insights into imaging approach. *Insights Imaging*. (2021) 12:45. doi: 10.1186/s13244-021-00995-7
- Weckbach S, Wielpütz MO, von Stackelberg O. Patient-centered, value-based management of incidental findings in radiology. *Radiologie*. (2023) 63:657–4. doi: 10.1007/s00117-023-01200-0
- Weckbach S, editor. *Incidental Radiological Findings*, 1st Edn. Cham: Springer International Publishing (2018). doi: 10.1007/978-3-319-42581-8
- Wolf SM, Lawrenz FP, Nelson CA, Kahn JP, Cho MK, Clayton EW, et al. Managing incidental findings in human subjects research: analysis and recommendations. *J Law Med Ethics*. (2008) 36:219–48, 211. doi: 10.1111/j.1748-720X.2008.00266.x
- Sconfienza LM, Mauri G, Muzzupappa C, Poloni A, Bandirali M, Esseridou A, et al. Relevant incidental findings at abdominal multi-detector contrast-enhanced computed tomography: a collateral screening? *World J Radiol*. (2015) 7:350–6. doi: 10.4329/wjrv.v7.i10.350
- Parazzini F, Esposito G, Tozzi L, Noli S, Bianchi S. Epidemiology of endometriosis and its comorbidities. *Eur J Obstet Gynecol Reprod Biol*. (2017) 209:3–7. doi: 10.1016/j.ejogrb.2016.04.021
- Horne AW, Missmer SA. Pathophysiology, diagnosis, and management of endometriosis. *BMJ*. (2022) 379:e070750. doi: 10.1136/bmj-2022-070750
- Harth S, Roller FC, Zeppernick F, Meinhold-Heerlein I, Krombach GA. Feasibility of periprocedural decision on the administration of intravenous contrast media in MRI for endometriosis. *Eur J Radiol*. (2023) 165:110949. doi: 10.1016/j.ejrad.2023.110949
- Tong A, VanBuren WM, Chamié L, Feldman M, Hindman N, Huang C, et al. Recommendations for MRI technique in the evaluation of pelvic endometriosis: consensus statement from the Society of Abdominal Radiology endometriosis disease-focused panel. *Abdom Radiol*. (2020) 45:1569–86. doi: 10.1007/s00261-020-02483-w
- Bazot M, Bharwani N, Huchon C, Kinkel K, Cunha TM, Guerra A, et al. European society of urogenital radiology (ESUR). Guidelines: MR imaging of pelvic endometriosis. *Eur Radiol*. (2017) 27:2765–75. doi: 10.1007/s00330-016-4673-z
- Cutaia G, Tosto G, Cannella R, Bruno A, Leto C, Salvaggio L, et al. Prevalence and clinical significance of incidental findings on multiparametric prostate MRI. *Radiol Med*. (2020) 125:204–13. doi: 10.1007/s11547-019-01106-9
- Sadowski EA, Thomassin-Naggara I, Rockall A, Matusen KE, Forstner R, Jha P, et al. O-RADS MRI risk stratification system: guide for assessing adnexal lesions from the ACR O-RADS committee. *Radiology*. (2022) 303:35–47. doi: 10.1148/radiol.204371
- Sahin H, Panico C, Ursprung S, Simeon V, Chiodini P, Frary A, et al. Non-contrast MRI can accurately characterize adnexal masses: a retrospective study. *Eur Radiol*. (2021) 31:6962–73. doi: 10.1007/s00330-021-07737-9
- Teede HJ, Tay CT, Laven JJE, Dokras A, Moran LJ, Piltonen TT, et al. Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Eur J Endocrinol*. (2023) 189:G43–64. doi: 10.1093/ajeendo/lvad096
- Kubik-Huch RA, Weston M, Nougaret S, Leonhardt H, Thomassin-Naggara I, Horta M, et al. European Society of Urogenital Radiology (ESUR). Guidelines: MR imaging of leiomyomas. *Eur Radiol*. (2018) 28:3125–37. doi: 10.1007/s00330-017-5157-5
- Bookwalter CA, VanBuren WM, Neisen MJ, Bjarnason H. Imaging appearance and nonsurgical management of pelvic venous congestion syndrome. *Radiographics*. (2019) 39:596–608. doi: 10.1148/rg.2019180159
- Valente C, Haefliger L, Favre J, Omoumi P. Ossification of the acetabular rim: a highly prevalent finding in asymptomatic non-osteoarthritic hips of all ages. *Eur Radiol*. (2021) 31:6802–9. doi: 10.1007/s00330-021-07750-y
- Modic MT, Steinberg PM, Ross JS, Masaryk TJ, Carter JR. Degenerative disk disease: assessment of changes in vertebral body marrow with MR imaging. *Radiology*. (1988) 166:193–9. doi: 10.1148/radiology.166.1.3336678
- Fardon DF, Williams AL, Dohring EJ, Murtagh FR, Gabriel Rothman SL, Sze GK, et al. Lumbar disc nomenclature: version 20: Recommendations of the combined task forces of the North American Spine Society the American Society of Spine Radiology and the American Society of Neuroradiology. *Spine J*. (2014) 24:2525–45. doi: 10.1016/j.spinee.2014.04.022
- Hutchins J, Hebelka H, Lagerstrand K, Brisby H. A systematic review of validated classification systems for cervical and lumbar spinal foraminal stenosis based on magnetic resonance imaging. *Eur Spine J*. (2022) 31:1358–69. doi: 10.1007/s00586-022-07147-5
- Diekhoff T, Lambert R, Hermann KG. MRI in axial spondyloarthritis: understanding an 'ASAS-positive MRI' and the ASAS classification criteria. *Skeletal Radiol*. (2022) 51:1721–30. doi: 10.1007/s00256-022-04018-4
- Fernbach SK, Maizels M, Conway JJ. Ultrasound grading of hydronephrosis: introduction to the system used by the Society for Fetal Urology. *Pediatr Radiol*. (1993) 23:478–80. doi: 10.1007/BF02012459
- Choi HJ, Kim SH, Seo S-S, Kang S, Lee S, Kim JY, et al. MRI for pretreatment lymph node staging in uterine cervical cancer. *AJR Am J Roentgenol*. (2006) 187:W538–43. doi: 10.2214/AJR.05.0263
- Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. (2009) 45:228–47. doi: 10.1016/j.ejca.2008.10.026
- Sherrer RL, Lai WS, Thomas JV, Nix JW, Rais-Bahrami S. Incidental findings on multiparametric MRI performed for evaluation of prostate cancer. *Abdom Radiol*. (2018) 43:696–701. doi: 10.1007/s00261-017-1237-x
- Lambrechts MJ, Pitchford C, Hogan D, Li J, Fogarty C, Rawat S, et al. Lumbar spine intervertebral disc desiccation is associated with medical comorbidities linked to systemic inflammation. *Arch Orthop Trauma Surg*. (2023) 143:1143–53. doi: 10.1007/s00402-021-04194-3
- Brinjikji W, Diehn FE, Jarvik JG, Carr CM, Kallmes DF, Murad MH, et al. MRI findings of disc degeneration are more prevalent in adults with low back pain than in asymptomatic controls: a systematic review and meta-analysis. *AJNR Am J Neuroradiol*. (2015) 36:2394–9. doi: 10.3174/ajnr.A4498
- Rajasekaran S, Dilip Chand Raja S, Pushpa BT, Ananda KB, Ajay Prasad S, Rishi MK, et al. The catastrophization effects of an MRI report on the patient and surgeon and the benefits of 'clinical reporting': results from an RCT and blinded trials. *Eur Spine J*. (2021) 30:2069–81. doi: 10.1007/s00586-021-06809-0
- Patel MD, Ascher SM, Horrow MM, Pickhardt PJ, Poder L, Goldman M, et al. Management of incidental adnexal findings on CT and MRI: a white paper of the ACR Incidental Findings Committee. *J Am Coll Radiol*. (2020) 17:248–54. doi: 10.1016/j.jacr.2019.10.008
- Herts BR, Silverman SG, Hindman NM, Uzzo RG, Hartman RP, Israel GM, et al. Management of the incidental renal mass on CT: a white paper of the ACR Incidental Findings Committee. *J Am Coll Radiol*. (2018) 15:264–73. doi: 10.1016/j.jacr.2017.04.028
- Hoang JK, Hoffman AR, González RG, Wintermark M, Glenn BJ, Pandharipande PV, et al. Management of incidental pituitary findings on CT, MRI, and 18F-fluorodeoxyglucose PET: a white paper of the ACR Incidental Findings Committee. *J Am Coll Radiol*. (2018) 15:966–72. doi: 10.1016/j.jacr.2018.03.037
- Munden RF, Carter BW, Chiles C, MacMahon H, Black WC, Ko JP, et al. Managing incidental findings on thoracic CT: mediastinal and cardiovascular findings. A white paper of the ACR Incidental Findings Committee. *J Am Coll Radiol*. (2018) 15:1087–96. doi: 10.1016/j.jacr.2018.04.029
- Gore RM, Pickhardt PJ, Mortelet KJ, Fishman EK, Horowitz JM, Fimmel CJ, et al. Management of incidental liver lesions on CT: a white paper of the ACR Incidental Findings Committee. *J Am Coll Radiol*. (2017) 14:1429–37. doi: 10.1016/j.jacr.2017.07.018
- Mayo-Smith WW, Song JH, Boland GL, Francis IR, Israel GM, Mazzaglia PJ, et al. Management of incidental adrenal masses: a white paper of the ACR Incidental Findings Committee. *J Am Coll Radiol*. (2017) 14:1038–44. doi: 10.1016/j.jacr.2017.05.001
- Megibow AJ, Baker ME, Morgan DE, Kamel IR, Sahani DV, Newman E, et al. Management of incidental pancreatic cysts: a white paper of the ACR Incidental Findings Committee. *J Am Coll Radiol*. (2017) 14:911–23. doi: 10.1016/j.jacr.2017.03.010

42. Hoang JK, Langer JE, Middleton WD, Wu CC, Hammers LW, Cronan JJ, et al. Managing incidental thyroid nodules detected on imaging: white paper of the ACR incidental thyroid findings committee. *J Am Coll Radiol.* (2015) 12:143–50. doi: 10.1016/j.jacr.2014.09.038
43. Heller MT, Harisinghani M, Neitlich JD, Yeghiayan P, Berland LL. Managing incidental findings on abdominal and pelvic CT and MRI, part 3: white paper of the ACR Incidental Findings Committee II on splenic and nodal findings. *J Am Coll Radiol.* (2013) 10:833–9. doi: 10.1016/j.jacr.2013.05.020
44. Khosa F, Krinsky G, Macari M, Yucel EK, Berland LL. Managing incidental findings on abdominal and pelvic CT and MRI, Part 2: white paper of the ACR Incidental Findings Committee II on vascular findings. *J Am Coll Radiol.* (2013) 10:789–94. doi: 10.1016/j.jacr.2013.05.021
45. Patel MD, Ascher SM, Paspulati RM, Shanbhogue AK, Siegelman ES, Stein MW, et al. Managing incidental findings on abdominal and pelvic CT and MRI, part 1: white paper of the ACR Incidental Findings Committee II on adnexal findings. *J Am Coll Radiol.* (2013) 10:675–81. doi: 10.1016/j.jacr.2013.05.023
46. Sebastian S, Araujo C, Neitlich JD, Berland LL. Managing incidental findings on abdominal and pelvic CT and MRI, Part 4: white paper of the ACR Incidental Findings Committee II on gallbladder and biliary findings. *J Am Coll Radiol.* (2013) 10:953–6. doi: 10.1016/j.jacr.2013.05.022
47. Berland LL, Silverman SG, Gore RM, Mayo-Smith WW, Megibow AJ, Yee J, et al. Managing incidental findings on abdominal CT: white paper of the ACR Incidental Findings Committee. *J Am Coll Radiol.* (2010) 7:754–73. doi: 10.1016/j.jacr.2010.06.013
48. Munden RF, Black WC, Hartman TE, MacMahon H, Ko JP, Dyer DS, et al. Managing incidental findings on thoracic CT: lung findings. A white paper of the ACR Incidental Findings Committee. *J Am Coll Radiol.* (2021) 18:1267–79. doi: 10.1016/j.jacr.2021.04.014
49. Hegedüs, P., and Stackelberg, O. von, Neumann C, Selder S, Werner N, Erdmann P, et al. How to report incidental findings from population whole-body MRI: view of participants of the German National Cohort. *Eur Radiol.* (2019). 29:5873–8. doi: 10.1007/s00330-019-06077-z
50. Booth TC, Waldman AD, Wardlaw JM, Taylor SA, Jackson A. Management of incidental findings during imaging research in “healthy” volunteers: current UK practice. *Br J Radiol.* (2012) 85:11–21. doi: 10.1259/bjr/73283917
51. Chamberlin JH, Smith C, Schoepf UJ, Nance S, Elojeimy S, O’Doherty J, et al. A deep convolutional neural network ensemble for composite identification of pulmonary nodules and incidental findings on routine PET/CT. *Clin Radiol.* (2023) 78:e368–76. doi: 10.1016/j.crad.2023.01.014
52. Venkadesh KV, Setio AAA, Schreuder A, Scholten ET, Chung K, Wille W, et al. (2021). Deep learning for malignancy risk estimation of pulmonary nodules detected at low-dose screening CT. *Radiology.* (2021). 300:438–47. doi: 10.1148/radiol.2021204433
53. Maskell G. Incidental findings in medical imaging. *Br J Radiol.* (2023) 96:20211352. doi: 10.1259/bjr.20211352
54. Brady A. Incidentalomas, SPEW, and VOMIT-radiological dyspepsia? *Eur Radiol.* (2020) 30:4968–73. doi: 10.1007/s00330-020-06844-3
55. Munro MG, Critchley HOD, Broder MS, Fraser IS. FIGO classification system (PALM-COEIN). For causes of abnormal uterine bleeding in nonpregnant women of reproductive age. *Int J Gynaecol Obstet.* (2011) 113:3–13. doi: 10.1016/j.ijgo.2010.11.011
56. Keckstein J, Saridogan E, Ulrich UA, Sillem M, Oppelt P, Schweppe KW, et al. The #Enzian classification: A comprehensive non-invasive and surgical description system for endometriosis. *Acta Obstet Gynecol Scand.* (2021) 100:1165–75. doi: 10.1111/aogs.14099



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# Comparing ovarian reserve parameters after laparoscopic endometrioma resection in the follicular vs. luteal phase: a prospective cohort study

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**Objective:** To evaluate whether performing laparoscopic endometrioma surgery in the follicular or luteal phase affects changes in ovarian reserve.

**Methods:** This prospective cohort study was conducted in a university-affiliated hospital. A total of 55 women who underwent unilateral laparoscopic endometrioma removal were included in the study. Of these, 28 were in the follicular phase of the menstrual cycle (follicular group), and 27 were in the luteal phase (luteal group). The primary outcomes were the rates of decreased anti-Müllerian hormone (AMH) levels and antral follicle counts (AFC) 6 months after the surgery, which were compared between the groups.

**Results:** The groups were similar in patient characteristics and endometrioma sizes. AMH levels and AFCs were significantly lower in the post-operative 6th month compared with their pre-operative values ( $p < 0.05$  for both groups). The rate of decrease in AMH levels 6 months after the surgery was not significantly different between the follicular and luteal groups (24.5 and 19.5%, respectively,  $p > 0.05$ ). Similarly, the rate of decrease in AFCs 6 months after the surgery did not differ between the groups (13.4 and 14.3%, respectively,  $p > 0.05$ ).

**Conclusion:** Performing laparoscopic endometrioma surgery, whether in the follicular or luteal phase, does not seem to affect the changes in the ovarian reserve.

**Clinical trial registration:** <https://clinicaltrials.gov/study/NCT03484546>, identifier NCT034845.

## KEYWORDS

endometrioma removal, ovarian damage, anti-Müllerian hormone, endometriosis, ovarian reserve

## Introduction

Many studies have shown that endometrioma surgery decreases ovarian reserve (1–3). However, the definitive treatment of endometrioma-related dysmenorrhea, dyspareunia, and suspicious adnexal masses is still the surgical removal of the endometriomas (4). Although there is controversy regarding the indications for endometrioma removal, it remains a frequently performed surgical procedure to relieve symptoms.



Since endometrioma negatively affects ovarian reserve and endometriosis is a known cause of infertility, preserving ovarian reserve during endometrioma surgery may be more critical than other ovarian cyst surgeries. Furthermore, endometrioma patients have a higher risk of recurrence and the necessity for repetitive surgeries compared to other ovarian cysts.

Studies indicate that surgical technique and the surgeon's experience are important factors in reducing the negative effect of endometrioma surgery on ovarian reserve (5). Alternative surgical techniques have been described in these studies. The main goal of these techniques is to minimize damage to healthy follicles during hemostatic procedures. Some of these techniques include suturing instead of using electrocoagulation, using bipolar electrosurgical instruments instead of monopolar, vasopressin injection, and the use of hemostatic sealants (6–8). Although these techniques are partially effective, they still do not seem to be sufficient to reduce the changes in ovarian reserve.

Few studies have investigated other factors affecting the changes in ovarian reserve. One of these factors is the menstrual cycle phase during endometrioma surgery. Studies have observed differences in the histological and vascular findings of ovaries based on the menstrual cycle phases (9, 10). A study recently published by Wu et al. (11) found that performing endometrioma surgery during the late luteal phase reduces changes in ovarian reserve.

In our study, we investigated whether the extent of damage to the ovarian reserve is affected by the menstrual cycle phases in laparoscopic endometrioma removal.

## Material methods

This prospective study was conducted in the Department of Gynecology of Acibadem Mehmet Ali Aydinlar University Maslak Hospital from 28 March 2018 through 1 October 2021 and approved by the Institutional Ethics Committee (ATADEK, ID no: 2018–4/18). Written informed consent was obtained from all participants. The study was registered at [Clinicaltrials.gov](https://clinicaltrials.gov/study/NCT03484546) (ID No: NCT03484546). <https://clinicaltrials.gov/study/NCT03484546>

All procedures followed the ethical standards of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

## Study design

Patients diagnosed with unilateral endometrioma by ultrasound, aged between 18 and 40 years, with regular menstrual periods, and with indications for endometrioma removal were enrolled in the study. Indications for the surgery were dysmenorrhea, dyspareunia, increase in the size of cyst diameter and the suspicion adnexal mass other than endometrioma. The exclusion criteria were as follows: the presence of non-endometrioma cyst (according to pathology results), bilateral or multiple cyst removal, an additional surgical procedure in the same session, a history of previous ovarian surgery, pre- or post-operative hormonal medication use (oral contraceptives, gonadotropin analogs/antagonists, progestins), chronic anticoagulant use (possible excessive hemostatic intervention), deep infiltrating endometriosis and the presence of dense adhesions between endometrioma and intraabdominal structures (Severe—Stage IV Revised-ASRM

TABLE 1 Patient exclusion criteria.

| Exclusion criteria                                                                                                                                              |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| • Non-endometrioma cyst (according to final pathology report)                                                                                                   |
| • Bilateral and/or multiple cyst removal                                                                                                                        |
| • Additional surgical procedure in the same session                                                                                                             |
| • Previous ovarian surgery                                                                                                                                      |
| • Oral contraceptive, gonadotrophic releasing hormone agonists or progestin use in pre and post-operative period                                                |
| • Chronic anti-coagulant use before operation                                                                                                                   |
| • Deep infiltrating endometriosis and severe adhesions between endometrioma and intraabdominal structures (Severe Stage IV r-ASRM endometriosis classification) |
| • Pregnancy within 6 months after the surgery                                                                                                                   |
| • Irregular menstrual periods whose menstrual phases cannot be determined                                                                                       |
| • Post-menopausal status, premature ovarian failure                                                                                                             |

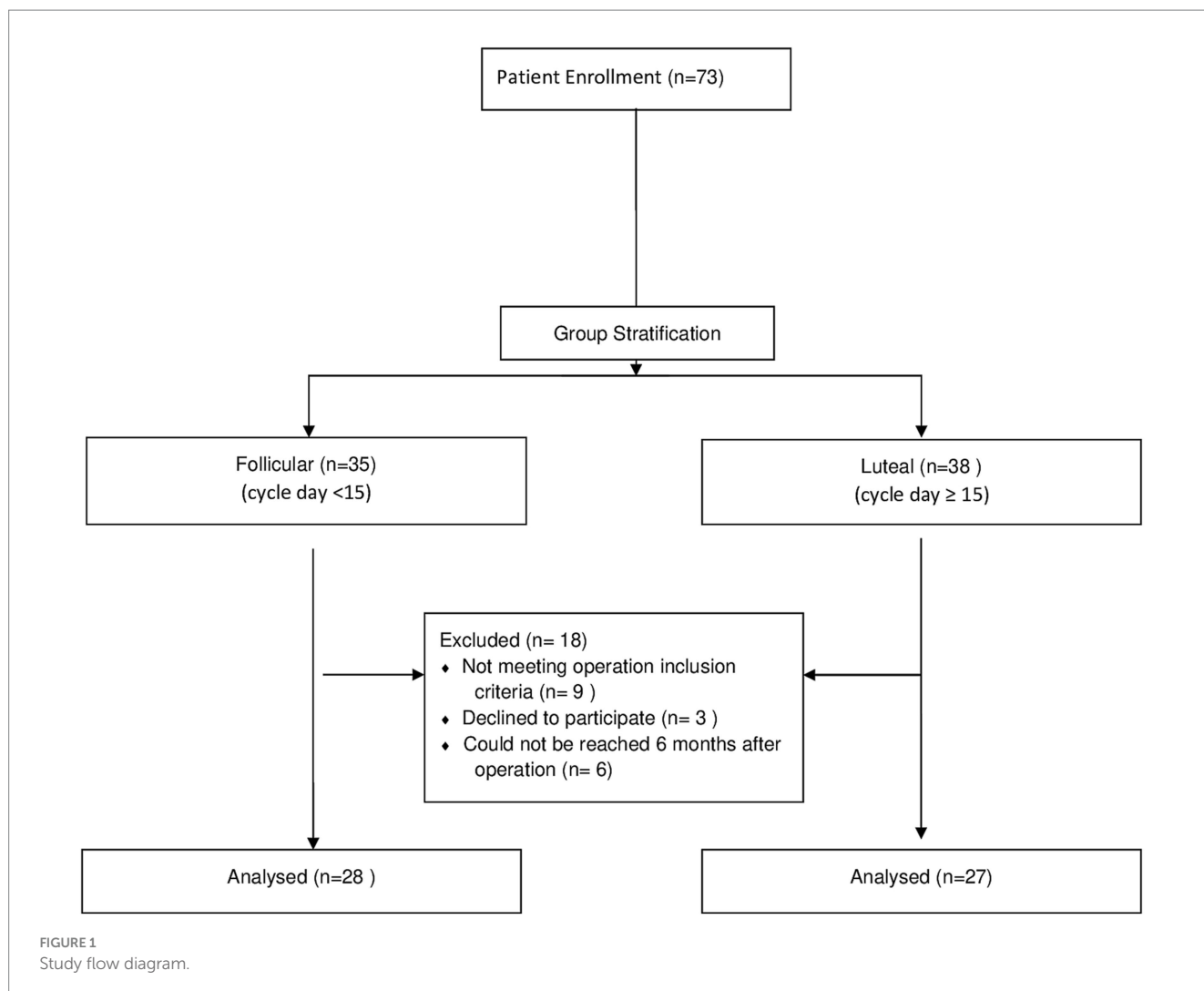
endometriosis classification (12)), pregnancy within 6 months after surgery, having irregular menstrual periods, premature ovarian failure and post-menopausal status (Table 1).

The menstrual day of the patients on the operation day was calculated by adjusting to a 28-day cycle using a formula described by Ramakrishnan et al. (13) and Song et al. (14). The adjusted day of the menstrual cycle =  $(14 \times \text{day of the cycle at the time of surgery}) / (\text{cycle length of the patient} - 14)$ . Patients whose adjusted cycle day <15 were grouped as follicular, while those with an adjusted cycle day  $\geq 15$  were grouped as luteal (Figure 1).

When our study was designed, there were no comparable studies in the existing literature. In determining the sample size for power of our study, we examined other studies exploring the impact of different surgical techniques in endometrioma cystectomy on ovarian reserve. In the study conducted by Song et al. (7), the preoperative and postoperative percentage changes in AMH levels were evaluated based on two surgical techniques (Bipolar Coagulation group: 42.2%; Suture group: 24.6%). The calculation yielded an effect size of  $d = 0.799$ , and it was determined that a minimum of 26 individuals per group is necessary to achieve 80% power at the 0.05 significance level. The G\*Power program (Heinrich Heine Universität Düsseldorf, 2020) was used to calculate the sample size.

Diameter measurements of endometriomas and antral follicle counts of the same ovary were performed using transvaginal ultrasound and recorded in the outpatient service. Ultrasonography were performed vaginally by the same physician (OT) with GE Voluson S8 (GE Health Care, Chicago, United States) via wideband microconvex endocavitary 180° 2.9–9.7 MHz probe. The operation day was scheduled based on operating room availability and patient preferences. The assignment of participants to study groups based on surgery during the follicular vs. luteal phase was nonrandomized, thus our study is not a randomized controlled study. On the surgery day, blood samples were collected for pre-operative AMH levels in the inpatient service. The serum was separated by centrifugation and stored at  $-70^{\circ}\text{C}$ .

All operations were performed using the same technique by two experienced surgeons (OT, MG) in minimally invasive gynecologic surgery. Operations were performed via four abdominal ports (10 mm umbilical, 5 mm right, left, and suprapubic ports). Ovarian surfaces were incised, and endometrioma cysts were removed using the stripping technique. Hemostasis was achieved with bipolar forceps coagulation adjusted to 30 W power. No suturing or hemostatic agents



were used. Cysts were removed from the abdominal cavity in a contained bag system. Operating time was defined as the time from the first skin incision to the end of skin closure. Estimated blood loss (EBL) was calculated as the difference in fluid volume between irrigation and suction.

Patients whose diagnosis of endometrioma was confirmed in the pathology reports were asked to be followed up 6 months after the surgery. Antral follicle counts were performed on the operated ovary with ultrasonography. Subsequently, blood samples were collected for post-operative AMH levels. The serum was separated and stored at  $-70^{\circ}\text{C}$ .

After patient enrollment was completed, serum samples were thawed. AMH levels were measured with an enzyme immunoassay kit (Ansh Labs, Webster, TX, United States) according to the manufacturer's instructions. Patient characteristics (age, BMI, gravida, endometrioma diameter, antral follicle count) and surgery data (operation time, EBL) were obtained from the study data records.

## Outcome measures

The primary outcome was the decrease rate in AMH levels and AFCs 6 months after the surgery. The decrease rate of serum AMH

levels was defined as:  $\text{Rate of decline (\%)} = 100 \times (\text{Preoperative AMH level} - \text{Postoperative AMH level}) / \text{Preoperative AMH level}$ . The decrease rate of AFC was calculated as:  $\text{Rate of decline (\%)} = 100 \times (\text{Preoperative AFC} - \text{Postoperative AFC}) / \text{Preoperative AFC}$ . The secondary outcome was the estimated blood loss in the surgery.

## Statistical analysis

All analyses were conducted in NCSS (Number Cruncher Statistical System) 2020 Statistical Software (NCSS LLC, Kaysville, Utah, United States). Quantitative variables were shown with mean, standard deviation, median, and quartile values, and qualitative variables were shown with descriptive statistical methods such as frequency and percentage. The assumption of normality was made with Shapiro–Wilk and Kolmogorov–Smirnov tests. Normal distributed data were evaluated with the Student *T*-Test, while non-normally distributed data was assessed with the Mann–Whitney *U* and Wilcoxon Signed Rank test. Decrease rate of AMH and AFC were compared with Mann–Whitney *U* test. Spearman's rank correlation coefficient was used to assess the correlation between menstrual cycle day and the decreased rate of AMH levels.

$p$  value of  $<0.05$  was considered as the threshold for statistical significance.

## Results

Of the 73 enrolled patients, 18 were excluded because they did not meet the inclusion criteria [kissing ovaries (5), non-endometrial cysts (3), bilateral cystectomy (1), declined to participate (3), could not be reached after the operation (6)]. Fifty-five patients met the inclusion criteria; 28 were in the follicular group, and 27 were in the luteal group (Figure 1).

Age, body mass index (BMI), gravida, endometrioma diameter, operative time, and estimated blood loss did not differ statistically between the groups (Table 2). The median pre-operative AMH levels were comparable between the groups (2.4 ng/mL (1.3–4.6) and 2.8 ng/mL (1.9–4.0) respectively;  $p=0.67$ ). Post-operative AMH levels at 6 months were significantly lower than pre-operative AMH levels for both the follicular and luteal groups ( $p=0.001$  for both groups). Additionally, post-operative AFCs at 6 months were significantly lower than pre-operative AFC for both groups ( $p=0.03$  and  $p=0.002$ , respectively).

The rates of decrease in AMH levels for the follicular and luteal groups (24.5 and 19.5%, respectively) were comparable ( $p=0.52$ ).

The changes in AFC 6 months after the surgery were similar between the groups, as well [13.4% (−9.4–25.0) and 14.3 (0–28.6), respectively,  $p=0.54$ ] (Table 3).

In addition, AMH difference rates did not correlate with menstrual cycle day for both the follicular and luteal groups ( $p=0.68$ ,  $p=0.43$ ,  $r=-0.08$ ,  $r=-0.15$ , respectively).

## Discussion

In our study, we prospectively evaluated the effect of being in the follicular or luteal phase on the day of surgery on ovarian reserve in patients who underwent laparoscopic endometrioma surgery. This study concluded that the menstrual phase itself did not significantly affect the extent of changes in ovarian reserve, measured through AMH levels and AFCs. The estimated blood loss was also not affected by the menstrual phase during surgery. In addition, there was no correlation between the cycle day and the degree of changes in ovarian reserve.

TABLE 2 Patient characteristics.

|                                       | Follicular<br>( $n=28$ ) | Luteal<br>( $n=27$ ) | $p$               |
|---------------------------------------|--------------------------|----------------------|-------------------|
| Age                                   | 29.96 ± 7.01             | 31.07 ± 6.57         | <sup>a</sup> 0.54 |
| BMI <sup>c</sup> (kg/m <sup>2</sup> ) | 23.3 ± 3.7               | 22.01 ± 3.45         | <sup>a</sup> 0.17 |
| Gravida                               | 0 (0–1)                  | 0 (0–0)              | <sup>b</sup> 0.93 |
| Endometrioma Diameter (cm)            | 6 (4.6–7.8)              | 6 (5–7)              | <sup>b</sup> 0.47 |
| Operation time (min)                  | 62.04 ± 12.93            | 57.96 ± 15.77        | <sup>a</sup> 0.29 |
| EBL <sup>d</sup> (ml)                 | 47.5 (40–53.8)           | 50 (45–55)           | <sup>b</sup> 0.32 |

Data are given as mean ± standard deviation, median (Q1–Q3), Q1–Q3: %25–%75 percentiles.

<sup>a</sup>Student- $t$  Test; <sup>b</sup>Mann Whitney- $U$  Test; <sup>c</sup>Body Mass Index; <sup>d</sup>Estimated blood loss.

Damage to healthy follicles in endometrioma surgery occurs in two stages: the excision of the healthy cortical tissue while removing the cyst and the injury to healthy follicles during hemostasis. Alternative surgical techniques have been recommended to minimize the damage to healthy follicles. Suturing instead of electro-coagulation and using bipolar energy instead of monopolar for hemostasis are some of recommended techniques (6, 15). In addition, using hemostatic sealants can also be beneficial (16).

A limited number of studies have investigated variables other than surgical technique to reduce ovarian damage in women undergoing laparoscopic ovarian surgery. One possible variable that may alter the extent of ovarian damage is the menstrual cycle phase on the day of surgery.

Some studies claimed that operating on different days of the menstrual cycle can change the amount of blood loss during surgery. Paraskevaidis et al. found increased blood loss when the loop electrosurgical excision procedure (LEEP) was performed in the luteal phase of the menstrual cycle (17). Similarly, Sariguney et al. and Findikcioglu et al. observed a significant increase in blood loss when mastopexy and rhinoplasty were performed in the luteal phase (18, 19). In contrast, other studies found no significant relationship between the menstrual cycle phase on the day of surgery and blood loss (20–23).

In our opinion, it is less likely that non-gynecologic organs are affected by the phase of the menstrual cycle. However, gynecological organs, whose functions and structures differ during the menstrual cycle, may be affected by the menstrual cycle changes. It has been well documented that the blood flow of the uterus and ovaries varies with cyclic hormonal changes (24). Sladkevicius et al. showed that the pulsatility index and time-averaged maximum velocity were lower during the menstrual period in the dominant ovary (25). In a study using Doppler ultrasonography throughout the cycle conducted by Tan et al., FSH levels and the blood supply of the ovary with the dominant follicle increased, while there was no change in Doppler findings in the non-dominant ovary (24). These studies suggest that ovarian blood flow varies within the different cycle phases.

In the study by Song et al., medical records of 155 patients were reviewed. They retrospectively concluded that the menstrual cycle phase during surgery did not affect ovarian damage and was not an essential factor in determining the optimal time for ovarian cystectomy (14). This study had some limitations. The patients had different gynecologic conditions (dermoid, endometrioma, other). The cases that underwent bilateral cystectomy were also enrolled in this study, which might have caused more injury to the ovarian reserve than those undergoing unilateral cystectomy. Another limitation was that post-operative AMH levels were measured 3 months after the operations. In studies that measured AMH levels after ovarian surgery, it was found that AMH levels recovered in the 6th month after surgery. Thus, in our study, post-operative AMH levels were measured 6 months after the operations (3).

Wu et al. conducted a randomized controlled study on the subject. They found that performing laparoscopic endometrioma removal in the late luteal phase significantly reduces ovarian damage. In their study, patients were given oral contraceptives (OC) to determine the late luteal and early follicular phase groups. Although the prospective randomized design strengthens this study, administering OC could

TABLE 3 Comparison of pre-post operative AMH, AFC, and decrease rates.

|                            | Pre-operative AMH (ng/ml) | Post-operative AMH (ng/ml) | P(for pre-post AMH) | Pre-operative AFC       | Post-operative AFC      | P(for pre-post AFC) | Decrease rate of AMH (%) | Decrease rate of AFC (%) |
|----------------------------|---------------------------|----------------------------|---------------------|-------------------------|-------------------------|---------------------|--------------------------|--------------------------|
| <b>Follicular (n = 28)</b> | 2.4<br>(1.3–4.6)          | 2.01<br>(0.9–3.6)          | <b>*0.001</b>       | 7 (6–8)                 | 6.5 (5–7)               | <b>*0.03</b>        | 24.5<br>(12.8–48.3)      | 13.4<br>(–9.4–25.0)      |
| <b>Luteal (n = 27)</b>     | 2.8<br>(1.9–4.0)          | 2.07<br>(1.2–3.9)          | <b>*0.001</b>       | 7 (6–8)                 | 6 (5–7)                 | <b>*0.002</b>       | 19.5<br>(7.9–35.0)       | 14.3<br>(0–28.6)         |
| <b>p</b>                   | <b><sup>b</sup>0.67</b>   | <b><sup>b</sup>0.82</b>    |                     | <b><sup>b</sup>0.67</b> | <b><sup>b</sup>0.83</b> |                     | <b><sup>b</sup>0.52</b>  | <b><sup>b</sup>0.54</b>  |

Data are given as median (Q1–Q3), Q1–Q3: %25–%75 percentiles.

<sup>a</sup>Wilcoxon Signed Rank test; <sup>b</sup>Mann Whitney-*U* Test.

inhibit ovulation, potentially preventing regular physiological changes in the ovaries related to blood flow and histology. In our study, patients were grouped based on their natural cycles.

Our study has strengths and limitations. On the positive side, our study was prospective and high-powered. All surgeries were performed by the same surgeons using a standard technique, and patients' cycle phases were determined by optimizing their natural menstrual cycles. However, our study also has limitations. We could not confirm that the operated ovary led to the dominant follicle when the surgery was performed, as no ultrasound follow-up or ovulation tests were done during that menstrual cycle. Additionally, we did not consider in which phase of the cycle the patients' preoperative and postoperative AFCs were performed. The main reason for this was that AMH levels are not affected by cycle variations. While suturing is recommended over electro-coagulation, it was not performed in our study's surgical technique. Nevertheless, we believe our study results were not affected since the same technique was consistently used for all patients. In addition, changes in AMH levels and AFC are used for assessment of ovarian reserve changes, they are not always concordant with clinical ovarian reserve. Furthermore, although our study had high power, the sample size was relatively small.

In conclusion, the menstrual phase on the day of surgery does not significantly affect ovarian reserve damage during laparoscopic endometrioma removal. It suggests that surgeons may not need to consider menstrual cycle phases when scheduling these surgeries, allowing for more flexibility and convenience for both patients and physicians.

## Data availability statement

The study's raw data is available at: <https://osf.io/mry73/>.

## Ethics statement

The studies involving humans were approved by the Acibadem University and Acibadem Healthcare Institutions Medical Research Ethics Committee [Acibadem Üniversitesi ve Acibadem Sağlık Kuruluşları Tıbbi Araştırma Etik Kurulu (ATADEK)]. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

OT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing. MA: Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. MC: Conceptualization, Data curation, Formal analysis, Writing – review & editing. GU: Data curation, Investigation, Software, Writing – review & editing. MG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Supervision, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## References

- Donnez J, Nisolle M, Gillet N, Smets M, Bassil S, Casanas-Roux F. Large ovarian endometriomas. *Hum Reprod.* (1996) 11:641–5. doi: 10.1093/humrep/11.3.641
- Hemmings R, Bissonnette F, Bouzayen R. Results of laparoscopic treatments of ovarian endometriomas: laparoscopic ovarian fenestration and coagulation. *Fertil Steril.* (1998) 70:527–9. doi: 10.1016/s0015-0282(98)00219-2
- Chang HJ, Han SH, Lee JR, Jee BC, Lee BI, Suh CS, et al. Impact of laparoscopic cystectomy on ovarian reserve: serial changes of serum anti-Müllerian hormone levels. *Fertil Steril.* (2010) 94:343–9. doi: 10.1016/j.fertnstert.2009.02.022
- Hart RJ, Hickey M, Maouris P, Buckett W. Excisional versus ablative surgery for ovarian endometriomata. *Cochrane Database Syst Rev.* (2008) 2:CD004992. doi: 10.1002/14651858.CD004992.pub3
- Falcone T, Flyckt R. Clinical Management of Endometriosis. *Obstet Gynecol.* (2018) 131:557–71. doi: 10.1097/AOG.0000000000002469
- Ata B, Turkogeldi E, Seyhan A, Urman B. Effect of hemostatic method on ovarian reserve following laparoscopic endometrioma excision; comparison of suture, hemostatic sealant, and bipolar desiccation. A systematic review and meta-analysis. *J Minim Invasive Gynecol.* (2015) 22:363–72. doi: 10.1016/j.jmig.2014.12.168
- Song T, Kim WY, Lee KW, Kim KH. Effect on ovarian reserve of hemostasis by bipolar coagulation versus suture during laparoendoscopic single-site cystectomy for ovarian endometriomas. *J Minim Invasive Gynecol.* (2015) 22:415–20. doi: 10.1016/j.jmig.2014.11.002
- Song T, Lee SH, Kim WY. Additional benefit of hemostatic sealant in preservation of ovarian reserve during laparoscopic ovarian cystectomy: a multi-center, randomized controlled trial. *Hum Reprod.* (2014) 29:1659–65. doi: 10.1093/humrep/deu125
- Sladkevicius P, Valentin L, Marsal K. Blood flow velocity in the uterine and ovarian arteries during menstruation. *Ultrasound Obstet Gynecol.* (1994) 4:421–7. doi: 10.1046/j.1469-0705.1994.04050421.x
- Jokubkiene L, Sladkevicius P, Rovas L, Valentin L. Assessment of changes in volume and vascularity of the ovaries during the normal menstrual cycle using three-dimensional power Doppler ultrasound. *Hum Reprod.* (2006) 21:2661–8. doi: 10.1093/humrep/del211
- Wu Q, Yang Q, Lin Y, Wu L, Lin T. The optimal time for laparoscopic excision of ovarian endometrioma: a prospective randomized controlled trial. *Reprod Biol Endocrinol.* (2023) 21:59. doi: 10.1186/s12958-023-01109-2
- Canis M, Donnez JG, Guzik DS, Halme JK, Rock JA, Schenken RS, et al. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril.* (1997) 67:817–21. doi: 10.1016/s0015-0282(97)81391-x
- Ramakrishnan R, Khan SA, Badve S. Morphological changes in breast tissue with menstrual cycle. *Mod Pathol.* (2002) 15:1348–56. Epub 2002/12/14. doi: 10.1097/01.MP.0000039566.20817.46
- Song T, Kim MK, Kim ML, Jung YW, Yun BS, Seong SJ. Effect of menstrual phase on the surgical treatment of ovarian cysts. *J Obstet Gynaecol.* (2017) 37:919–23. doi: 10.1080/01443615.2017.1312313
- Asgari Z, Rouholamin S, Hosseini R, Sepidarkish M, Hafizi L, Javaheri A. Comparing ovarian reserve after laparoscopic excision of endometriotic cysts and hemostasis achieved either by bipolar coagulation or suturing: a randomized clinical trial. *Arch Gynecol Obstet.* (2016) 293:1015–22. doi: 10.1007/s00404-015-3918-4
- Araujo R, Maia SB, Baracat CME, Fernandes C, Ribeiro H, Ribeiro P. Ovarian function following use of various hemostatic techniques during treatment for unilateral endometrioma: a randomized controlled trial. *Int J Gynaecol Obstet.* (2022) 157:549–56. doi: 10.1002/ijgo.13912
- Paraskevaidis E, Davidson EJ, Koliopoulos G, Alamanos Y, Lolis E, Martin-Hirsch P. Bleeding after loop electrosurgical excision procedure performed in either the follicular or luteal phase of the menstrual cycle: a randomized trial. *Obstet Gynecol.* (2002) 99:997–1000. doi: 10.1016/s0029-7844(02)02003-3
- Sariguney Y, Demirtas Y, Findikcioglu F, Ayhan S, Latifoglu O, Cenetoglu S, et al. Proper timing of breast reduction during the menstrual cycle. *Ann Plast Surg.* (2004) 53 PMID: 15602247:528–31. doi: 10.1097/01.sap.0000134753.15282.cd
- Kemal Findikcioglu FF, Demirtas Y, Yavuzer R, Ayhan S, Atabay K. Effect of the menstrual cycle on intraoperative bleeding in rhinoplasty patients. *Eur J Plast Surg.* (2009) 32:77–81. doi: 10.1007/s00238-008-0313-4
- Lin Z, Moonasar N, Wu RH, Seemongal-Dass RR. The impact of the menstrual cycle on perioperative bleeding in vitreoretinal surgery. *J Ophthalmol.* (2017) 2017:9549284–4. doi: 10.1155/2017/9549284
- Findikcioglu K, Findikcioglu F, Sezgin B, Demirtas Y, Yavuzer R. The impact of the menstrual cycle on intra-operative and postoperative bleeding in abdominoplasty patients. *J Plast Reconstr Aesthet Surg.* (2012) 65:e338–43. doi: 10.1016/j.bjps.2012.09.001
- Baron DA, Hardie T, Leventhal JL, Della Badia CR. Timing of hysterectomy surgery during the menstrual cycle--impact of menstrual cycle phase on rate of complications: preliminary study. *J Am Osteopath Assoc.* (1999) 99:25–7. doi: 10.7556/jaoa.1999.99.1.25
- Kurdi MS, Ramaswamy AH. Does the phase of the menstrual cycle really matter to anaesthesia? *Indian J Anaesth.* (2018) 62:330–6. doi: 10.4103/ija.IJA\_139\_18;
- Tan SL, Zaidi J, Campbell S, Doyle P, Collins W. Blood flow changes in the ovarian and uterine arteries during the normal menstrual cycle. *Am J Obstet Gynecol.* (1996) 175:625–31. doi: 10.1053/ob.1996.v175.a73865
- Sladkevicius P, Valentin L, Marsal K. Blood flow velocity in the uterine and ovarian arteries during the normal menstrual cycle. *Ultrasound Obstet Gynecol.* (1993) 3:199–208. doi: 10.1046/j.1469-0705.1993.03030199.x





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# The causal role of gastroesophageal reflux disease in endometriosis: a bidirectional Mendelian randomization study

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Observational studies have reported an association between gastroesophageal reflux disease (GERD) and endometriosis. We conducted a two-sample and bidirectional Mendelian randomization analysis to determine whether those associations are causal. Two-sample and bidirectional MR analyses were performed using summary statistics from the European Individual Genome-Wide Association Study (GWAS). The inverse variance weighting (IVW) method is used as the main analysis method to evaluate causality. Sensitivity analyses were performed to assess heterogeneity, horizontal versatility, and stability. The results showed no significant causal association between GERD in women with endometriosis in the UK Bank database [ratio (OR)  $\approx 0$ , 95% adjusted interval (CI) 1.0007~1.0044,  $P = 0.006$ ] and Finn databases [ratio (OR) = 1.29, 95% adjusted interval (CI) 0.99~1.67,  $P = 0.06$ ]. However, when studying the Finn database only for endometriosis, which is confined to the uterus, a significant increase in GERD was limited to the risk of endometriosis in the uterus [ratio (OR) = 1.47, 95% adjusted interval (CI) 1.00~2.17,  $P = 0.05$ ]. Sensitivity analysis showed that the results were robust and did not detect multi efficacy or heterogeneity. Meanwhile, reverse MR analysis showed that endometriosis did not increase the risk of GERD. This MR study supports a causal relationship between GERD and an increased risk of endometriosis confined to the uterus. Therefore, patients with gastric esophageal reflux should be treated with gynecological examination to avoid and prevent the development of endometriosis.

## KEYWORDS

bidirectional Mendelian randomization (MR) analysis, gastroesophageal reflux disease, risk of developing endometriosis, endometriosis confined to the uterus, endometriosis

## 1 Introduction

Endometriosis is a common benign disease in gynecology, affecting approximately 10% (190 million) of women and girls of childbearing age worldwide (1). It is a chronic disease that is affected by estrogen regulation and is associated with dysmenorrhea, sexual intercourse, bowel pain and/or urination pain, chronic pelvic pain, bloating, nausea, and fatigue, and some patients also suffer from depression, anxiety and infertility (2).

Patients bear a severe burden of life and psychology, with enormous social and economic burdens (3–6). In addition, endometriosis sufferers often experience symptoms of intestinal or bladder irritation due to chronic pain comorbidities, which overlap with other diseases, leading to significant delays in the diagnosis of endometriosis after the onset of symptoms (7). Therefore, it is important to explore the factors associated with endometriosis to guide the early diagnosis and treatment of the disease.

Gastroesophageal reflux disease (GERD) refers to the reflux of gastroduodenal contents into the esophagus causing acid reflux, heartburn and other symptoms. Reflux can cause tissue damage to the mouth, throat, and bronchial tract and other tissue damage near the esophagus. Esophageal manifestations include asthma, chronic cough, idiopathic pulmonary fibrosis, hoarseness, chronic sore throat and tooth erosion (8). These nonspecific symptoms can cause overlap or confusion with other diseases (8). A clinical report in the United States showed that after long-term gastric esophageal reflux treatment, patients with GERD had a history of endometriosis and endometriosis resection and showed continued progression of symptoms of dysphagia, vomiting and reflux, and weight loss, with unknown causes and complications (9). The American Gastroenterological Society study also suggests that intestinal endometriosis can present with acute abdominal pain and small intestinal obstruction on CT. Therefore, when women of childbearing age have acute abdominal pain, the possibility of endometriosis involving the gastrointestinal tract should be considered (10, 11). Over the past five years, the American Gastrointestinal Association has also reported a possible association between a history of GERD and a history of hysterectomy in women (12). In addition, in recent new drug reports, domestic and foreign research institutes and companies have reported the invention of novel prevention and treatment drugs for both endometriosis and gastrointestinal diseases, which have synergistic effects (13–15). Although the underlying mechanisms of these phenomena are unclear, some evidence may support the potential of endometriosis to cause GERD, which in turn can lead to elevated levels of inflammation, leading to the development of endometriosis.

Although clinical observations and some current evidence suggest a possible association between GERD and endometriosis, it has not been possible to establish a causal link between them. Mendelian randomization (MR) is an innovative approach to optimizing observational epidemiology and can be used to investigate the causal effects of altered exposure on health outcomes (16).

The method introduces instrumental variables that affect exposure only, independent of potential confounding factors associated with outcomes and exposure outcomes, and will use single nucleotide polymorphisms (SNPs), which are highly correlated with exposure and randomized genetic variation, as instrumental variables to assess the causal relationship between the variable exposure and the outcome (17). SNPs have characteristics that precede disease occurrence and are unaffected by the outcome and the correlation between many confusing exposures and outcomes; thus, MR studies can reduce the risk of potential bias from confounding factors and reverse causation and effectively evaluate the causal relationship between exposure and outcome (18). To date, only one study of the correlation between GERD and endometriosis using MR has been retrieved (19). However, the study did not address the correlation between endometriosis

and GERD at different sites. Therefore, this study explores the causal relationship between GERD and endometriosis through MR and further explores the correlation between endometriosis at different sites. It seeks a new research direction for exploring the pathogenesis of endometriosis at different sites and provides a theoretical basis for endometriosis screening and early accurate diagnosis of endometriosis in patients with GERD.

## 2 Materials and methods

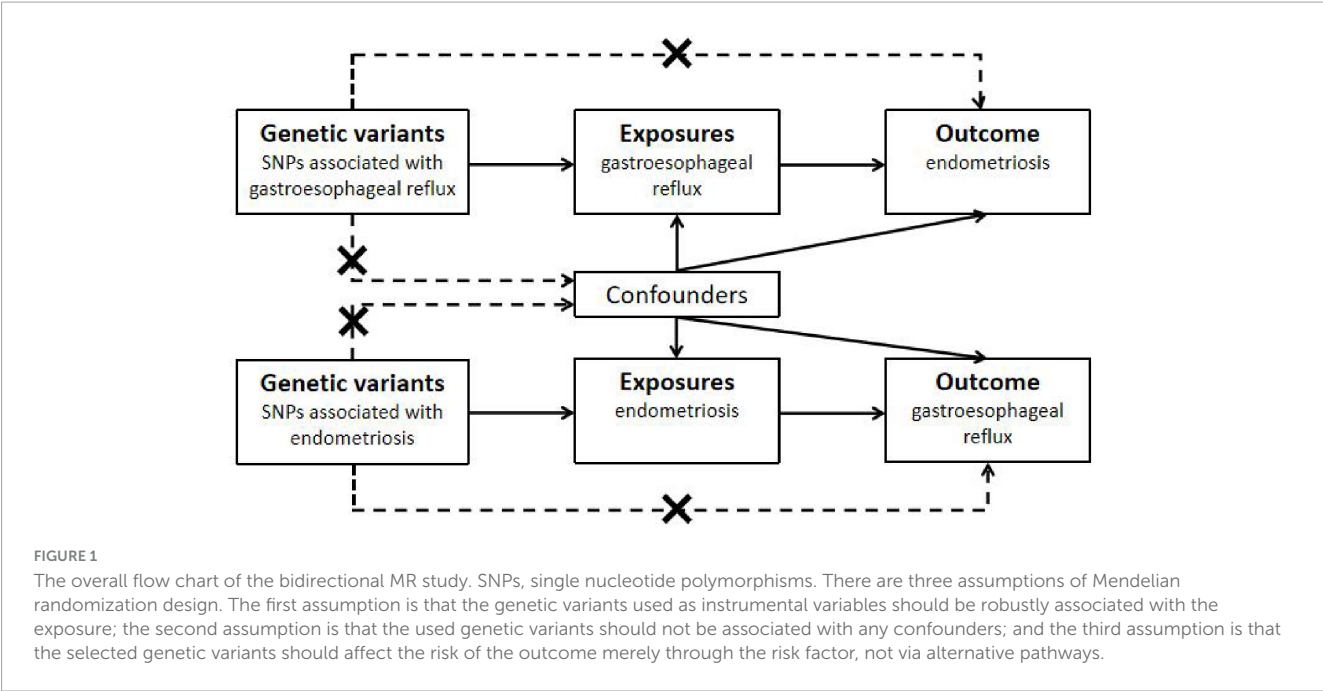
### 2.1 GWAS summary-level data of GERD and endometriosis

The overall flow chart of the bidirectional MR study is shown in Figure 1. A genome-wide association study (GWAS) meta-analysis was used to study European GERD data, which included 78,707 patients with GERD in Europe and 288,734 healthy controls (20). These data are available in the GWAS Catalog project database (21). In addition, the aggregate GWAS statistics for endometriosis are from the FinnGen database (8,288 cases of endometriosis and 9,972 cases of healthy controls) and the UK Bank database (1,496 cases of endometriosis) (22). Among them, the FinnGen database includes subsets of endometriosis occurring in fallopian tubes (116 cases of endometriosis and 146 cases of healthy controls), the uterus (2,372 cases of endometriosis and 1,600 cases of healthy controls), the pelvic peritoneum (2,953 cases of endometriosis and 3,940 cases of healthy controls), the ovaries (3,231 cases of endometriosis and 3,865 cases of healthy controls), the rectal vaginal and vaginal compartments (1,360 cases of endometriosis and 1,570 cases of healthy controls) and the intestines (117 cases of endometriosis and 375 cases of healthy controls). Table 1 provides details of the GWAS summary level data of exposure and outcome analyzed in this MR study. All data analyzed in this study were obtained from publicly available databases in which ethical approval was obtained for each cohort, and informed consent was obtained from all participants prior to participation. Figure 2 shows endometriosis at different sites. The specific analysis process (Example: GERD as an exposure/endometriosis as an outcome example) is shown in Figure 3.

### 2.2 Selection of instrumental variables

Mendelian randomization is a method of studying the causal relationship between exposure and outcome using genetic variation as an instrumental variable in medical research observations (23). Instrumental variable (IV) selection satisfies correlation with exposure, and IVs should be independent of any confusion associated with the exposure result, which means that there are no causal pathways from IVs to results, except through exposure (24, 25).

The selection of gene variants involves controlling genome-wide significance thresholds ( $p < 5 \times 10^{-8}$ ) and screening SNPs as IVs for MR analysis (16). Consideration of chained unbalanced SNPs had an impact on the resulting effect values by removing SNPs with  $r^2 < 0.001$  to the most significant SNP in the 10, 000 kb range of chromosomes to satisfy near-perfect chained equilibrium

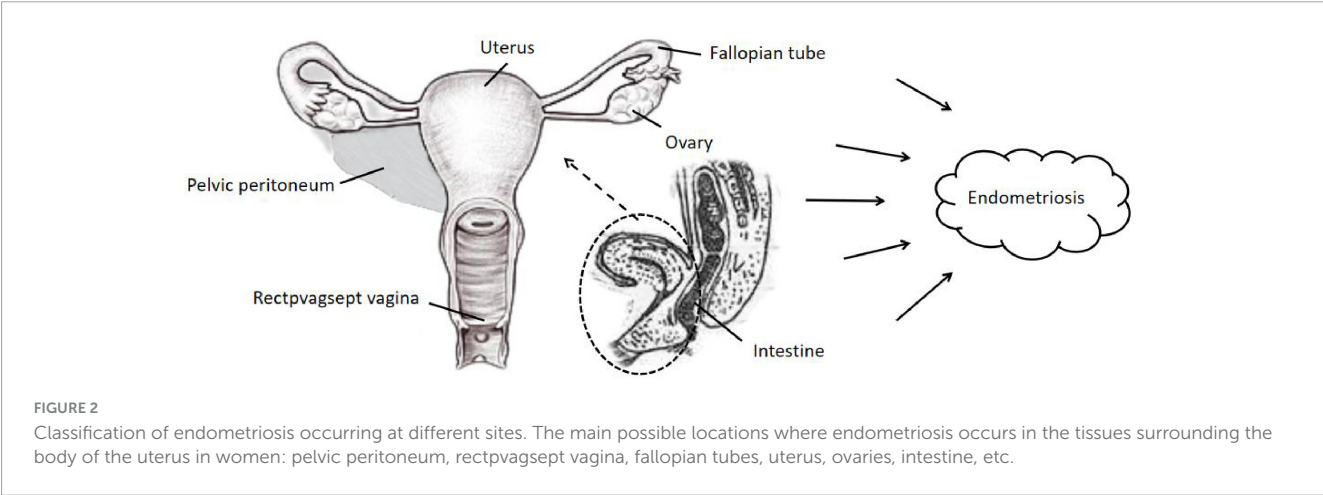


**FIGURE 1**  
The overall flow chart of the bidirectional MR study. SNPs, single nucleotide polymorphisms. There are three assumptions of Mendelian randomization design. The first assumption is that the genetic variants used as instrumental variables should be robustly associated with the exposure; the second assumption is that the used genetic variants should not be associated with any confounders; and the third assumption is that the selected genetic variants should affect the risk of the outcome merely through the risk factor, not via alternative pathways.

**TABLE 1** Details of the GWAS summary-level data.

| Traits                            | N case  | N control | Population | Data accession address                                                            |
|-----------------------------------|---------|-----------|------------|-----------------------------------------------------------------------------------|
| GERD                              | 129,080 | 473,524   | European   | <a href="https://gwas.mrcieu.ac.uk/">https://gwas.mrcieu.ac.uk/</a>               |
| ENDOMETRIOSIS (UK Bank database)  | 1,496   | 446,991   | European   | <a href="http://www.nealelab.is/uk-biobank">http://www.nealelab.is/uk-biobank</a> |
| ENDOMETRIOSIS (FinnGen database)  | 8,288   | 68,969    | European   | <a href="https://r9.finngen.fi/">https://r9.finngen.fi/</a>                       |
| ENDOMETRIOSIS_FALLOPIAN_TUBE      | 116     | 68,969    | European   | <a href="https://r9.finngen.fi/">https://r9.finngen.fi/</a>                       |
| ENDOMETRIOSIS_UTERUS              | 2,372   | 68,969    | European   | <a href="https://r9.finngen.fi/">https://r9.finngen.fi/</a>                       |
| ENDOMETRIOSIS_PELVICPERITONEUM    | 2,953   | 68,969    | European   | <a href="https://r9.finngen.fi/">https://r9.finngen.fi/</a>                       |
| ENDOMETRIOSIS_OVARY               | 3,231   | 68,969    | European   | <a href="https://r9.finngen.fi/">https://r9.finngen.fi/</a>                       |
| ENDOMETRIOSIS_RECTPVAGSEPT_VAGINA | 1,360   | 68,969    | European   | <a href="https://r9.finngen.fi/">https://r9.finngen.fi/</a>                       |
| ENDOMETRIOSIS_INTESTINE           | 117     | 68,969    | European   | <a href="https://r9.finngen.fi/">https://r9.finngen.fi/</a>                       |

GERD, gastroesophageal reflux disease.



**FIGURE 2**  
Classification of endometriosis occurring at different sites. The main possible locations where endometriosis occurs in the tissues surrounding the body of the uterus in women: pelvic peritoneum, rectpvagsept vagina, fallopian tubes, uterus, ovaries, intestine, etc.

between the two SNPs and to ensure the independence of each instrumental variable. Additionally, palindromic SNPs, outcome-associated SNPs ( $p < 0.05$ ), and SNPs not present in the resultant

GWAS pooled data were removed. The extent of weak instrumental bias was assessed according to the  $f$ -statistic formula, and IVs with  $F > 10$  were retained to avoid bias caused by weak IVs (26).

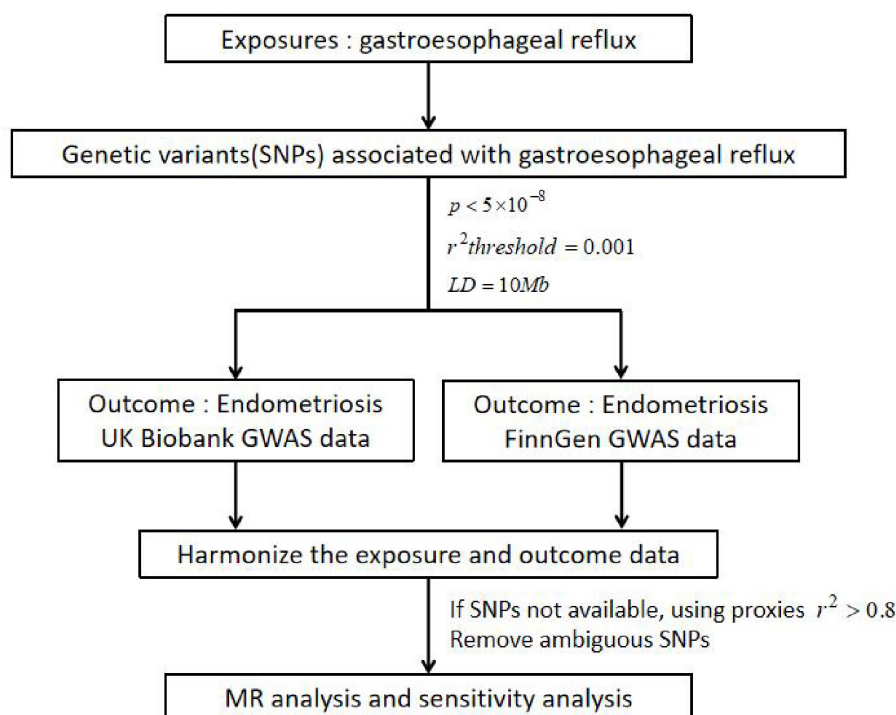


FIGURE 3

Specific analysis process. Example: GERD as a exposure/endometriosis as an outcome example; GERD, gastroesophageal reflux disease; SNPs, single nucleotide polymorphisms;  $p$ , statistical  $p$ -value;  $r^2$ , correlation index for evaluating the effect of the fitted regression;  $LD$ , linkage disequilibrium; GWAS, genome-wide association study. GERD was selected as the exposure and endometriosis was selected as the outcome. The screened instrumental variable (SNPs) was associated with exposure, fulfilling the following three conditions:  $p < 5 \times 10^{-8}$ ,  $r^2_{threshold} = 0.001$  and  $LD = 10Mb$ . In addition, the aggregate GWAS statistics for endometriosis are from FinnGen database (8,288 cases of endometriosis and 9,972 cases of health control) and UK Bank database (1,496 cases of endometriosis). If SNPs not available, using proxies  $r^2 > 0.8$ , remove ambiguous SNPs and harmonize the exposure and outcome data. Finally, MR analysis and sensitivity analysis were performed.

Body mass index, height, depression and anxiety, menarche, reproductive history, back pain, and the influence of economic factors may be potential confounders affecting GERD and endometriosis (22, 27–34). To increase the credibility of the findings, SNPs associated with these confounders ( $p < 5 \times 10^{-8}$ ) were retrieved from the IEU Open GWAS program database and excluded, and the number of these confounding accessions is shown in Table 2.

## 2.3 Statistical methods

The MR study relied on three core instrumental variable assumptions (correlation with exposure, independence from confounders, and exclusion of restrictions unrelated to outcome) to test the causal effect of exposure on outcome (16). Inverse variance weighted (IVW) analysis was used to estimate the causal effect of exposure and outcome using the Wald ratio estimator based on the principles of meta-analysis (35). To demonstrate the stability and directionality of the results, in addition to the IVW method, two other MR methods [MR-Egger method and weighted median method] were used to assess causality. The MR-Egger method estimates the causal effect of genes on traits by fitting a linear regression model that relates the effect of genetic variation on traits to the effect of genetic variation on gene expression. It also provides unbiased estimates, detecting

TABLE 2 Sources of confounding factors.

| Confounding factors               | Sources                                                                                                                        |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Height                            | <a href="https://doi.org/10.1093/humrep/dex207">https://doi.org/10.1093/humrep/dex207</a> (27)                                 |
| Body mass index                   | <a href="https://doi.org/10.1016/j.ejogrb.2004.11.019">https://doi.org/10.1016/j.ejogrb.2004.11.019</a> (28)                   |
| Depression and anxiety            | <a href="https://doi.org/10.2147/vhrm.s147173">https://doi.org/10.2147/vhrm.s147173</a> (29)                                   |
| Depression and anxiety            | <a href="https://doi.org/10.1001/jamanetworkopen.2022.51214">https://doi.org/10.1001/jamanetworkopen.2022.51214</a> (22)       |
| Age at menarche                   | <a href="https://doi.org/10.1007/s00404-022-06541-0">https://doi.org/10.1007/s00404-022-06541-0</a> (30)                       |
| Age at menarche                   | <a href="https://doi.org/10.1016/j.fertnstert.2012.05.035">https://doi.org/10.1016/j.fertnstert.2012.05.035</a> (31)           |
| Reproductive history              | <a href="https://doi.org/10.1097/01.AOG.0000142714.54857.f8">https://doi.org/10.1097/01.AOG.0000142714.54857.f8</a> (32)       |
| Back pain                         | <a href="https://doi.org/10.3389/fncel.2020.590823">https://doi.org/10.3389/fncel.2020.590823</a> (33)                         |
| The influence of economic factors | <a href="https://doi.org/10.1093/oxfordjournals.epirev.a036270">https://doi.org/10.1093/oxfordjournals.epirev.a036270</a> (34) |

and correcting for propensity and reverse causation bias in causal effect estimates (36). The weighted median method weights the causal effects of different genetic variants on a trait and then takes the weighted median as the final causal effect estimate. This method is robust and can reduce bias due to deviations in the estimates of certain genetic variants. However, the criterion for using the weighted median method is that at least 50%

TABLE 3 MR analysis results.

| Exposure | Outcome                           | <i>n</i> SNP | Method          | OR (95% CI)       | <i>P</i> -value |
|----------|-----------------------------------|--------------|-----------------|-------------------|-----------------|
| GERD     | ENDOMETRIOSIS (UK Bank database)  | 33           | IVW             | 1.001–1.004       | 0.006           |
|          |                                   | 33           | MR-Egger        | 0.983–1.018       | 0.96            |
|          |                                   | 33           | Weighted median | 0.999–1.004       | 0.20            |
| GERD     | ENDOMETRIOSIS (FinnGen database)  | 31           | IVW             | 0.992–1.666       | 0.06            |
|          |                                   | 31           | MR-Egger        | 0.202–29.965      | 0.49            |
|          |                                   | 31           | Weighted median | 0.820–1.555       | 0.46            |
| GERD     | ENDOMETRIOSIS_FALLOPIAN_TUBE      | 31           | IVW             | 0.155–4.328       | 0.81            |
|          |                                   | 31           | MR-Egger        | 0.000–7.18e+08    | 0.58            |
|          |                                   | 31           | Weighted median | 0.098–1.01e+01    | 1.00            |
| GERD     | ENDOMETRIOSIS_UTERUS              | 31           | IVW             | 0.999–2.166       | 0.05            |
|          |                                   | 31           | MR-Egger        | 0.009–13.542      | 0.57            |
|          |                                   | 31           | Weighted median | 0.926–2.638       | 0.09            |
| GERD     | ENDOMETRIOSIS_PELVICPERITONEUM    | 31           | IVW             | 0.764–1.753       | 0.49            |
|          |                                   | 31           | MR-Egger        | 0.072–211.151     | 0.51            |
|          |                                   | 31           | Weighted median | 0.579–1.630       | 0.91            |
| GERD     | ENDOMETRIOSIS_OVARY               | 31           | IVW             | 0.850–1.895       | 0.24            |
|          |                                   | 31           | MR-Egger        | 0.200–412.233     | 0.27            |
|          |                                   | 31           | Weighted median | 0.695–1.787       | 0.65            |
| GERD     | ENDOMETRIOSIS_RECTPVAGSEPT_VAGINA | 31           | IVW             | 0.630–1.757       | 0.85            |
|          |                                   | 31           | MR-Egger        | 0.157–2,533.167   | 0.24            |
|          |                                   | 31           | Weighted median | 0.401–1.602       | 0.53            |
| GERD     | ENDOMETRIOSIS_INTESTINE           | 31           | IVW             | 0.263–3.886       | 0.99            |
|          |                                   | 31           | MR-Egger        | 8.07e–06–1.07e+06 | 0.87            |
|          |                                   | 31           | Weighted median | 0.135–4.779       | 0.81            |

of the SNPs must satisfy the prerequisite of valid IVs (37). A significance threshold of  $p < 0.05$  was set, and the causal association results were expressed as odds ratios (ORs) and 95% confidence intervals (95% CIs).

## 2.4 Reverse MR analysis

Reverse MR analysis was performed to assess whether endometriosis affects GERD, and screening instrumental variables, Mendelian randomization analysis, and sensitivity analysis were performed sequentially. Instrumental variables were selected as described in Section “2.2 Selection of instrumental variables,” and statistical methods were selected as described in Section “2.3 Statistical methods.”

## 3 Results

### 3.1 Results of MR analysis using IVs based on genome-wide significance screening

MR results were based on instrumental variables screened at the genome-wide significance threshold ( $p < 5 \times 10^{-8}$ ), and a

total of 44 SNPs associated with confounding factors (body mass index, height, depression and anxiety, menarche, reproductive history, back pain, and the influence of economic factors) were excluded. The causal effect of GERD on endometriosis and on endometriosis occurring in different locations was assessed based on 33 instrumental variables after removing the palindromic SNPs, outcome-associated SNPs ( $p < 0.05$ ), and SNPs that were not present in the outcome GWAS pooled data. Detailed information on the confounding SNPs associated with the results is provided in [Supplementary Table 1](#), and detailed information on the instrumental variables for MR and the results of the analyses are provided in [Supplementary Table 2](#).

In all endometriosis databases, the *f*-statistics of all IVs were greater than 10, ranging from 29.75–45.55, which excluded the interference of weak instrumental variables on the results. In addition, the results of MR analysis for IVs screened based on genome-wide significance thresholds are shown in [Table 3](#). The MR results indicated that there was no significant causal relationship between GERD and the occurrence of endometriosis (UK Bank: OR  $\approx$  0, 95% CI 1.0007–1.0044,  $P = 0.006$ ; FinnGen: OR = 1.29, 95% CI 0.99–1.67,  $P = 0.06$ ). In addition, MR results, occurring in the subdatabases of fallopian tubes, pelvic peritoneum, ovaries, rectovaginal septum with vagina and intestines, yielded the same



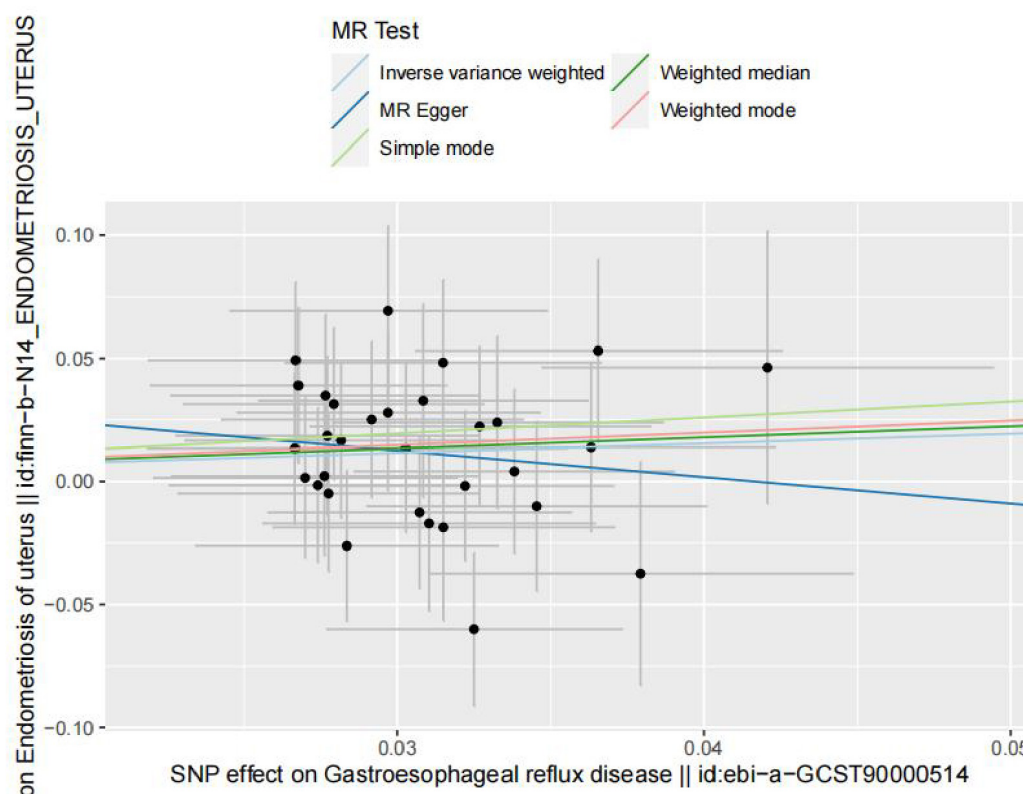


FIGURE 4

Scatterplot of genetic correlations between exposure (endometriosis occurring at the uterus) and outcome (gastroesophageal reflux disease) based on IVs screened at genome-wide significance thresholds.

conclusions as described above, and the results of MR analysis are shown in Table 3.

However, in the subdatabase of endometriosis confined to the uterine corpus, MR results demonstrated a causal relationship between GERD and the development of endometriosis. Specifically, MR results in IVW indicated that GERD significantly increased the risk of endometriosis (OR = 1.47, 95% CI 1.00–2.17,  $P = 0.05$ ) (Table 3). In addition, two other MR methods yielded similar causal estimates, including MR-Egger and weighted median (Table 3 and Figure 4). Sensitivity analyses were conducted to assess the robustness of the MR results. The MR Steiger test indicated that the inferred causal direction between exposure (GERD) and outcome (endometriosis) was in the “right direction” ( $p < 0.05$ ). The Cochran’s Q test indicated that there was no heterogeneity between IVs ( $p > 0.05$ ) (Table 4). The results of the MR-Egger intercept test and the MRPRESSO global test indicated that the MR analyses were not potentially affected by any level of pleiotropy ( $p > 0.05$ ) (Table 5). Leave-one-out sensitivity analyses confirmed the robustness of the MR results, as there were no prior SNPs that severely affected the results upon exclusion (Supplementary Figure 1).

### 3.2 Reverse MR results

Reverse MR analysis of the UK Bank database and endometriosis occurring at the uterus with no valid IVs after

removal of the palindromic SNPs, outcome-associated SNPs ( $p < 0.05$ ), SNPs not present in the resultant GWAS pooled data, and SNPs associated with confounders. The FinnGen database assessed the causal effect of endometriosis on GERD based on five IVs. Detailed information on the IVs for reverse MR analysis is shown in Supplementary Tables 3, 4. None of the MR methods showed a causal relationship between endometriosis and GERD ( $p > 0.05$ ) (Table 6). The Cochran’s Q test showed that reverse MR analysis was affected by heterogeneity ( $p < 0.05$ ) (Table 7). In addition, the MR-Egger intercept test and MR-PRESSO global test showed that the reverse MR analysis was not affected by horizontal pleiotropy ( $p > 0.05$ ) (Table 8). Finally, leave-one-out sensitivity analysis confirmed the robustness of the reverse MR results (Supplementary Figure 2).

## 4 Discussion

In this study, bidirectional MR analysis was performed using a variety of MR methods, and the results showed that from the entire endometriosis dataset, no significant causality with GERD was found in either forward or reverse MR analysis (even though a significant causality was shown in the UK bank database, the OR was approximately equal to 1, suggesting that the occurrence of GERD did not significantly increase the risk of developing endometriosis). These associations were robust in sensitivity analyses, with no detectable heterogeneity

TABLE 4 Heterogeneity results of Cochran’s Q test.

| Exposure | Outcome              | Method   | Cochran’s Q test |      |        |
|----------|----------------------|----------|------------------|------|--------|
|          |                      |          | Q                | Q_df | Q_pval |
| GERD     | ENDOMETRIOSIS_UTERUS | IVW      | 20.54            | 29   | 0.875  |
| GERD     | ENDOMETRIOSIS_UTERUS | MR-Egger | 21.15            | 30   | 0.883  |

TABLE 5 Results of MR-Egger intercept test and MR-PRESSO global test for horizontal multivariate validity.

| Exposure | Outcome              | MR-Egger intercept test |       |         | MR-PRESSO global test |         |
|----------|----------------------|-------------------------|-------|---------|-----------------------|---------|
|          |                      | Intercept               | SE    | P-value | RSS obs               | P-value |
| GERD     | ENDOMETRIOSIS_UTERUS | 0.045                   | 0.057 | 0.44    | 22.66                 | 0.883   |

TABLE 6 Reverse MR results of causality of occurrence and endometriosis at the uterus on GERD.

| Exposure                         | Outcome | n SNP | Method          | OR (95% CI) | P-value |
|----------------------------------|---------|-------|-----------------|-------------|---------|
| ENDOMETRIOSIS (FinnGen database) | GERD    | 5     | IVW             | 0.965–1.077 | 0.49    |
|                                  |         | 5     | MR-Egger        | 0.842–1.794 | 0.36    |
|                                  |         | 5     | Weighted median | 0.979–1.074 | 0.30    |

TABLE 7 Heterogeneity results of Cochran’s Q test in reverse MR analysis.

| Exposure                         | Outcome | Method   | Cochran’s Q test |      |          |
|----------------------------------|---------|----------|------------------|------|----------|
|                                  |         |          | Q                | Q_df | Q_pval   |
| ENDOMETRIOSIS (FinnGen database) | GERD    | IVW      | 29.86            | 7    | 1.01e-04 |
|                                  |         | MR-Egger | 29.81            | 6    | 4.28e-05 |

TABLE 8 Horizontal multivariate results of MR-Egger intercept test and MR-PRESSO global test in MR reverse analysis.

| Exposure                         | Outcome | MR-Egger intercept test |      |         | MR-PRESSO global test |         |
|----------------------------------|---------|-------------------------|------|---------|-----------------------|---------|
|                                  |         | Intercept               | SE   | P-value | RSS obs               | P-value |
| ENDOMETRIOSIS (FinnGen database) | GERD    | 0.002                   | 0.02 | 0.923   | 36.84                 | < 0.001 |

or pleiotropy. The above results were largely consistent in MR analysis using IVs screened based on genome-wide significance thresholds from databases in different countries, adding more confidence to the results. Our findings are consistent with the results of previous reports on this type of disease by Adewuyi et al. (19). Surprisingly, however, when we analyzed the data using the Finn database, which provides subdatasets of endometriosis occurring in different locations, and when analyzing each subdataset individually, we found that genetically predicted GERD significantly increased the risk of endometriosis occurring in the uterine corpus, while at the same time, the reverse MR analysis revealed that confinement to the body of the uterus of the endometriosis did not appear to be causally related to GERD.

Previously reported observational studies have hinted at a possible relationship between GERD and endometriosis. Seaman et al. (38) found that endometriosis may coexist with the manifestation of gastrointestinal symptoms compared to healthy controls. Smorgick et al. (39) noted that the associations were closer relative to adolescents and young women, particularly in the adult female subgroup. Similarly, a cross-sectional cohort study involving Danish women found an association between gastrointestinal symptoms in patients with endometriosis, with cause and effect unknown

(40). El Moaen and Carpentier (9) performed a clinical report showing the complex impact of a previous history of endometriosis on gastroesophageal reflux disease. In addition, the severity of gastroesophageal reflux can contribute to the development of endometriosis. For example, Mysior et al. (10) and Dasari et al. (11) reported that endometriosis involving the gastrointestinal tract should be considered when identifying acute abdominal pain in women of childbearing age. Although these observational studies do not explain causality, they provide sufficient evidence for an association between GERD and endometriosis.

Endometriosis confined to the uterine corpus, also known as adenomyosis, is a diffuse or confined lesion formed by the invasion of endometrial glands and mesenchyme into the myometrium, and its pathogenesis and pathophysiology have not yet been clarified, although it has been reported to have some genetic homology with endometriosis in other sites. The association of the study population with other diseases is shown in Table 9, from which it can be seen that the overlap between patients with adenomyosis and those with intestinal endometriosis was 2.82%, and with other gastroesophageal and gastrointestinal diseases was less than 2%. There was about 7% overlap between these study participants and those identified as being in stages 1,2 of endometriosis American Society for Reproductive Medicine

TABLE 9 Association of study groups with other diseases.

| Disease type                                                            | Case overlap<br>N (Jaccard<br>index) |
|-------------------------------------------------------------------------|--------------------------------------|
| Endometriosis of intestine                                              | 162 (2.82)                           |
| Diverticular disease of intestine                                       | 850 (1.95)                           |
| Other diseases of intestines                                            | 1,898 (1.89)                         |
| Diseases of esophagus, stomach and duodenum                             | 1,321 (1.75)                         |
| Gastro-oesophageal reflux disease                                       | 707 (1.85)                           |
| Gastrointestinal diseases                                               | 3,711 (1.41)                         |
| Other noninfective gastroenteritis and colitis                          | 191 (1.23)                           |
| Diarrhea and gastroenteritis of presumed<br>infectious origin           | 543 (1.29)                           |
| Intestinal stricture                                                    | 212 (1.22)                           |
| Paralytic ileus and intestinal obstruction                              | 186 (1.17)                           |
| Other functional intestinal disorders                                   | 457 (1.75)                           |
| Intestinal infectious diseases                                          | 770 (1.35)                           |
| Any gastric operation                                                   | 3,206 (1.65)                         |
| Benign leiomyoma with endometriosis                                     | 1,985 (21.49)                        |
| Endometriosis of rectovaginal septum and vagina                         | 369 (4.63)                           |
| Deep endometriosis                                                      | 487 (5.79)                           |
| Endometriosis diagnosis and infertility diagnosis<br>occurring together | 689 (7.85)                           |
| Unspecified/other endometriosis                                         | 560 (6.47)                           |
| Endometriosis of pelvic peritoneum                                      | 888 (7.68)                           |
| Endometriosis ASRM stages 1,2                                           | 913 (7.80)                           |
| Endometriosis of ovary                                                  | 825 (6.95)                           |
| Endometriosis ASRM stages 3,4                                           | 1,033 (7.50)                         |
| Endometriosis                                                           | 5,319 (28.11)                        |

N indicates the number of overlapping individuals between two diseases (or study endpoints). The Jaccard index is used to measure the similarity of two sets with a value between 0 and 1, where 0 means completely different and 1 means completely the same, where the index is taken as a percentage value. Only disease types with index percentile values greater than 1 and associated with intestinal endometriosis are listed in the table; other diseases with less overlap with patient use are not listed.

(ASRM) and stages 3,4 of endometriosis ASRM. Notably, the patients with adenomyosis studied had an overlap of more than 20% with benign uterine fibroids with endometriosis and endometriosis. Meanwhile, Table 10 provides the drug use in the study population, which shows that the overlap between the adenomyosis patients and the concomitant drug group was less than 2% in all cases, with the exception of the “Triptan medication for migraine,” which also had an approximate overlap of 2%. Therefore, the conclusion of our study that gastroesophageal reflux may somewhat increase the risk of developing adenomyosis was less affected by the medications used by the study subjects, and it can be ruled out that these patients were not at pathogenic risk due to the use of medications. In the present MR study, GERD can lead to an increased risk of adenomyosis without a significant causal relationship with other sites of endometriosis, which explores the possible different pathogenesis of adenomyosis

TABLE 10 Drug use in the Finnish patient group.

| Drug type                                                                          | Case overlap<br>N (Jaccard<br>index) |
|------------------------------------------------------------------------------------|--------------------------------------|
| Use of eye-antiallergens (taken as indicator of<br>allergic/atopic conjunctivitis) | 533 (1.73)                           |
| Benzodiazepine use                                                                 | 981 (1.65)                           |
| Use of pramipexole                                                                 | 195 (1.50)                           |
| Care involving use of rehabilitation procedures                                    | 572 (1.56)                           |
| Use of hypnotics and sedatives                                                     | 1,421 (1.69)                         |
| Medicines for HER+breast cancer                                                    | 282 (1.55)                           |
| Other (not insulin) diabetes medications                                           | 845 (1.11)                           |
| ILD medications: immunosuppressive drugs                                           | 445 (1.27)                           |
| ILD medications                                                                    | 1,562 (1.67)                         |
| Diabetes medication                                                                | 891 (1.05)                           |
| ILD medications: prednisolone,<br>methylprednisolone, prednisone                   | 1,463 (1.73)                         |
| Triptan medication for migraine, single purchase<br>ok                             | 1,148 (2.08)                         |
| Second line medication for Crohn’s disease                                         | 386 (1.26)                           |
| Codeine or tramadol medication                                                     | 1,499 (1.91)                         |
| First line medication for Crohn’s disease                                          | 1,675 (1.67)                         |
| Depression medications                                                             | 2,352 (1.69)                         |
| Statin medication                                                                  | 2,127 (1.29)                         |
| Antihypertensive medication–note that there are<br>other indications               | 3,321 (1.32)                         |
| Paracetamol of NSAID medication                                                    | 5,150 (1.29)                         |

N indicates the number of overlapping individuals between two diseases (or study endpoints). The Jaccard index is used to measure the similarity of two sets with a value between 0 and 1, where 0 means completely different and 1 means completely the same, where the index is taken as a percentage value. Only medication types with Index percentile values > 1 are listed in the table; other medications with low overlap of use with patients are not listed.

and other endometriosis from another new angle and provides a new direction for further pathologic studies.

MR research is an innovative approach to inferring causality. Compared to traditional observational studies, MR studies eliminate confounding variables and reverse causation. Compared to randomized controlled trials, MR studies are more effective, and there are no ethical restrictions on their implementation. The MR results showed that GERD significantly increased the risk of endometriosis confined to the uterus (IVW: OR = 1.47, 95% CI 1.00–2.17,  $P = 0.05$ ) using IVs based on genome-wide significance threshold screening. In addition, the extrapolation of the weighted median approach was consistent with the results of IVW (37). Subsequently, various sensitivity tests further demonstrated the validity of the results.

Meanwhile, the reason for analyzing the slight difference with the results of Adewuyi et al. (19) is most likely related to the exclusion of confounders in the instrumental variables, and the literature on MR analysis of GERD and endometriosis was cited in the latest review of the causal relationship between different exposures and endometriosis using Mendelian randomization, which referred to the association between GERD and depression,

and GERD may play a role as a mediating variable in depression and anxiety affecting the occurrence of endometriosis, implying that previous studies did not exclude the interference of depression and anxiety as a confounding factor on the research results, and can also indicate that this paper based on the results of the latest research progress to exclude the new confounding factors may get different analytical conclusions of research and practical significance, and can provide a new reference value (41). On the basis of previous studies and conclusions, we continue to dig deeper into endometriosis occurring in different locations and find that GERD may increase the risk of adenomyosis, which can also prove the results of previous studies to a certain extent.

Several hypotheses could explain the increased risk of adenomyosis caused by GERD. First, previous studies imply that other syndromes of the intestinal tract are closely associated with endometriosis conditions (15), both showing a tendency to increase the overall level of chronic inflammation (39, 42, 43). In this context, the activation of mast cells and their degranulation, followed by the release of lymphokines, tumor necrosis factor- $\alpha$ , and the presence of proinflammatory cytokines in mesenchymal tissues, promotes the persistence of a chronic inflammatory situation (42, 44–47). Considering the pathophysiologic mechanisms shared by GERD and endometriosis, the possible diagnosis of both pathologies needs to be investigated in the presence of severe pelvic pain. Second, depression and anxiety may mediate GERD-induced endometriosis (22, 29). Since GERD episodes may lead to elevated levels of central nervous system inflammation, which may trigger depression and anxiety, patients with GERD often suffer from more severe anxiety-depression (48, 49). At the same time, anxiety depression produces estrogen disorders, which exacerbate depression anxiety and make endometriosis, which is regulated by estrogen, possible (2). Therefore, it is necessary to maintain psychological support for patients with GERD to maintain estrogen stability and reduce the risk of subsequent endometriosis.

The current study has some strengths. First, this is an MR investigation assessing the causal relationship between GERD and endometriosis, which obtained not exactly the same conclusions as previous studies and did not find a significant causal relationship between GERD and endometriosis in both the positive and negative directions. Second, the MR analyses in this paper were performed using separate pooled-level data from large-scale GWAS in different countries, which improves the confidence of inferences due to the large sample sizes and different populations, and many MR methods and sensitivity analyses were used to improve the confidence of the results. Third, thanks to the Finn database, which breaks down endometriosis occurring in different locations, the present study enriches and completes the findings of previous studies and is highly likely to imply a causal association between GERD and specific sites of endometriosis occurrence. However, this study has some limitations. First, the original GWAS pooled data analyzed in this study were from a European population; therefore, the findings may not be applicable to other ethnicities. Second, due to the limitations of the GWAS pooled data, it was not possible to stratify the analysis for general factors such as age and gender. Third, it is difficult to ensure that the results are completely independent of horizontal polymorphism effects. Therefore, we performed a series of sensitivity analyses to demonstrate the reliability of the results.

## 5 Conclusion

Evidence is provided that genetically predicted GERD increases the risk of adenomyosis. Therefore, symptomatic treatment of patients with GERD should be complemented by gynecological examination to avoid and prevent the development of endometriosis.

## Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: the datasets analyzed during the current study are available in the GWAS database, <https://www.ebi.ac.uk/gwas>.

## Ethics statement

The studies involving humans were approved by the Medical Ethics Review Board of West China Second Hospital of Sichuan University, and informed consent was obtained from the subjects (Date: March 8, 2019). The datasets analyzed in this study were publicly available summary statistics, and informed consent was obtained from all individuals participating in the study. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## Author contributions

ZS: Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization, Writing – review & editing, Writing – original draft. ZL: Supervision, Validation, Methodology, Formal analysis, Data curation, Conceptualization, Writing – review & editing. KW: Validation, Supervision, Methodology, Funding acquisition, Writing – review & editing. FY: Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing, Validation.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2024.1440157/full#supplementary-material>

### SUPPLEMENTARY FIGURE 1

Funnel plots of the causal effect between GERD and endometriosis confined to the uterine corpus.

### SUPPLEMENTARY FIGURE 2

Funnel plots of the causal effect between endometriosis confined to the uterine corpus and GERD.

## References

- Laganà A, Garzon S, Götte M, Viganò P, Franchi M, Ghezzi F, et al. The pathogenesis of endometriosis: Molecular and cell biology insights. *Int J Mol Sci.* (2019) 20:5615. doi: 10.3390/ijms20225615
- Koninckx P, Ussia A, Adamyan L. Pathogenesis of endometriosis: The genetic/epigenetic theory. *Fertil Steril.* (2019) 111:327–40. doi: 10.1016/j.fertnstert.2018.10.013
- Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. *Nat Rev Endocrinol.* (2019) 15:666–82. doi: 10.1038/s41574-019-0245-z
- Hämmerli S, Kohl Schwartz A, Geraedts K, Imesch P, Rauchfuss M, Wölfler M, et al. Does endometriosis affect sexual activity and satisfaction of the man partner? A comparison of partners from women diagnosed with endometriosis and controls. *J Sex Med.* (2018) 15:853–65. doi: 10.1016/j.jsxm.2018.03.087
- Soliman A, Coyne K, Gries K, Castelli-Haley J, Snabes M, Surrey E. The effect of endometriosis symptoms on absenteeism and presenteeism in the workplace and at home. *J Manag Care Spec Pharm.* (2017) 23:745–54. doi: 10.18553/jmcp.2017.23.7.745
- Soliman A, Yang H, Du E. The direct and indirect costs associated with endometriosis: A systematic literature review. *Hum Reprod.* (2016) 31:712–22. doi: 10.1093/humrep/dev335
- Saunders P, Horne A. Endometriosis: Etiology, pathobiology, and therapeutic prospects. *Cell.* (2021) 184:2807–24. doi: 10.1016/j.cell.2021.04.041
- Katz P, Dunbar K, Schnoll-Sussman F. ACG clinical guideline for the diagnosis and management of gastroesophageal reflux disease. *Am J Gastroenterol.* (2021) 117:27–56. doi: 10.14309/ajg.0000000000001538
- El Moaen T, Carpentier SG. resulting in superior mesenteric artery syndrome: An unfortunate outcome. *Am J Gastroenterol.* (2018) 113:S1399–401. doi: 10.14309/00000434-201810001-02522
- Mysior J, Sreenivasan P, Mangla R, Schwender B. Intestinal endometriosis as an unusual cause of small bowel obstruction and abdominal pain. *Am J Gastroenterol.* (2011) 106:S236. doi: 10.14309/00000434-201110002-00619
- Dasari V, Grossman E, Dubrovskaya V. Ileocecal valve endometriosis as a cause of acute intestinal obstruction. *Am J Gastroenterol.* (2012) 107:S383. doi: 10.14309/00000434-201210001-00936
- Salas Noain J, Gudipally S, Zheng S, Amaral R. 1525 Atypical presentation of colon cancer: Colon-Vaginal fistula. *Am J Gastroenterol.* (2019) 114:S844–844. doi: 10.14309/01.ajg.0000595628.12916.9a
- Griffiths MJ, Horne AW, Gibson DA, Roberts N, Saunders PTK. Endometriosis: recent advances that could accelerate diagnosis and improve care. *Trends Mol Med.* (2024) 30:875–89. doi: 10.1016/j.molmed.2024.06.008
- Yang F, Wu Y, Hockey R, Doust J, Mishra GD, Montgomery GW, et al. Evidence of shared genetic factors in the etiology of gastrointestinal disorders and endometriosis and clinical implications for disease management. *Cell Rep Med.* (2023) 4:101250. doi: 10.1016/j.xcrm.2023.101250
- Viganò D, Zara F, Usai P. Irritable bowel syndrome and endometriosis: New insights for old diseases. *Digest Liver Dis.* (2018) 50:213–9. doi: 10.1016/j.jld.2017.12.017
- Skrivankova V, Richmond R, Woolf B, Davies N, Swanson S, VanderWeele T, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomisation (STROBE-MR): Explanation and elaboration. *BMJ.* (2021) 375:n2233. doi: 10.1136/bmj.n2233
- Richmond R, Davey Smith G. Mendelian randomization: Concepts and scope. *Cold Spring Harb Perspect Med.* (2021) 12:a040501. doi: 10.1101/cshperspect.a040501
- Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol.* (2016) 27:3253–65. doi: 10.1681/ASN.2016010098
- Adewuyi EO, Mehta D, Sapkota Y, Auta A, Yoshihara K, Nyegaard M, et al. Genetic analysis of endometriosis and depression identifies shared loci and implicates causal links with gastric mucosa abnormality. *Hum Genet.* (2020) 140:529–52. doi: 10.1007/s00439-020-02223-6
- Ong J-S, An J, Han X, Law MH, Nandakumar P, Schumacher J, et al. Multitrait genetic association analysis identifies 50 new risk loci for gastro-oesophageal reflux, seven new loci for Barrett's oesophagus and provides insights into clinical heterogeneity in reflux diagnosis. *Gut.* (2021) 71:1053–61. doi: 10.1136/gutjnl-2020-323906
- GWAS Catalog. *The NHGRI-EBI catalog of human genome-wide association studies.* (2023). Available online at: <https://www.ebi.ac.uk/gwas> (accessed November 17, 2023).
- Koller D, Pathak G, Wendt F. Epidemiologic and genetic associations of endometriosis with depression, anxiety, and eating disorders. *JAMA Netw Open.* (2023) 6:e2251214. doi: 10.1001/jamanetworkopen.2022.51214
- Burgess S, Small D, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. *Stat Methods Med Res.* (2015) 26:2333–55. doi: 10.1177/0962280215597579
- Greenland S. An introduction to instrumental variables for epidemiologists. *Int J Epidemiol.* (2000) 29:722–9. doi: 10.1093/ije/29.4.722
- Martens E, Pestman W, de Boer A. Instrumental variables. *Epidemiology.* (2006) 17:260–7. doi: 10.1097/01.ede.0000215160.88317.cb
- Pierce B, Ahsan H, Vanderweele T. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. *Int J Epidemiol.* (2010) 40:740–52. doi: 10.1093/ije/dyq151
- Farland L, Missmer S, Bijon A, Gusto G, Gelot A, Clavel-Chapelon F, et al. Associations among body size across the life course, adult height and endometriosis. *Hum Reprod.* (2017) 32:1732–42. doi: 10.1093/humrep/dex207
- Ferrero S, Anserini P, Remorgida V, Ragni N. Body mass index in endometriosis. *Eur J Obstet Gynecol Reprod Biol.* (2005) 121:94–8. doi: 10.1016/j.ejogrb.2004.11.019
- Bussotti M, Sommaruga M. Anxiety and depression in patients with pulmonary hypertension: Impact and management challenges. *Vasc Health Risk Manag.* (2018) 14:349–60. doi: 10.2147/vhrm.s147173
- Lu M, Niu J, Liu B. The risk of endometriosis by early menarche is recently increased: A meta-analysis of literature published from 2000 to 2020. *Arch Gynecol Obstet.* (2022) 307:59–69. doi: 10.1007/s00404-022-06541-0
- Nnoaham K, Webster P, Kumbang J, Kennedy S, Zondervan K. Is early age at menarche a risk factor for endometriosis? A systematic review and meta-analysis of case-control studies. *Fertil Steril.* (2012) 98:702–712.e6. doi: 10.1016/j.fertnstert.2012.05.035
- Missmer S, Hankinson S, Spiegelman D, Barbieri R, Malspeis S, Willett W, et al. Reproductive history and endometriosis among premenopausal women. *Obstet Gynecol.* (2004) 104:965–74. doi: 10.1097/01.aog.0000142714.54857.f8
- Maddern J, Grundy L, Castro J, Brierley S. Pain in Endometriosis. *Front Cell Neurosci.* (2020) 14:590823. doi: 10.3389/fncel.2020.590823



34. Houston D. Evidence for the risk of pelvic endometriosis by age, race and socioeconomic status. *Epidemiol Rev.* (1984) 6:167–91. doi: 10.1093/oxfordjournals.epirev.a036270
35. Pagoni P, Dimou N, Murphy N, Stergiakouli E. Using Mendelian randomisation to assess causality in observational studies. *Evid Based Ment Health.* (2019) 22:67–71. doi: 10.1136/ebmental-2019-300085
36. Burgess S, Thompson S. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol.* (2017) 32:377–89. doi: 10.1007/s10654-017-0255-x
37. Bowden J, Davey Smith G, Haycock P, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40:304–14. doi: 10.1002/gepi.21965
38. Seaman H, Ballard K, Wright J, De Vries C. Endometriosis and its coexistence with irritable bowel syndrome and pelvic inflammatory disease: Findings from a national case-control study-Part 2. *BJOG.* (2008) 115:1392–6. doi: 10.1111/j.1471-0528.2008.01879.x
39. Smorgick N, Marsh C, As-Sanie S, Smith Y, Quint E. Prevalence of pain syndromes, mood conditions, and asthma in adolescents and young women with endometriosis. *J Pediatr Adolesc Gynecol.* (2013) 26:171–5. doi: 10.1016/j.jpag.2012.12.006
40. Schomacker M, Hansen K, Ramlau-Hansen C, Forman A. Is endometriosis associated with irritable bowel syndrome? A cross-sectional study. *Eur J Obstet Gynecol Reprod Biol.* (2018) 231:65–9. doi: 10.1016/j.ejogrb.2018.10.023
41. McGrath I, Montgomery G, Mortlock S. Insights from Mendelian randomization and genetic correlation analyses into the relationship between endometriosis and its comorbidities. *Hum Reprod Update.* (2023) 29:655–74. doi: 10.1093/humupd/dmad009
42. Polak G, Barczyński B, Bednarek W. Increased levels of proteins of the acute inflammatory phase in the peritoneal fluid of women with advanced stages of endometriosis. *Polish Gynaecol.* (2015) 86:414–8. doi: 10.17772/gp/2416
43. Sinagra E, Pompei G, Tomasello G, Cappello F, Morreale G, Amvrosiadis G, et al. Inflammation in irritable bowel syndrome: Myth or new treatment target? *World J Gastroenterol.* (2016) 22:2242–55. doi: 10.3748/wjg.v22.i7.2242
44. Héron A, Dubayle D. A focus on mast cells and pain. *J Neuroimmunol.* (2013) 264:1–7. doi: 10.1016/j.jneuroim.2013.09.018
45. Buhner S, Schemann M. Mast cell-nerve axis with a focus on the human gut. *Biochim Biophys Acta.* (2012) 1822:85–92. doi: 10.1016/j.bbdis.2011.06.004
46. Walker M. *Are conditions of immune dysregulation risk factors for functional gastrointestinal disorders? Findings from a random population based study.* Berlin: Morrester (2016).
47. Kempuraj D, Papadopoulou N, Stanford E, Christodoulou S, Madhappan B, Sant G, et al. Increased numbers of activated mast cells in endometriosis lesions positive for corticotropin-releasing hormone and urocortin. *Am J Reprod Immunol.* (2004) 52:267–75. doi: 10.1111/j.1600-0897.2004.00224.x
48. You Z, Perng C, Hu L. Risk of psychiatric disorders following gastroesophageal reflux disease: A nationwide population-based cohort study. *Eur J Intern Med.* (2015) 26:534–9. doi: 10.1016/j.ejim.2015.05.005
49. Zeng Y, Cao S, Yang H. The causal role of gastroesophageal reflux disease in anxiety disorders and depression: A bidirectional Mendelian randomization study. *Front Psychiatry.* (2023) 14:1135923. doi: 10.3389/fpsy.2023.1135923



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# Commentary: The causal role of gastroesophageal reflux disease in endometriosis: a bidirectional Mendelian randomization study

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## KEYWORDS

Mendelian randomization (MR), gastroesophageal reflux disease (GERD), endometriosis, causal inference, genetic variants

## A Commentary on

[The causal role of gastroesophageal reflux disease in endometriosis: a bidirectional Mendelian randomization study](#)

by Shi, Z., Li, Z., Wang, K., and Yang, F. (2024). *Front. Med.* 11:1440157.  
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## 1 Introduction

Endometriosis is the most common benign gynecological condition, affecting approximately 10% of women and girls of reproductive age worldwide (1). This is a chronic disorder affected by estrogen regulation and presents with symptoms such as dysmenorrhea, pelvic pain, and psychological distress that impose a great social and economic burden on affected individuals (2). This timely diagnosis is further complicated by the overlapping symptoms of intestinal or bladder irritation, thus delaying the identification of the condition (3, 4). At the same time, in connection with gastroesophageal reflux disease (GERD), non-specific symptoms include acid reflux and chronic cough, which create diagnostic confusion with other conditions (5). Clinical observations have pointed out a probable interrelationship between GERD and endometriosis (6), reporting that women with GERD may experience exacerbation of symptoms after endometriosis treatment (7). Although these clinical associations offer exciting prospects, it is very hard to establish a cause-and-effect relationship. Mendelian randomization (MR) will be a new strategy to fill this gap in the literature by using genetic variants as instrumental variables in estimating the question of causality without the confounding inherent in observational studies (8). Discussions by Shi et al. (9) on the cause-and-effect relationship between GERD and endometriosis via MR reveal new insights into the pathogenesis of endometriosis and further improves the early diagnosis and interventional strategy for patients with endometriosis suffering from GERD. This study, however, has certain limitations in some aspects.

## 2 Comments and analysis

### 2.1 Advantages

#### 2.1.1 Innovativeness

This study uses an innovative bidirectional MR analysis to investigate the causal relationship between GERD and endometriosis. MR is a relatively new research approach that uses genetic variants as instrumental variables, helping to mitigate the influence of confounding factors and thereby improving the accuracy of causal inferences. Traditional observational studies often face challenges in establishing causality, whereas MR provides a more rigorous framework for such investigations. This is the first application of MR analysis to the relationship between GERD and endometriosis, paving the way for exploring associations between complex diseases and marking a significant advance in causal inference research.

#### 2.1.2 Methodological rigor

The study employs multiple MR analysis methods—including inverse variance weighting (IVW), MR-Egger regression, and weighted median approaches—to enhance the robustness of causal inferences. By incorporating various methods, the research validates the reliability of its results from different perspectives. It also utilizes multiple sensitivity tests, such as Cochran's Q test, and the MR-PRESSO test, to assess the issues of pleiotropy and heterogeneity. Using these tests strengthens the scientific rigor of the study, helps control potential biases, and ensures the statistical robustness of the findings. This methodological validation provides stronger support for the credibility of the conclusions.

#### 2.1.3 Reliability of data sources

The research data are obtained from large genome-wide association study (GWAS) databases, including the UK Biobank and FinnGen databases in Europe. These databases have undergone strict ethical and data quality reviews, demonstrating high reliability. The large sample size offers a wealth of genetic information, providing statistical support for the analyses and enhancing the efficacy and representativeness of the results. The credibility of the data sources not only boosts the rigor of the study but also increases the applicability of its conclusions in European populations.

### 2.2 Limitations

Although bidirectional MR analysis was used, the lack of additional methods for result verification limits the robustness of causal inferences. In the reverse causal analysis, the small sample size and insufficient number of effective instrumental variables (single nucleotide polymorphisms (SNPs), especially in the analysis of localized endometriosis, which only included five SNPs, diminish the statistical power of the reverse analysis results. Furthermore, the study did not investigate the dose-response relationship between the severity or duration of GERD and endometriosis, leading to a somewhat one-sided understanding of the causal relationship.

#### 2.2.1 Inadequacies in data analysis

The study relies on *p*-values to determine statistical significance without a detailed interpretation of effect sizes and confidence intervals. Although the association between GERD and endometriosis is statistically significant ( $OR = 1.47$ ,  $P = 0.05$ ), the effect size is small and the confidence interval approaches 1, indicating a weak actual impact (10). Additionally, the insufficient effectiveness of the instrumental variables in the reverse MR analysis may lead to weak instrument bias, affecting the accuracy of causal inferences. The study also inadequately controls for pleiotropy and heterogeneity, increasing the risk of bias in data analysis.

#### 2.2.2 Limitations in sample sources

While the study utilizes high-quality European datasets (UK Biobank and FinnGen), the limited ethnic diversity of these samples raises important concerns about the generalizability of findings. Significant interethnic variations exist in both disease prevalence (GERD: 18.1%–27.8% in European populations vs. 2.5%–7.8% in East Asian cohorts) and genetic architecture (e.g., differential effect sizes for risk loci such as rs1799964) (11). To address this limitation in future research, we recommend (1) incorporating trans-ethnic GWAS consortia (e.g., Biobank Japan, China Kadoorie Biobank) with standardized phenotyping protocols, (2) implementing genetic ancestry principal components as covariates to account for population stratification, and (3) conducting stratified analyses by key demographic variables (age tertiles, body mass index categories, and menopausal status) to evaluate effect heterogeneity (12, 13). Such approaches would enable differentiation between genetically driven and environmentally mediated mechanisms while improving the clinical applicability of findings across diverse populations.

#### 2.2.3 Logical contradictions in causal inference

The results of the forward and reverse MR analyses are inconsistent. The forward MR analysis indicates that GERD may increase the risk of endometriosis, while the reverse analysis shows no significant impact of endometriosis on GERD. This unidirectional causal relationship lacks biological support and does not adequately explain why GERD affects endometriosis without reciprocal effects. This logical contradiction remains unresolved, undermining the scientific rigor of the study.

#### 2.2.4 Insufficient biological plausibility

While this study establishes a statistically significant association between GERD and endometriosis ( $OR = 1.47$ ,  $P = 0.05$ ), the biological mechanisms underlying this relationship remain insufficiently explored. Current evidence suggests multiple potential pathways: (1) GERD-induced gastric acid reflux may promote chronic systemic inflammation, with elevated cytokines (IL-6 and TNF- $\alpha$ ) activating pelvic macrophages and facilitating ectopic endometrial growth (3, 6); (2) acid reflux-associated gut microbiota dysbiosis (14) and subsequent immune dysregulation may disrupt the gut-endometrial axis,

creating a bidirectional pathological loop (15); and (3) vagus nerve activation by chronic reflux could alter the uterine microenvironment through neurogenic inflammation (16). However, the modest effect size and the lack of mechanistic validation limit clinical interpretation. Future research should employ liquid biopsies to track inflammatory markers and animal models to experimentally verify these pathways, particularly focusing on the microbiome-immune interface and neuroendocrine crosstalk.

## 2.3 Impact on the field

This study pioneers the use of bidirectional MR to investigate the causal relationship between GERD and endometriosis, utilizing large-scale GWAS data from European populations. The methodological rigor, including IVW, MR-Egger, and sensitivity analyses, enhances the reliability of causal inferences.

Limitations include the lack of ethnic diversity in samples, potential weak instrument bias in reverse MR analysis, and insufficient exploration of biological mechanisms. While the study provides novel insights, its clinical applicability is constrained by small effect sizes ( $OR = 1.47$ ) and unresolved bidirectional inconsistency. Future research should integrate multi-ethnic cohorts (e.g., Asian or African populations) and experimental models to validate these findings, as suggested by recent microbiome studies (14, 15).

## 2.4 Improvement suggestions

### 2.4.1 Validation with additional methods

To strengthen causal inference, future studies should implement a multi-method validation framework that combines MR with complementary approaches. First, Bayesian MR methods should be used to quantify posterior probabilities of causal effects while incorporating prior biological knowledge about GERD-endometriosis pathways. Second, sensitivity analyses using different pleiotropy-robust methods (e.g., weighted median and MR-PRESSO) should be systematically compared through heterogeneity metrics ( $I^2 < 25\%$  indicating consistency). For dose-response evaluation, researchers should (1) stratify GERD exposure by clinically validated severity indices (e.g., Los Angeles classification grades) and treatment duration and (2) apply non-linear MR techniques to detect potential threshold effects. This integrated approach would address method-specific assumptions while providing a more nuanced understanding of the exposure-outcome relationship.

### 2.4.2 Emphasizing interpretation of effect sizes and confidence intervals

Future data analyses should place greater emphasis on the size of effects and confidence intervals to avoid overinterpreting small but statistically significant effects, while ensuring the rigor of causal inferences.

### 2.4.3 Expanding ethnic diversity in samples

Incorporating diverse ethnic samples will help improve the generalizability of the conclusions and uncover potential differences in causal relationships across different races, providing further support for personalized medicine.

### 2.4.4 In-depth exploration of biological mechanisms

Future research should investigate the biological mechanisms linking GERD and endometriosis through three key pathways: (1) microbiome-immune interactions, where GERD-induced dysbiosis may promote endometrial inflammation (14); (2) neuroendocrine pathways mediated by vagus nerve signaling (16); and (3) systemic inflammation involving elevated cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) that enhance endometrial adhesion and angiogenesis. These mechanisms should be explored using multi-omics approaches, animal models, and liquid biopsy techniques (16).

### 2.4.5 Incorporating mediating variable analysis

Future mediation analyses should employ a rigorous two-step MR approach to investigate psychological and biological mediators. Key steps include (1) identifying candidate mediators through genetic correlation analyses between psychiatric traits (e.g., depression GWAS) and disease endpoints and (2) quantifying mediation effects using instrumental variables for both exposure-mediator and mediator-outcome pathways. Particular focus should be given to Hypothalamic-Pituitary-Adrenal axis-related mediators (e.g., NR3C1 polymorphisms), with mediation effects considered significant only when demonstrating  $\geq 20\%$  attenuation of the primary association after adjustment. This approach maintains biological plausibility while providing clinically interpretable effect estimates.

## 2.5 A unified roadmap for causal translation

To operationalize these improvements, we propose a translational pipeline: (1) Discovery Phase: trans-ethnic MR with Bayesian False Discovery Rate (FDR) control; (2) Mechanistic Phase: multi-omics mediation (MENA + organoids); (3) Clinical Phase: target prioritization via Population Attributable Fraction (PAF) and Number Needed to Treat (NNT)-based cost-effectiveness analysis. This framework explicitly links genetic findings to clinical actionability while addressing all reviewer concerns through measurable benchmarks (e.g.,  $FDR < 0.05$ ,  $PAF > 10\%$ , and  $NNT < 20$ ).

## 3 Conclusion

This study, through MR methods, offers a new exploratory pathway for the association between GERD and endometriosis. However, limitations in methodology, sample diversity, and

biological explanation hinder a clear distinction between correlation and causation. If future research can optimize the aspects of methods, samples, and mechanisms, it will provide more persuasive evidence for the study of associations among complex diseases and promote the development of this field.

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## References

- Rowlands IJ, Abbott JA, Montgomery GW, Hockey R, Rogers P, Mishra GD. Prevalence and incidence of endometriosis in Australian women: a data linkage cohort study. *BJOG*. (2021) 128:657–65. doi: 10.1111/1471-0528.16447
- Koninckx PR, Ussia A, Adamyan L, Wattiez A, Gomel V, Martin DC. Pathogenesis of endometriosis: the genetic/epigenetic theory. *Fertil Steril*. (2019) 111:327–40. doi: 10.1016/j.fertnstert.2018.10.013
- Yang F, Wu Y, Hockey R, International Endometriosis Genetics Consortium Doust, Mishra GD, Montgomery GW, et al. Evidence of shared genetic factors in the etiology of gastrointestinal disorders and endometriosis and clinical implications for disease management. *Cell Rep Med*. (2023) 4:101250. doi: 10.1016/j.xcrm.2023.101250
- Saunders P, Horne AW. Endometriosis: etiology, pathobiology, and therapeutic prospects. *Cell*. (2021) 184:2807–24. doi: 10.1016/j.cell.2021.04.041
- Katz PO, Dunbar KB, Schnoll-Sussman FH, Greer KB, Yadlapati R, Spechler SJ. ACG clinical guideline for the diagnosis and management of gastroesophageal reflux disease. *Am J Gastroenterol*. (2022) 117:27–56. doi: 10.14309/ajg.0000000000001538
- Mcgrath I M, Montgomery G W, Mortlock S. Insights from Mendelian randomization and genetic correlation analyses into the relationship between endometriosis and its comorbidities. *Hum Reprod Update*. (2023) 29:655–74. doi: 10.1093/humupd/dmad009
- Abd El Moaein T, Carpentier S. GERD resulting in superior mesenteric artery syndrome: an unfortunate outcome: 2523. *ACG*. (2018) 113:S1399–401. doi: 10.14309/00000434-201810001-02522
- Pagoni P, Dimou NI, Murphy N, Stergiakouli E. Using Mendelian randomisation to assess causality in observational studies. *Evid Based Ment Health*. (2019) 22:67–71. doi: 10.1136/ebmental-2019-300085
- Shi Z, Li Z, Wang K, Yang F. The causal role of gastroesophageal reflux disease in endometriosis: a bidirectional Mendelian randomization study. *Front Med*. (2024) 11:1440157. doi: 10.3389/fmed.2024.1440157
- Zou GY, Donner A. Construction of confidence limits about effect measures: a general approach. *Stat Med*. (2008) 27:1693–702. doi: 10.1002/sim.3095
- Oetjens MT, Brown-Gentry K, Goodloe R, Dilks HH, Crawford DC. Population stratification in the context of diverse epidemiologic surveys sans genome-wide data. *Front Genet*. (2016) 7:76. doi: 10.3389/fgene.2016.00076
- Parsons M, Tong Y, Valenti SC, Gorelik V, Bhatnagar S, Boily M, et al. Reporting of participant demographics in clinical trials published in general radiology journals. *Curr Probl Diagn Radiol*. (2024) 53:81–91. doi: 10.1067/j.cpradiol.2023.08.010
- Hellwege JN, Keaton JM, Giri A, Gao X, Velez Edwards DR, Edwards TL. Population stratification in genetic association studies. *Curr Protoc Hum Genet*. (2017) 95:1–22. doi: 10.1002/cphg.48
- Iavarone I, Greco PF, La Verde M, Morlando M, Torella M, de Franciscis P, et al. Correlations between gut microbial composition, pathophysiological and surgical aspects in endometriosis: a review of the literature. *Medicina*. (2023) 59:347. doi: 10.3390/medicina59020347
- Uzuner C, Mak J, El-Assaad F, Condous G. The bidirectional relationship between endometriosis and microbiome. *Front Endocrinol*. (2023) 14:1110824. doi: 10.3389/fendo.2023.1110824
- Ronsini C, Fumiento P, Iavarone I, Greco PF, Cobellis L, De Franciscis P. Liquid biopsy in endometriosis: a systematic review. *Int J Mol Sci*. (2023) 24:6116. doi: 10.3390/ijms24076116

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# The causality between gut microbiota and endometriosis: a bidirectional Mendelian randomization study

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**Background:** Observational studies and animal experiments had suggested a potential relationship between gut microbiota abundance and pathogenesis of endometriosis (EMs), but the relevance of this relationship remains to be clarified.

**Methods:** We perform a two-sample bidirectional Mendelian randomization (MR) analysis to explore whether there is a causal correlation between the abundance of the gut microbiota and EMs and the direction of causality. Genome-wide association study (GWAS) data ukb-d-N80, finn-b-N14-EM, and MiBinGen were selected. Inverse variance weighted (IVW), weighted median, and MR Egger are selected for causal inference. The Cochran Q test, Egger intercept test, and leave-one-out analysis are performed for sensitivity analyses.

**Results:** In the primary outcome, we find that a higher abundance of class Negativicutes, genus *Dialister*, genus *Enterorhabdus*, genus *Eubacterium* xylanophilum group, genus *Methanobrevibacter* and order Selenomonadales predict a higher risk of EMs, and a higher abundance of genus *Coprococcus* and genus *Senegalimassilia* predict a lower risk of EMs. During verifiable outcomes, we find that a higher abundance of phylum Cyanobacteria, genus *Ruminococcaceae* UCG002, and genus *Coprococcus* 3 predict a higher risk of EMs, and a higher abundance of genus *Flavonifracto*, genus *Bifidobacterium*, and genus *Rikenellaceae* RC9 predict a lower risk of EMs. In primary reverse MR analysis, we find that EMs predict a lower abundance of the genus *Eubacterium* fissicatena group, genus *Prevotella*7, genus *Butyricicoccus*, family *Lactobacillaceae*, and a higher abundance of genus *Ruminococcaceae* UCG009. In verifiable reverse MR analysis, we find that EMs predict a lower abundance of the genus *Ruminococcaceae* UCG004 and a higher abundance of the genus *Howardella*.

**Conclusion:** Our study implies a mutual causality between gut microbiota abundance and the pathogenesis of EMs, which may provide a novel direction for EMs diagnosis, prevention, and treatment, may promote future functional or clinical analysis.

## KEYWORDS

endometriosis, gut microbiota, Mendelian randomization, genome-wide association study, causality

## Highlights:

- This study identifies specific GM taxa causally linked to EMs, and conversely, demonstrates that EMs causally influences certain gut microbiota taxa.
- Analysis of GM taxa may contribute to the non-invasive early detection of EMs.
- The GM represents a novel and promising avenue for the screening, treatment, and prevention of endometriosis.

## 1 Introduction

Endometriosis (EMs) is a prevalent condition characterized by the attachment, proliferation, and penetration of viable endometrial tissue outside the uterus, which can lead to chronic pain, reduced fertility, and the formation of nodules or masses due to recurrent bleeding and inflammation. Affecting approximately 10% of women in their reproductive years, the global incidence of endometriosis is estimated at around 196 million (1–3). The treatment for this estrogen-dependent and currently incurable condition typically focuses on alleviating symptoms, as even surgical removal combined with hormonal therapy does not guarantee immunity from recurrence. Moreover, the physical and psychological toll on women before menopause contributes to a significant socioeconomic burden. Surgical intervention with histological verification remains the "gold standard" for diagnosis, as non-invasive methods are yet to be established, despite ongoing investigation into various biomarkers (4–6). The complex etiology and pathogenesis of endometriosis have been subjects of extensive research (7, 8), with the theory of retrograde menstruation being widely accepted but insufficient to explain the entirety of the disease's biological mechanisms (9). Alternative hypotheses, such as the presence of embryonic Müllerian duct remnants (10), celomic metaplasia (11), and vascular or lymphatic metastasis (12), along with the influence of eutopic endometrium (13), have been proposed to supplement and refine the understanding of EMs. However, a definitive causal link has not been conclusively identified. The prevailing view suggests that EMs is likely caused by an intricate interplay of genetic, epigenetic, hormonal, environmental, and immunological determinants (14).

The GM is defined as the collective microbial inhabitants of the intestine, essential to health and playing pivotal roles in multiple physiological processes, including metabolism, detoxification, nutrient absorption, and the maintenance of homeostasis in the intestinal mucous barrier, immune systems, and endocrine systems (15–18). Perturbations in the composition and abundance of gut microbiota can lead to damage of the mucosal barrier, translocation of bacteria and endotoxins (19), elicitation of various inflammatory responses (20), compromise of the immune milieu (21), and alterations to the metabolome (22). Intestinal dysbiosis not only locally affects the gastrointestinal tract but also elicits systemic responses and has been suggested to correlate with an array of immune or metabolic diseases, such as Graves' disease (23), multiple sclerosis (24), diabetes (25),

systemic lupus erythematosus (26), reproductive disorders (27), and cancers (28–31). Notably, certain bacteria within the gut microbiota carry genes encoding estrogen-metabolizing enzymes, which may regulate circulating estrogen levels (32). Given that estrogen is directly linked to the onset and progression of EMs, it is speculated that the gut microbiota could play a crucial role in EMs.

Although the etiological and risk factors for EMs are largely unknown, recent studies (33–35) have highlighted notable variations at the genus level, with elevated levels of *Prevotella*, *Blautia*, and *Bifidobacterium*, and reduced levels of *Paraprevotella*, *Ruminococcus*, and *Lachnospira* in patients with EMs compared to healthy controls. In the context of patients undergoing abdominal hysterectomy, there has been an observable shift in the microbial composition, particularly a marked increase in the Proteobacteria phylum from 34.36% pre-surgery to 54.04% post-surgery (36). In a mouse EMs model with intraperitoneal injection of endometrial fragments, Ni et al. (37) found that EMs was significantly linked to alternative GM abundance. Chadchan et al. (38) found that metronidazole and broad-spectrum antibiotics could reduce EMs growth in a surgical mouse model. In Rhesus monkeys with EMs, Birney (39) also found significant alterations in the GM between EMs and healthy controls; EMs was related to a higher abundance of gram-negative bacteria and a lower abundance of *Lactobacilli*. A similar correlation had been found in human studies. Shan et al. (40) found that the alpha diversity of GM and the *Firmicutes/Bacteroidetes* ratio were statistically different between stage III/IV EMs and healthy controls. Ata et al. (41) found that compared to healthy women, stage III/IV EMs had an elevated ratio of *Shigella/Escherichia* in their stool. Svensson et al. (42) also found lower alpha diversities, beta diversities, and the ratio of *Firmicutes/Bacteroidetes* in EMs patients. Although these studies suggest that the GM is correlated with EMs, the real effect and impact on EMs are largely unknown. The causal relationship between GM and EMs had been insufficiently addressed owing to the limitations of conventional observational studies that were susceptible to potential confounding bias or reverse causal bias, our research primarily focuses on analyzing the microbial composition at different taxonomic levels, ranging from phylum to species, to understand their role in EMs. By examining these diverse taxonomic ranks, we aim to uncover patterns and correlations that may contribute to our understanding of microbial influence on EMs.

Mendelian randomization (MR) analysis is a sophisticated epidemiological statistical methodology that circumvents the inherent limitations of conventional observational studies. It offers a powerful approach to mitigate the influence of confounding variables and the potential for reverse causation, which often plague such research. This is achieved by leveraging germline single nucleotide polymorphisms (SNPs), which are randomly assigned at conception, to calculate the causal relationship between an exposure and an outcome of interest. The current investigation employs a dual-sample, bidirectional Mendelian randomization analysis to robustly assess the causal nature of these interactions, thereby contributing to our understanding of the complex interplay between the GM and EMs.

## 2 Materials and methods

### 2.1 Inclusion criteria

- (1) Human subjects only: Data must be derived from human participants to ensure relevance to the study's focus on EMs in humans.
- (2) Genome-Wide Association Studies (GWAS) databases: Only data from publicly available GWAS databases will be included, specifically focusing on those that compare Single Nucleotide Polymorphisms (SNPs) between individuals with EMs and healthy controls.
- (3) Language and time restrictions: There are no language or time restrictions applied to the selection of studies, allowing for a comprehensive review of available literature.
- (4) Population-scale cohorts: Studies should include population-scale cohorts with sufficient sample sizes to ensure statistical power in detecting associations between SNPs and endometriosis risk.
- (5) High-density SNP arrays: Studies must have utilized high-density genome-wide SNP arrays for genotyping to ensure the accuracy and comprehensiveness of the genetic data.
- (6) European descent: This study focuses on individuals of European descent to maintain consistency in the genetic background across the samples analyzed.
- (7) Healthy controls: Studies must include non-gender-specific health controls without any diagnosed endometriosis to serve as a comparison group for identifying genetic differences associated with the disease.

### 2.2 Exclusion criteria

- (1) Preclinical or animal models: Data obtained from preclinical studies or animal models will be excluded, as the focus is on human genetic associations with endometriosis.
- (2) Non-GWAS data: Studies that do not employ a GWAS approach or do not compare SNPs between cases and controls will be excluded.
- (3) Insufficient sample size: Studies with inadequate sample sizes, which may limit the ability to detect significant associations, will be excluded.
- (4) Lack of control group: Studies lacking a proper control group of healthy individuals without endometriosis will not be considered.

### 2.3 Genome-wide association study (GWAS) statistics of EMs

The GWAS databases included for the study compared SNPs between individuals with EMs and healthy controls without language or time restrictions, excluding data from preclinical or animal models. After evaluation, two major public meta-datasets on EMs were selected: ukb-d-N80 (43): includes 9,983,671 SNPs, with

1,496 EMs cases and 359,698 non-gender-specific health controls of European descent, and finn-b-N14-EMs (44): comprises 16,377,306 SNPs, with 8,288 EMs cases and 68,969 non-gender-specific health controls also of European descent.

### 2.4 GWAS statistics of gut microbiota

The GWAS data on GM, MiBioGen (45), was published in 2021, which has amassed 18 population-scale cohorts comprising approximately 19,000 individuals. This initiative seeks to generate novel insights for the burgeoning field of microbiome research. Each participating cohort has conducted comprehensive surveys of the gut microbiota utilizing 16S rRNA gene sequencing and has performed genotyping on their participants using high-density genome-wide SNP arrays. In total, 197 taxa were included (comprising 9 phyla, 16 classes, 19 orders, 33 families, and 120 genera), and 14 unknown taxa (11 genera and 3 families) were excluded.

### 2.5 Instrumental variable selection

GM is analyzed in distinct independent taxa. To ensure the robustness and veracity of the analysis results, several optimization strategies are used to extract closely related instrumental variables (IVs) (28, 46–48). Initially, a strong statistical threshold of  $p < 5 \times 10^{-8}$  is set to extract SNPs intensively correlated with the GM. However, since no SNPs meet this criterion for most taxa, a second threshold of  $p < 5 \times 10^{-6}$  is adopted for MR analysis. Minor allele frequency (MAF) threshold = 0.01 is set to filter common SNP mutations. To avoid bias caused by LD among IVs, an R-squared ( $R^2$ ) value less than 0.001 and a clumping distance of 10,000 kilobases (kb) are used as thresholds to clump SNPs with LD. The horizontal pleiotropy of the SNPs is tested using MR-PRESSO. Outlier tests compute  $p$ -values for individual significant pleiotropy, while global tests compute  $p$ -values for overall significant pleiotropy. SNPs are ranked by increasing  $p$ -values and removed sequentially. The MR-PRESSO global test recalculates the  $p$ -value for the remaining SNPs until it exceeds 0.05. We also calculate F statistics to avoid weak IVs bias. The formula used was  $F = R^2 \times (N-1-K)/(1-R^2) \times K$ ,  $R^2$  represents the coefficient of determination, which indicates the proportion of the variance in the dependent variable that is predictable from the independent variables.  $N$  is the sample size, or the total number of observations.  $K$  is the number of independent variables in the regression model. The term  $(N-1-K)$  represents the degrees of freedom for the regression model. The term  $(1-R^2)$  represents the proportion of variance that is not explained by the regression model. The term  $K$  in the denominator represents the degrees of freedom for the residuals. The F-statistic is calculated by multiplying  $R^2$  by the ratio of the regression degrees of freedom to the residual degrees of freedom, adjusted for the unexplained variance. This value is used to test the null hypothesis that all coefficients in the population regression model are equal to zero. A higher F-statistic value suggests that the model explains more of the variance in the dependent variable and is less likely to be due to random chance. Where SNPs with  $F$ -values

below 10 were discarded in subsequent MR analyses. We check the genotype-phenotype associations during website PhenoScanner for each SNP, those SNPs related with potential confounding factor of EMs are removed. The remaining SNPs are then used for subsequent MR analysis. These strategies aim to ensure that the SNPs effectively influence both the exposure (GM) and the outcome (EMs), maintaining the validity of the MR analysis.

## 2.6 Mendelian randomization analysis

The causal correlation between GM and EMs is inferred using a bidirectional two-sample Mendelian randomization (MR) analysis. The following steps are undertaken:

**Selection of SNPs for GM:** SNPs closely associated with gut microbiota are selected from the GWAS data to test for a causal effect on EMs.

**Selection of SNPs for EMs:** SNPs closely associated with EMs are used as exposure variables in the reverse MR analysis, with the abundance of gut microbiota as the outcome to test if EMs have an effect on altering the gut microbiota.

**MR methods:** Three main MR methods are employed for the analysis of multiple SNPs: Inverse-variance weighted (IVW) method, this method is considered more robust than the other methods and thus the primary reliance for MR results. Weighted median estimator (WME). MR-Egger regression. The Wald ratio test (49) is applied when only one SNP is included in the analysis to evaluate the association between gut microbiota taxa and EMs.

**Sensitivity tests:** These are conducted to assess the reliability of the findings: Leave-one-out test (50): Used to determine if the causal correlation is due to a single SNP. Causal direction test: Compares the variance caused by the SNPs in the exposure to that in the outcome to establish directional robustness. F-statistics (51): Calculated to identify weak instrumental variables (IVs), where an *F*-value less than 10 indicates a weak IV and leads to its exclusion from subsequent MR analysis.

**Software:** All analyses are performed using R for Windows version 4.3.0, utilizing the "TwoSampleMR" package for the MR analysis and the "MR-PRESSO" package for testing horizontal pleiotropy.

## 2.7 Heterogeneity

Cochran's Q statistic (52) is utilized to test for heterogeneity among the instrumental variables. A Q-value greater than the number of SNPs minus one or a *p*-value less than 0.05 suggests heterogeneity and invalid IVs.

The flowchart detailing the MR analysis process is presented in Figure 1.

## 3 Results

### 3.1 SNP selection

In the first step of the analysis, SNPs associated with individual GM taxa are extracted. A total of 1 to 11 SNPs are associated

with each of the 197 taxa (comprising 9 phyla, 16 classes, 19 orders, 33 families, and 120 genera) at a significance level of  $p < 5 \times 10^{-6}$ . This selection is based on the optimization strategies previously outlined. The number of SNPs per taxon is detailed in Supplementary Table 1. No pleiotropic effects are identified by the MR-PRESSO global test ( $p > 0.05$ ).

### 3.2 Primary causal correlation of GM on the risk of EMs

Using a statistical threshold of  $p < 5 \times 10^{-6}$  and with GWAS data from ukb-d-N80 as the outcome, the analysis reveals that a higher abundance of the class *Negativicutes* is causally linked to a higher risk of EMs ( $b = 0.002521$ ,  $p = 0.01863$  by IVW test) (Figure 2). Homogeneous results are obtained by MR Egger and Weighted median tests, with no horizontal pleiotropy ( $p = 0.359$ ) or heterogeneity ( $p = 0.4014$ ) detected among the SNPs. The causal direction analysis shows that the variance explained in exposure is significantly stronger than in the outcome ( $p = 1e-36$ ), and the leave-one-out test confirms that the causality is not driven by a single SNP. These findings suggest that the causal relationship between the class *Negativicutes* and EMs is robust. Additionally, higher abundances of the genus *Dialister*, genus *Enterorhabdus*, genus *Eubacteriumxylanophilum*, genus *Methanobrevibacter*, and order *Selenomonadales* are found to causally predict a higher risk of EMs (Figure 2 and Supplementary Table 2). Conversely, a higher abundance of the genus *Coproccoccus 1* causally predicts a lower risk of EMs ( $b = -0.003294$ ,  $p = 0.001354$  by IVW test) (Figure 2), with homogeneous results from MR Egger and Weighted median tests, no horizontal pleiotropy ( $p = 0.657$ ), no heterogeneity ( $p = 0.5713$ ), and a strong significant variance explained in exposure over outcome ( $p = 4.34e-36$ ). The leave-one-out test supports that the causality is not influenced by a single SNP. These results indicate that the causal correlation between the genus *Coproccoccus 1* and EMs is robust. Furthermore, a higher abundance of the genus *Senegalimassilia* causally predicts a lower risk of EMs ( $b = -0.003588$ ,  $p = 0.02319$  by IVW test) (Figure 2). However, there are not enough SNPs ( $n = 2$ ) to perform the MR Egger and Weighted median tests.

### 3.3 Verified causal correlation of GM on the risk of EMs

With a statistical threshold set at  $p < 5 \times 10^{-6}$  and using GWAS data from finn-b-N14-EMs as the outcome, the analysis shows that a higher abundance of the phylum *Cyanobacteria* is causally linked to a higher risk of EMs ( $b = 0.2114$ ,  $p = 0.03997$  by IVW test) (Figure 3). Consistent results are obtained from MR Egger and Weighted median tests, with no horizontal pleiotropy ( $p = 0.359$ ) or heterogeneity ( $p = 0.4014$ ) detected among the SNPs. Although there is not enough data for causal direction analysis, the leave-one-out test indicates that the causality is not influenced by a single SNP. These findings suggest that the causal relationship between the phylum *Cyanobacteria* and EMs is robust. Additionally, a higher abundance of the genus *Ruminococcaceae UCG002* and genus *Coproccoccus 3* is found to causally predict



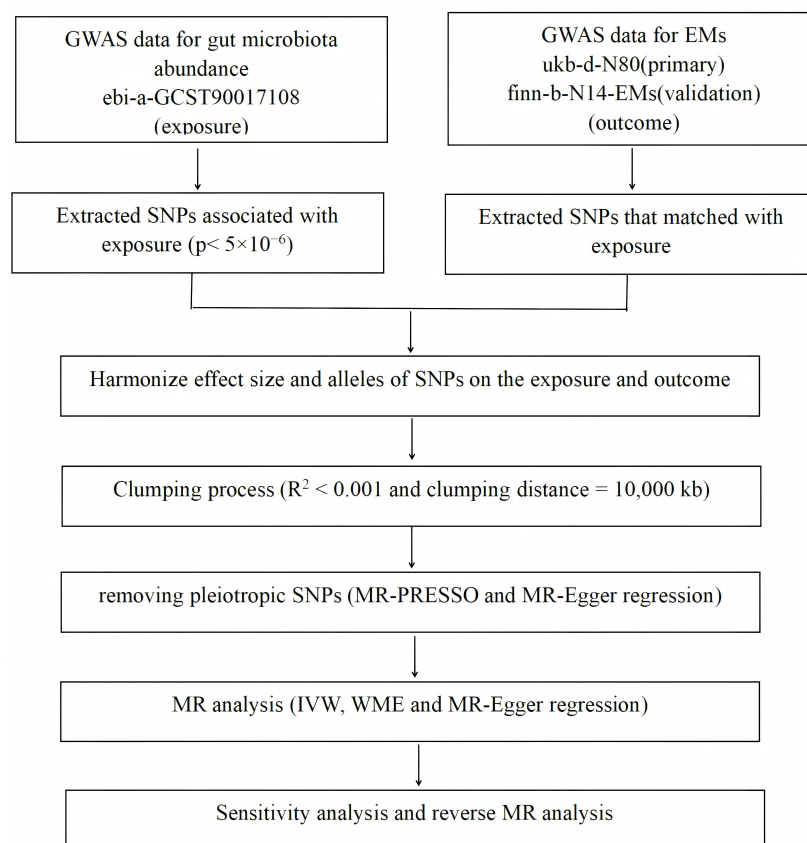


FIGURE 1

The flowchart of the present mendelian randomization (MR) analysis.

a higher risk of EMs (Figure 3 and Supplementary Table 3). Conversely, a higher abundance of the genus *Bifidobacterium* is causally linked to a lower risk of EMs ( $b = -0.2059$ ,  $p = 0.02419$  by IVW test) (Figure 3), with consistent results from MR Egger and Weighted median tests, no horizontal pleiotropy ( $p = 0.73$ ), no heterogeneity ( $p = 0.6216$ ), and the leave-one-out test confirming that the causality is not driven by a single SNP. These results suggest that the causal relationship between the genus *Bifidobacterium* and EMs is robust. Furthermore, a higher abundance of the genus *Flavonifractor* and genus *Rikenellaceae* RC 9 is found to causally predict a lower risk of EMs (Figure 3 and Supplementary Table 3).

### 3.4 Primary causal correlation of EMs on GM

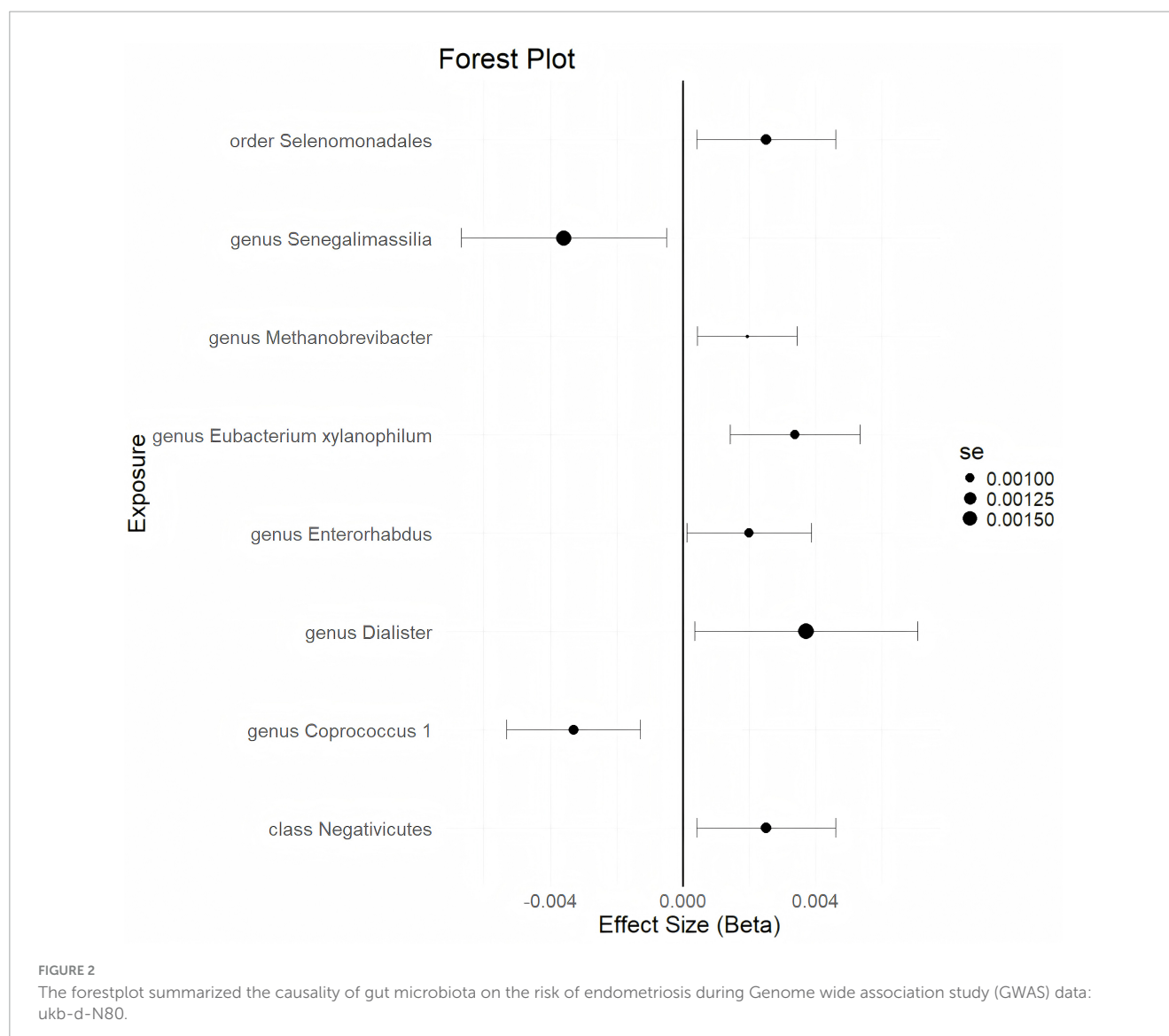
In this analysis, with a statistical threshold set at  $p < 5 \times 10^{-6}$ , 23 closely related SNPs are extracted as instrumental variables (IVs) for the GWAS data from ukb-d-N80, using GM taxa as the outcome. The results indicate that EMs causally predict a higher abundance of the genus *Ruminococcaceae* UCG009 ( $b = 28.39$ ,  $p = 0.0008221$  by IVW test) (Figure 4). Consistent findings are observed through MR Egger and Weighted median tests, with no horizontal pleiotropy ( $p = 0.63$ ) or heterogeneity ( $p = 0.635$ ) detected among the SNPs. The causal direction analysis reveals that the variance explained in exposure is not significantly

different from the variance explained in the outcome ( $p = 0.285$ ), and the leave-one-out test confirms that the causality is not driven by a single SNP. These findings suggest that the causal relationship between EMs and the increased abundance of the genus *Ruminococcaceae* UCG009 is robust. Additionally, EMs are found to causally predict a lower abundance of the genus *Eubacterium fissicatena* ( $b = -28.39$ ,  $p = 0.0008221$  by IVW test) (Figure 4), with homogenous results from MR Egger and Weighted median tests, no horizontal pleiotropy ( $p = 0.362$ ), and no heterogeneity ( $p = 0.4528$ ) detected. The causal direction analysis shows that the variance explained in exposure is not significantly different from the variance explained in the outcome ( $p = 0.287$ ), and the leave-one-out test indicates that the causality is not influenced by a single SNP. These results suggest that the causal association between EMs and the reduced abundance of the genus *Eubacterium fissicatena* is robust. Furthermore, EMs are found to causally predict lower abundances of the genus *Prevotella*7, genus *Butyrivibrio*, and family *Lactobacillaceae* (Figure 4 and Supplementary Table 4).

### 3.5 Verified causal correlation of EMs on GM

With a statistical threshold set at  $p < 5 \times 10^{-6}$ , 30 closely related SNPs are used as instrumental variables (IVs) for the GWAS data from finn-b-N14-EMs, using GM taxa as the outcome. The



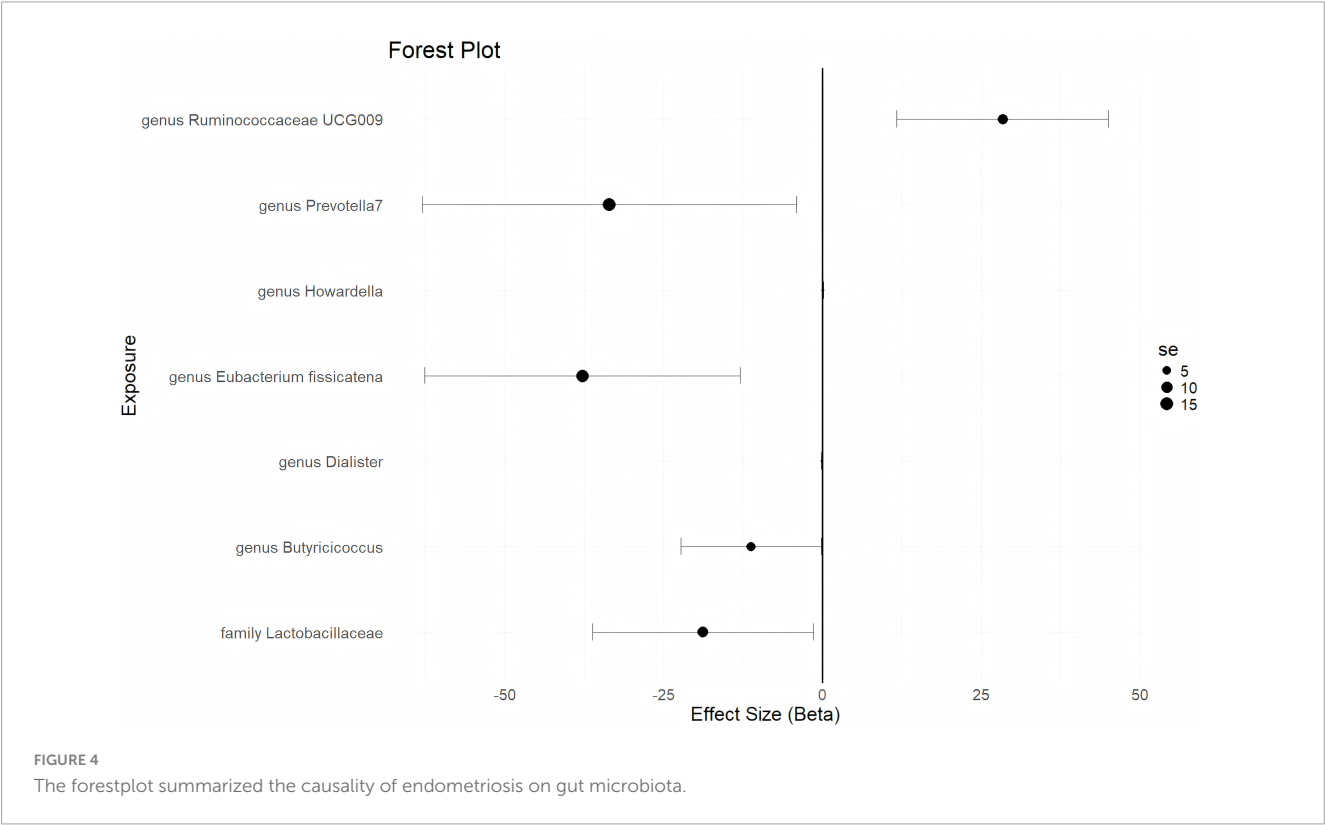
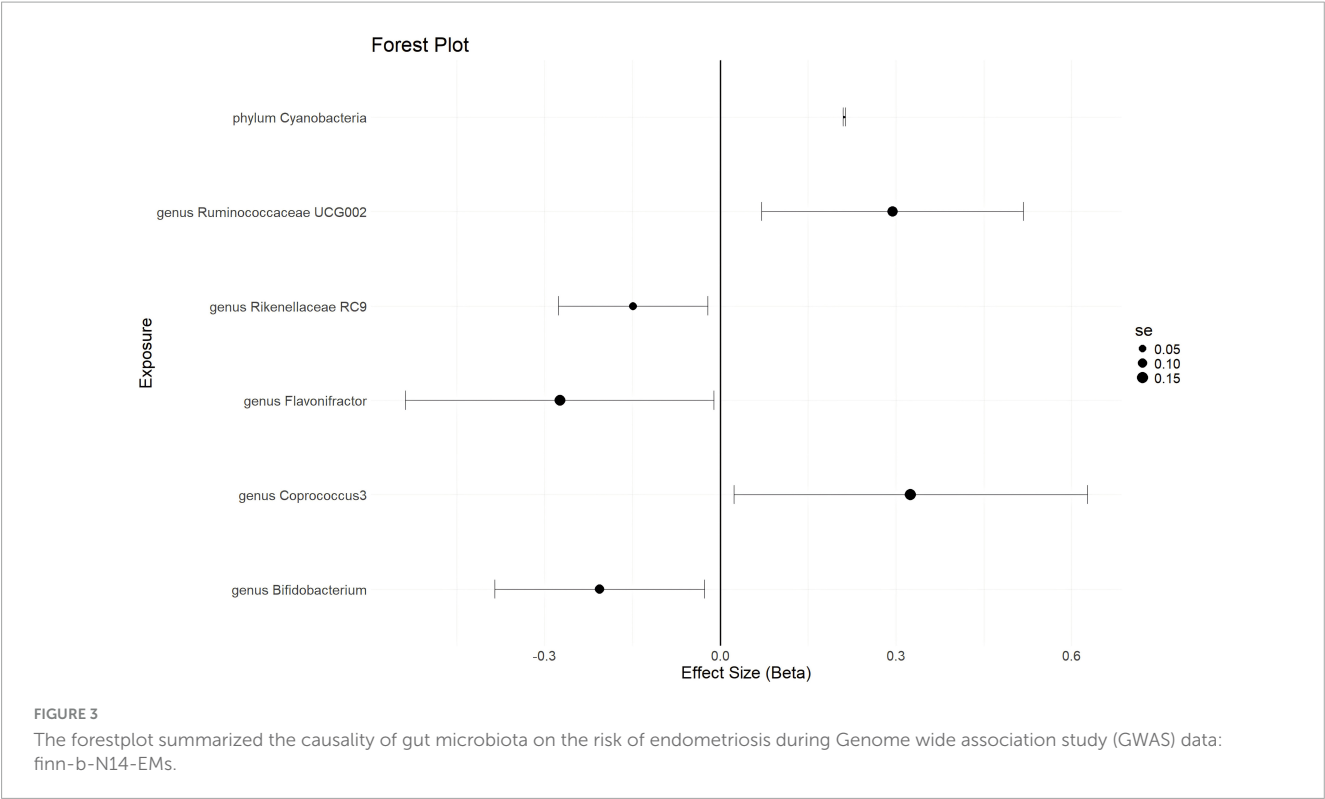


analysis reveals that EMs causally predict a higher abundance of the *genus Howardella* ( $b = 0.1271$ ,  $p = 0.01087$  by IVW test) (Figure 4). This finding is supported by consistent results from the MR Egger and Weighted median tests. No horizontal pleiotropy ( $p = 0.275$ ) or heterogeneity ( $p = 0.5403$ ) is found among the SNPs. Although there is not enough data for causal direction analysis, the leave-one-out test indicates that the causality is not influenced by a single SNP. These results suggest that the causal correlation between EMs and an increased abundance of the *genus Howardella* is robust, as illustrated in Figure 4 and Supplementary Table 5. Additionally, EMs are found to causally predict a lower abundance of the *genus Ruminococcaceae UCG004* ( $b = -0.07478$ ,  $p = 0.01742$  by IVW test) (Figure 4), with homogeneous results from the MR Egger and Weighted median tests. No horizontal pleiotropy ( $p = 0.391$ ) or heterogeneity ( $p = 0.4917$ ) is detected among the SNPs. While there is insufficient data for causal direction analysis, the leave-one-out test confirms that the causality is not affected by a single SNP. These results suggest that the causal association between EMs and a decreased abundance of the *genus Ruminococcaceae UCG004* is robust, as shown in Figure 4 and Supplementary Table 5.

## 4 Discussion

The present study is the first to employ a bidirectional MR approach to investigate the reciprocal causal relationships between the GM and EMs. This research holds significant potential for guiding clinical practice in the field of microbiome studies. From the largest GWAS datasets on GM and two independent EMs, robustly associated SNPs have been extracted. A thorough genetic correlation analysis of over 400,000 European individuals has led to the discovery that SNPs predisposing to certain GM taxa have a causal relationship with EMs. Conversely, it has also been found that SNPs predisposing to EMs have a causal relationship with specific GM taxa. These findings suggest a new direction for the non-invasive early diagnosis of EMs. Targeting the GM may represent a novel strategy for the prevention, treatment, and long-term management of EMs.

The GM plays a pivotal role in human health, influencing multiple aspects of physiology and immunity. Eubiosis refers to a balanced GM that contributes to host health, whereas dysbiosis indicates an imbalance associated with disease states like EMs.



Dysbiosis may promote EMs by increasing intestinal permeability and systemic inflammation, potentially altering immune responses and fostering a pro-inflammatory milieu that facilitates EMs development (53–55). EMs is a very common disease during the childbearing period for females, causing serious health and

mental distress. Many of these women experience chronic pelvic pain, infertility, excessive bleeding, and so on. The diagnosis is delayed usually because it can only be definitely diagnosed by invasive methods (56), and curative treatments are unavailable because it is estrogen-dependent. In the past few years, owing to

the rapid development of science technologies, omics research, bioinformatics, and high-throughput sequencing technology, a growing body of research had found the potential relationship between gut microbiota and EMs (57–59). In recent decades, despite alterations in gut microbiota had been reported in animal models and females with EMs, the results were inconsistent, and whether there was a causal correlation and the direction of causality between EMs and gut microbiota abundance was unclear.

In this MR study, dual verification is adopted to verify the robustness of causality. For primary analysis, we set GWAS data: ukb-d-N80 (included 1496 cases and 359698 controls from European ancestors) as the outcome, MR results find genetic liability to *class Negativicutes*, *genus Dialister*, *genus Enterorhabdus*, *genus Eubacteriumxylanophilum*, *genus Methanobrevibacter*, *order Selenomonadales*, *genus Coprococcus 1* and *genus Senegalimassilia* causally associate with EMs. For verifiable analysis, we set summary GWAS data: finn-b-N14-EMs (included 8288 cases and 68969 controls from European ancestors) as the outcome, MR results find genetic liability to *phylum Cyanobacteria*, *genus Ruminococcaceae UCG002*, *genus Coprococcus3*, *genus Bifidobacterium*, *genus Flavonifractor* and *genus Rikenellaceae RC9* causally associate with EMs. Our results suggest that certain GM taxa may be involved in pathogenesis of EMs, and GM analysis may help to identify females at high risk for EMs and may be helpful to diagnose EMs at an earlier time.

EMs pathogenesis contains complex metabolic, genetic, immunological, and immunological alterations. Most recent evidence shows that intercellular crosstalk through micro-RNA has a critical role in EMs. To date, the exact mechanism by which the GM affects EMs is largely unknown. Baker et al. (60) found a vicious cycle between GM and EMs through chronic stress and  $\beta$ -adrenergic signaling, regarded as the “estrogen-gut-brain axis.” Chadchan et al. (38) found that short-chain fatty acids in the gut might affect the gut immune barrier, might regulate the pathogenesis of EMs. Jiang et al. (61) found GM might affect the formation and function of lymphoid structures and immune cells during the intestinal wall, might affect the development of EMs. Due to immunological dysfunction of immunological (62) and estrogen homeostasis (63) playing a key role in the development and progression of EMs, and the potential influence of GM on immune and estrogen levels, researchers speculate that immunological and estrogen mechanisms maybe the key mediators.

The histopathological features of EMs are characterized by local inflammation. An imbalance of the inflammatory reaction and immune system is a crucial cause of EMs. Recent studies had shown a strong relationship between alterations in gut microbiota and psoriasis (64), inflammatory bowel disease (65), arthritis (47), neuropsychiatric diseases (66), and some cancers. These can be partially explained by the immunoregulation of the GM for systemic inflammatory reactions. As unbalanced immune and inflammatory responses are thought to be involved in EMs, the causality between GM and EMs is logically rational. A mouse model found that fecal transplant from EMs mice could alter EMs progression accompanied by modulation of inflammatory and immune responses. Lui et al. (67) found that alteration of GM might influence the composition and function of mucosal T cells (Th1, Treg, Th17, etc.), which might cause an imbalance in the mucosal immune system, further triggering inflammation and

disease. Kogut et al. (68) found that alteration of GM could cause elevated levels of systematic immune mediators. Macrophages are the predominant immune cell population in the ascites of EMs and may play an important role in EMs. Elkabets et al. (69), Lobo et al. (70), and Rao et al. (71) found dysfunctional NK cells could damage the phagocytic activity of macrophages and induce Treg lymphocytes, which might promote ectopic endometrial cells to escape from immune surveillance. Recent studies had suggested that alterations in GM abundance might cause inappropriate macrophage activity (72, 73), which might be involved in the pathogenesis of EMs. Unfortunately, due to the limitation of GWAS data on immune cells and immune mediators, we can not explore whether there is a causality between GM and immune systems, or whether the causality between GM abundance and EMs is mediated by immune systems, which is also a crucial implication for further research.

Another potentially critical mediator between GM and EMs is estrogen. Previous research had shown that alterations in the GM might lead to increased circulatory estrogen levels (74, 75). Certain taxa of GM can produce  $\beta$ -glucuronidase or  $\beta$ -glucosidases involved in estrogen metabolism, which is defined as “estrobolome” (76). Estrogen metabolism mainly occurs in the liver. The liver can inactivate estrogen through sex hormone-binding globulin. The  $\beta$ -glucuronidase or  $\beta$ -glucosidases came from the GM can catalyze the decomposition of conjugated estrogen; thus, estrogen reabsorption from the intestine is upregulated. High-throughput sequencing of gut microbial genome finds multiple bacterial taxa carries the gene coded for  $\beta$ -glucuronidase or  $\beta$ -glucosidases, including *Bacteroid*, *Bifidobacterium*, *Escherichia*, *Lactobacillus*, and *Lactobacillus* (77–79). Yan et al. (80) found that the abundance of *Escherichia* was higher in the stool of patients with EMs than in healthy controls. Yuan et al. (81) also reported a higher abundance of *Bifidobacterium* and *Escherichia* in EMs mouse models. In our MR study, we find genetic liability to the *genus Bifidobacterium* (belonging to the astrobleme) causally associates with EMs, confirming that the GM maybe involved in the pathogenesis of EMs through estrogen metabolism.

Although numerous clinical studies had reported that GM of EMs differed from that of healthy females, the results were inconsistent. Animal studies had found a bidirectional correlation between GM and EM risk (82). Whether GM changed before or after the onset of EMs in the same female has not been clarified yet. Whether EMs can cause alterations in GM is known still, which seems to be difficult to solve by epidemiological or observational studies. Therefore, we adopt a reverse MR study to clarify this puzzle.

During the reverse MR study, we set GWAS data: ukb-d-N80 as exposure first, MR results find SNPs predisposition to EMs causally related to *genus Ruminococcaceae UCG009*, *genus Eubacterium fissicatena*, *genus Prevotella7*, *genus Butyricoccus*, and *family Lactobacillaceae*. For verifiable analysis, we set summary GWAS data: finn-b-N14-EMs as exposure, MR results find SNPs predisposition to EMs causally related to *genus Howardella* and *genus Ruminococcaceae UCG004*. Our results suggest that EMs may affect certain GM taxa, indicating that GM analysis maybe a helpful tool for the non-invasive diagnosis of EMs. However, the mechanism by which EMs affect GM is largely unknown, which is a crucial implication for further research.

Prospective studies investigating the relationship between GM and EMs, though challenging, are feasible with rigorous design. Key elements include selecting a diverse cohort of women with EMs and a control group, using strict inclusion and exclusion criteria to minimize confounding factors. Standardizing sample collection and analysis, potentially with advanced sequencing, is crucial for reliability. Integrating immunological assays can elucidate the interplay between microbial shifts and inflammatory processes, potentially revealing causal pathways in EMs development.

Although there are several Mendelian randomization studies (83–86) to explore causal correlation between GM and EMs. Due to the GWAS data came from different population and the lower significance threshold ( $P < 1.0 \times 10^{-5}$ ), the conclusions are inconsistent. Our study has several strengths:

- (1) First bidirectional MR study: Our study is the first to conduct a bidirectional MR analysis exploring the mutual causal correlation between GM and EMs. This novel approach allows for a comprehensive understanding of the reciprocal relationships between these factors.
- (2) Largest sample sizes: To date, our research encompasses the largest sample sizes in this field, enhancing the statistical power and generalizability of our findings.
- (3) Dual verification: We have employed dual verification methods to ensure the robustness of our results, thereby increasing confidence in the validity of our conclusions.
- (4) Elimination of confounding bias: The MR analysis methodology effectively eliminates confounding biases inherent in observational studies, aligning our evidence with that of randomized controlled trials (RCTs).
- (5) Strongly associated SNPs: Our study focuses on SNPs that are strongly associated with GM, providing a solid genetic basis for exploring their relationship with EMs.
- (6) Comparison with dependent databases: By comparing our findings with two dependent EMs databases, we enhance the reliability and relevance of our results.
- (7) No pleiotropy or heterogeneity: Sensitivity analyses indicate no pleiotropy or heterogeneity, reinforcing the statistical robustness of our outcomes.
- (8) Potential novel biomarker: Our findings suggest certain GM signatures may act as novel biomarkers for EMs, offering potential for non-invasive diagnostic methods.
- (9) Consistency with existing literature: Our findings resonate with existing literature, particularly the review by Iavarone et al. (87), which highlights correlations between GM composition and EMs. Our study further supports the notion that specific microbial signatures could be indicative of pathophysiological states.
- (10) Therapeutic implications: Given the accessibility of treatments for GM dysbiosis through prebiotics or probiotics, our results pave the way for novel therapeutic strategies beyond traditional medicines and surgery for EMs management.

Despite the significant contributions of our research, several limitations must be acknowledged.

- (1) The sequencing methodology employed relied on 16S rRNA gene analysis, which, while informative, does not

provide species-level resolution of GM. This constraint potentially obscures critical details within the endometrial microbial communities that could be pertinent to the pathogenesis of EMs. Achieving species-level resolution through advanced techniques such as shotgun metagenomics or targeted PCR assays could significantly enhance our comprehension by identifying specific microbial taxa associated with EMs and elucidating the underlying pathogenic mechanisms. Future studies should therefore adopt these high-resolution sequencing technologies to delve deeper into the GM composition.

- (2) The population utilized in our study is of European descent, raising concerns about the generalizability of our findings to other ethnicities and geographical regions. Ethnic and geographical variations are known to influence GM composition, potentially limiting the applicability of our results to more diverse populations. To address this, future research should include participants from multiple races and geographic locations to ensure broader relevance and validity.
- (3) The use of summary data in our GWAS analysis means that individual characteristics were not available for consideration, making it challenging to assess the impact of personalized confounding factors. The absence of individual-level data limits our ability to control for potential confounders that could affect the association between GM and EMs.
- (4) Our stringent inclusion criteria may have excluded genetic variants associated with GM that could contribute to EMs risk, potentially leading to missed opportunities for discovery. The rigorous thresholds applied at the IV selection stage might have inadvertently filtered out relevant genetic markers.
- (5) Although we analyzed over 200 taxa of GM, only a few showed statistical correlation with EMs. The possibility that these results occurred by chance cannot be entirely dismissed. Therefore, future investigations should aim to enroll larger sample sizes across diverse racial and geographical backgrounds to strengthen causal inferences. There is an urgent need for further in-depth mechanistic studies to understand the precise roles of GM alterations in the development of EMs. Additionally, exploring the diagnostic and therapeutic potential of targeting GM abundance in EMs requires comprehensive evaluation in subsequent research endeavors.

In this study, we have conducted a comprehensive assessment of the relationship between GM and EMs. Our findings indicate that there exists a causal correlation between specific GM taxa and EMs. We identified 14 GM taxa that are causally related to EMs, and conversely, EMs appear to be causally related to seven GM taxa. The bidirectional nature of these findings suggests a mutual causality between the GM and the pathogenesis of EMs. These results offer novel insights into the potential for GM as a diagnostic tool, as well as a target for the prevention and treatment of EMs. The implications of our study could pave the way for future functional and clinical analyses, potentially leading to the development of new therapeutic strategies that leverage the GM to combat EMs. These discoveries may also contribute to a deeper understanding of the complex interplay between the GM and EMs, providing a foundation for further research in this area.



## Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

## Author contributions

HY: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

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## Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2024.1434582/full#supplementary-material>

## References

- Allaire C, Bedaiwy MA, Yong PJ. Diagnosis and management of endometriosis. *CMAJ*. (2023) 195:E363–71.
- Garcia Garcia JM, Vannuzzi V, Donati C, Bernacchioni C, Bruni P, Petraglia F. Endometriosis: cellular and molecular mechanisms leading to fibrosis. *Reprod Sci*. (2023) 30:1453–61.
- Frayne J, Milroy T, Simonis M, Lam A. Challenges in diagnosing and managing endometriosis in general practice: a Western Australian qualitative study. *Aust J Gen Pract*. (2023) 52:547–55. doi: 10.31128/AJGP-10-22-6579
- Bjorkman S, Taylor HS. MicroRNAs in endometriosis: biological function and emerging biomarker candidates†. *Biol Reprod*. (2019) 100:1135–46.
- Ahn SH, Singh V, Tayade C. Biomarkers in endometriosis: challenges and opportunities. *Fertil Steril*. (2017) 107:523–32.
- Anastasiu CV, Moga MA, Elena Neculau A, Bălan A, Scărneciu I, Dragomir RM, et al. Biomarkers for the noninvasive diagnosis of endometriosis: state of the art and future perspectives. *Int J Mol Sci*. (2020) 21:1750. doi: 10.3390/ijms21051750
- Shafrir AL, Farland LV, Shah DK, Harris HR, Kvaskoff M, Zondervan K, et al. Risk for and consequences of endometriosis: a critical epidemiologic review. *Best Pract Res Clin Obstet Gynaecol*. (2018) 51:1–15.
- Asghari S, Valizadeh A, Aghebati-Maleki L, Nouri M, Yousefi M. Endometriosis: perspective, lights, and shadows of etiology. *Biomed Pharmacother*. (2018) 106:163–74. doi: 10.1016/j.biopha.2018.06.109
- Guo SW, Habiba M, Benagiano G. From retrograde menstruation to endometrial determinism and a brave new world of "Root Treatment" of endometriosis: destiny or a fanciful utopia? *Biomolecules*. (2023) 13:336. doi: 10.3390/biom13020336
- Lambrinoudaki I, Augoulea A, Creatsa M, Vlachos N, Christodoulakos G, Papadias C. Endometriosis and other benign and malignant müllerian lesions in pelvic and extrapelvic organs. *Anal Quant Cytol Histol*. (2009) 31:170–6.
- Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol*. (2014) 10:261–75.
- Jerman LF, Hey-Cunningham AJ. The role of the lymphatic system in endometriosis: a comprehensive review of the literature. *Biol Reprod*. (2015) 92:64. doi: 10.1095/biolreprod.114.124313
- Maia H Jr., Haddad C, Coelho G, Casoy J. Role of inflammation and aromatase expression in the eutopic endometrium and its relationship with the development of endometriosis. *Womens Health (Lond)*. (2012) 8:647–58. doi: 10.2217/whe.12.52
- Laganà AS, Garzon S, Götte M, Viganò P, Franchi M, Ghezzi F, et al. The pathogenesis of endometriosis: molecular and cell biology insights. *Int J Mol Sci*. (2019) 20:5615.
- Wiertsema SP, van Bergenhenegouwen J, Garssen J, Knippels LM. The interplay between the gut microbiome and the immune system in the context of infectious diseases throughout life and the role of nutrition in optimizing treatment strategies. *Nutrients*. (2021) 13:886. doi: 10.3390/nu13030886
- Shi N, Li N, Duan X, Niu H. Interaction between the gut microbiome and mucosal immune system. *Mil Med Res*. (2017) 4:14.
- Thaiss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. *Nature*. (2016) 535:65–74.
- Régner M, Van Hul M, Knauf C, Cani P. Gut microbiome, endocrine control of gut barrier function, and metabolic diseases. *J Endocrinol*. (2021) 248:R67–82.
- Ha CW, Martin A, Sepich-Poore GD, Shi B, Wang Y, Gouin K, et al. Translocation of viable gut microbiota to mesenteric adipose drives formation of creeping fat in humans. *Cell*. (2020) 183:666–83.e17. doi: 10.1016/j.cell.2020.09.009
- Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. *BMJ*. (2018) 360:j5145.
- Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, et al. Microbiota in health and diseases. *Signal Transduct Target Ther*. (2022) 7:135.
- Barko PC, McMichael MA, Swanson KS, Williams DA. The gastrointestinal microbiome: a review. *J Vet Intern Med*. (2018) 32:9–25.



23. Jiang W, Lu G, Gao D, Lv Z, Li D. The relationships between the gut microbiota and its metabolites with thyroid diseases. *Front Endocrinol (Lausanne)*. (2022) 13:943408. doi: 10.3389/fendo.2022.943408
24. Preiningerova JL, Jiraskova Zakostelska Z, Srinivasan A, Ticha V, Kovarova I, Kleinova P, et al. Multiple sclerosis and microbiome. *Biomolecules*. (2022) 12(3):433.
25. Del Chierico F, Rapini N, Deodati A, Matteoli MC, Cianfarani S, Putignani L. Pathophysiology of type 1 diabetes and gut microbiota role. *Int J Mol Sci*. (2022) 23:14650.
26. Zhang L, Qing P, Yang H, Wu Y, Liu Y, Luo Y. Gut microbiome and metabolites in systemic lupus erythematosus: link, mechanisms, and intervention. *Front Immunol*. (2021) 12:686501. doi: 10.3389/fimmu.2021.686501
27. Azpiroz MA, Orguilla L, Palacio MI, Malpartida A, Mayol S, Mor G, et al. Potential biomarkers of infertility associated with microbiome imbalances. *Am J Reprod Immunol*. (2021) 86:e13438. doi: 10.1111/aji.13438
28. Chen F, Dai X, Zhou CC, Li KX, Zhang YJ, Lou XY, et al. Integrated analysis of the fecal metagenome and serum metabolome reveals the role of gut microbiome-associated metabolites in the detection of colorectal cancer and adenoma. *Gut*. (2022) 71:1315–25. doi: 10.1136/gutjnl-2020-323476
29. Tong Y, Gao H, Qi Q, Liu X, Li J, Gao J, et al. High-fat diet, gut microbiome, and gastrointestinal cancer. *Theranostics*. (2021) 11:5889–910.
30. Park EM, Chelvanambi M, Bhutiani N, Kroemer G, Zitvogel L, Wargo JA. Targeting the gut and tumor microbiota in cancer. *Nat Med*. (2022) 28:690–703.
31. Zhao F, An R, Wang L, Shan J, Wang X. Specific gut microbiome and serum metabolome changes in lung cancer patients. *Front Cell Infect Microbiol*. (2021) 11:725284. doi: 10.3389/fcimb.2021.725284
32. Lephart ED, Naftolin F. Estrogen action and gut microbiome metabolism in dermal health. *Dermatol Ther (Heidelb)*. (2022) 12:1535–50. doi: 10.1007/s13555-022-00759-1
33. Chadchan SB, Naik SK, Popli P, Talwar C, Putluri S, Ambati CR, et al. Gut microbiota and microbiota-derived metabolites promotes endometriosis. *Cell Death Discov*. (2023) 9:28.
34. Xholli A, Cremonini F, Perugi I, Londero AP, Cagnacci A. Gut microbiota and endometriosis: exploring the relationship and therapeutic implications. *Pharmaceuticals (Basel)*. (2023) 16:1696. doi: 10.3390/ph16121696
35. Kobayashi H. Gut and reproductive tract microbiota: insights into the pathogenesis of endometriosis (Review). *Biomed Rep*. (2023) 19:43.
36. Le N, Cregger M, Brown V, Loret de Mola J, Bremer P, Nguyen L, et al. Association of microbial dynamics with urinary estrogens and estrogen metabolites in patients with endometriosis. *PLoS One*. (2021) 16:e0261362. doi: 10.1371/journal.pone.0261362
37. Ni Z, Sun S, Bi Y, Ding J, Cheng W, Yu J, et al. Correlation of fecal metabolomics and gut microbiota in mice with endometriosis. *Am J Reprod Immunol*. (2020) 84:e13307. doi: 10.1111/aji.13307
38. Chadchan SB, Cheng M, Parnell LA, Yin Y, Schriefer A, Mysorekar IU, et al. Antibiotic therapy with metronidazole reduces endometriosis disease progression in mice: a potential role for gut microbiota. *Hum Reprod*. (2019) 34:1106–16. doi: 10.1093/humrep/dez041
39. Birney E. Mendelian randomization. *Cold Spring Harb Perspect Med*. (2022) 12:a041302.
40. Shan J, Ni Z, Cheng W, Zhou L, Zhai D, Sun S, et al. Gut microbiota imbalance and its correlations with hormone and inflammatory factors in patients with stage 3/4 endometriosis. *Arch Gynecol Obstet*. (2021) 304:1363–73. doi: 10.1007/s00404-021-06057-z
41. Ata B, Yildiz S, Turkgeldi E, Brocal VP, Dinleyici EC, Moya A, et al. The Endobiota Study: comparison of vaginal, cervical and gut microbiota between women with stage 3/4 endometriosis and healthy controls. *Sci Rep*. (2019) 9:2204. doi: 10.1038/s41598-019-39700-6
42. Svensson A, Brunkwall L, Roth B, Orho-Melander M, Ohlsson B. Associations between endometriosis and gut microbiota. *Reprod Sci*. (2021) 28:2367–77.
43. Blass I, Sahar T, Shraibman A, Ofer D, Rappoport N, Linial M. Revisiting the risk factors for endometriosis: a machine learning approach. *J Pers Med*. (2022) 12:1114. doi: 10.3390/jpm12071114
44. Rusk N. The UK Biobank. *Nat Methods*. (2018) 15:1001.
45. Wang J, Kurilshikov A, Radjabzadeh D, Turpin W, Croitoru K, Bonder MJ, et al. Meta-analysis of human genome-microbiome association studies: the MiBioGen consortium initiative. *Microbiome*. (2018) 6:101. doi: 10.1186/s40168-018-0479-3
46. Xiang K, Wang P, Xu Z, Hu YQ, He YS, Chen Y, et al. Causal effects of gut microbiome on systemic lupus erythematosus: a two-sample Mendelian Randomization study. *Front Immunol*. (2021) 12:667097. doi: 10.3389/fimmu.2021.667097
47. Xu J, Zhang S, Tian Y, Si H, Zeng Y, Wu Y, et al. Genetic causal association between iron status and osteoarthritis: a two-sample Mendelian randomization. *Nutrients*. (2022) 14:3683.
48. Cao Z, Wu Y, Li Q, Li Y, Wu J. A causal relationship between childhood obesity and risk of osteoarthritis: results from a two-sample Mendelian randomization analysis. *Ann Med*. (2022) 54:1636–45. doi: 10.1080/07853890.2022.2085883
49. Gnona KM, Stewart WC. Revisiting the wald test in small case-control studies with a skewed covariate. *Am J Epidemiol*. (2022) 191:1508–18. doi: 10.1093/aje/kwac058
50. Cheng H, Garrick DJ, Fernando RL. Efficient strategies for leave-one-out cross-validation for genomic best linear unbiased prediction. *J Anim Sci Biotechnol*. (2017) 8:38.
51. Burgess S, Thompson SG, Crp Chd Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol*. (2011) 40:755–64.
52. Sanderson E, Davey Smith G, Windmeijer F, Bowden J. An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings. *Int J Epidemiol*. (2019) 48:713–27.
53. Zizolfi B, Foreste V, Gallo A, Martone S, Giampaolino P, Di Spiezio Sardo A. Endometriosis and dysbiosis: state of art. *Front Endocrinol (Lausanne)*. (2023) 14:1140774. doi: 10.3389/fendo.2023.1140774
54. Elkafas H, Walls M, Al-Hendy A, Ismail N. Gut and genital tract microbiomes: dysbiosis and link to gynecological disorders. *Front Cell Infect Microbiol*. (2022) 12:1059825. doi: 10.3389/fcimb.2022.1059825
55. Wei Y, Tan H, Yang R, Yang F, Liu D, Huang B, et al. Gut dysbiosis-derived  $\beta$ -glucuronidase promotes the development of endometriosis. *Fertil Steril*. (2023) 120(3 Pt. 2):682–94.
56. Horne AW, Missmer SA. Pathophysiology, diagnosis, and management of endometriosis. *BMJ*. (2022) 379:e070750.
57. Qin R, Tian G, Liu J, Cao L. The gut microbiota and endometriosis: from pathogenesis to diagnosis and treatment. *Front Cell Infect Microbiol*. (2022) 12:1069557. doi: 10.3389/fcimb.2022.1069557
58. Talwar C, Singh V, Kommagani R. The gut microbiota: a double-edged sword in endometriosis. *Biol Reprod*. (2022) 107:881–901.
59. Huang L, Liu B, Liu Z, Feng W, Liu M, Wang Y, et al. Gut microbiota exceeds cervical microbiota for early diagnosis of endometriosis. *Front Cell Infect Microbiol*. (2021) 11:788836. doi: 10.3389/fcimb.2021.788836
60. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen-gut microbiome axis: physiological and clinical implications. *Maturitas*. (2017) 103:45–53. doi: 10.1016/j.maturitas.2017.06.025
61. Jiang I, Yong PJ, Allaire C, Bedaiwy MA. Intricate connections between the microbiota and endometriosis. *Int J Mol Sci*. (2021) 22:5644.
62. Saunders PT, Horne AW. Endometriosis: etiology, pathobiology, and therapeutic prospects. *Cell*. (2021) 184:2807–24. doi: 10.1016/j.cell.2021.04.041
63. Rebordão MR, Galvão A, Szóstek A, Amaral A, Mateus L, Skarzynski DJ, et al. Physiopathologic mechanisms involved in mare endometriosis. *Reprod Domest Anim*. (2014) 49(Suppl. 4):82–7. doi: 10.1111/rda.12397
64. Buha? MC, Gavrilu? LI, Candrea R, Cătean A, Mocan A, Miere D, et al. Gut microbiota in psoriasis. *Nutrients*. (2022) 14:2970.
65. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat Microbiol*. (2019) 4:293–305.
66. Socała K, Doboszewska U, Szopa A, Serefko A, Włodarczyk M, Zielińska A, et al. The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. *Pharmacol Res*. (2021) 172:105840.
67. Lui JB, McGinn LS, Chen Z. Gut microbiota amplifies host-intrinsic conversion from the CD8 T cell lineage to CD4 T cells for induction of mucosal immune tolerance. *Gut Microbes*. (2016) 7:40–7. doi: 10.1080/19490976.2015.1117737
68. Kogut MH, Lee A, Santin E. Microbiome and pathogen interaction with the immune system. *Poult Sci*. (2020) 99:1906–13.
69. Elkabets M, Ribeiro VS, Dinarello CA, Ostrand-Rosenberg S, Di Santo JP, Apte RN, et al. IL-1 $\beta$  regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function. *Eur J Immunol*. (2010) 40:3347–57.
70. Lobo, TF, Borges CM, Mattar R, Gomes CP, de Angelo AG, Pendelowski KP, et al. Impaired treg and NK cells profile in overweight women with gestational diabetes mellitus. *Am J Reprod Immunol*. (2018) 79:1–5. doi: 10.1111/aji.12810
71. Rao VA, Kurian NK, Rao KA. Cytokines, NK cells, and regulatory T cell functions in normal pregnancy and reproductive failures. *Am J Reprod Immunol*. (2023) 89:e13667.
72. Krishnan S, Ding Y, Saedi N, Choi M, Sridharan GV, Sherr DH, et al. Gut microbiota-derived tryptophan metabolites modulate inflammatory response in hepatocytes and macrophages. *Cell Rep*. (2018) 23:1099–111.
73. Sun S, Xu X, Liang L, Wang X, Bai X, Zhu L, et al. Lactic acid-producing probiotic *saccharomyces cerevisiae* attenuates ulcerative colitis via suppressing macrophage

pyroptosis and modulating gut microbiota. *Front Immunol.* (2021) 12:777665. doi: 10.3389/fimmu.2021.777665

74. Kim S, Lee JY, Shin SG, Kim JK, Silwal P, Kim YJ, et al. ESRRA (estrogen-related receptor alpha) is a critical regulator of intestinal homeostasis through the activation of autophagic flux via the gut microbiota. *Autophagy.* (2021) 17:2856–75. doi: 10.1080/15548627.2020.1847460

75. He J, Xu S, Zhang B, Xiao C, Chen Z, Si F, et al. Gut microbiota and metabolite alterations associated with reduced bone mineral density or bone metabolic indexes in postmenopausal osteoporosis. *Aging (Albany NY).* (2020) 12:8583–604.

76. Ervin SM, Li H, Lim L, Roberts LR, Liang X, Mani S, et al. Gut microbial  $\beta$ -glucuronidases reactivate estrogens as components of the estrobolome that reactivate estrogens. *J Biol Chem.* (2019) 294:18586–99.

77. Edwinston AL, Yang L, Peters S, Hanning N, Jeraldo P, Jagtap P, et al. Gut microbial  $\beta$ -glucuronidases regulate host luminal proteases and are depleted in irritable bowel syndrome. *Nat Microbiol.* (2022) 7:680–94.

78. Tang W, Lu HY, Sun Q, Xu WM. [Characteristics of gut microbiota and its association with the activity of  $\beta$ -glucuronidase in neonates with hyperbilirubinemia]. *Zhongguo Dang Dai Er Ke Za Zhi.* (2021) 23:677–83.

79. Sui Y, Wu J, Chen J. The role of gut microbial  $\beta$ -glucuronidase in estrogen reactivation and breast cancer. *Front Cell Dev Biol.* (2021) 9:631552. doi: 10.3389/fcell.2021.631552

80. Yan S, Huang Y, Xu L, Dong X, Wang S, Jiao X, et al. *Escherichia coli* inhibits endometriosis by inducing M1 polarity of peritoneal macrophages and the IL-1 signaling pathway. *Mol Hum Reprod.* (2023) 29:gaad014. doi: 10.1093/molehr/gaad014

81. Yuan M, Li D, Zhang Z, Sun H, An M, Wang G. Endometriosis induces gut microbiota alterations in mice. *Hum Reprod.* (2018) 33:607–16.

82. Uzuner C, Mak J, El-Assaad F, Condous G. The bidirectional relationship between endometriosis and microbiome. *Front Endocrinol (Lausanne).* (2023) 14:1110824. doi: 10.3389/fendo.2023.1110824

83. Liang Y, Zeng W, Hou T, Yang H, Wu B, Pan R, et al. Gut microbiome and reproductive endocrine diseases: a Mendelian randomization study. *Front Endocrinol (Lausanne).* (2023) 14:1164186. doi: 10.3389/fendo.2023.1164186

84. Ji X, Yang Q, Zhu XL, Xu L, Guo JY, Rong Y, et al. Association between gut microbiota and endometriosis: a two-sample Mendelian randomization study. *Front Microbiol.* (2023) 14:1188458. doi: 10.3389/fmicb.2023.1188458

85. Dang C, Chen Z, Chai Y, Liu P, Yu X, Liu Y, et al. Assessing the relationship between gut microbiota and endometriosis: a bidirectional two-sample mendelian randomization analysis. *BMC Womens Health.* (2024) 24:123. doi: 10.1186/s12905-024-02945-z

86. Liu Z, Chen P, Luo L, Liu Q, Shi H, Yang X. Causal effects of gut microbiome on endometriosis: a two-sample Mendelian randomization study. *BMC Womens Health.* (2023) 23:637. doi: 10.1186/s12905-023-02742-0

87. Iavarone I, Greco PF, La Verde M, Morlando M, Torella M, de Franciscis P, et al. Correlations between gut microbial composition, pathophysiological and surgical aspects in endometriosis: a review of the literature. *Medicina (Kaunas).* (2023) 59:347. doi: 10.3390/medicina59020347



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# Epidemiology of endometriosis in Kazakhstan: a national population-based cohort analysis (2014–2019) using data from the national electronic healthcare system

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**Background:** Endometriosis is a condition affecting reproductive-age women and associated with dysmenorrhea, pelvic organs dysfunction, pelvic pain, and infertility. The real epidemiology of endometriosis remains underestimated. No data are available on prevalence of endometriosis in Kazakhstan. Therefore, the aim of this was to investigate the epidemiology, complications, surgical management approach, and outcomes of endometriosis in Kazakhstan by analyzing large-scale Kazakhstani healthcare data from the Unified Nationwide Electronic Health System (UNEHS).

**Methods:** A population-based study among women with endometriosis treated in any healthcare setting of the Republic of Kazakhstan during the period of 2014–2019 was performed. The International Classification of Diseases (ICD) 10th edition was used to retrieve data on endometriosis ("N80" and "N97"). ICD 9th edition's procedural codes were utilized to retrieve information on surgical procedures performed to manage patients with endometriosis.

**Results:** In total, 7,682 records of women diagnosed with endometriosis were analyzed from all Kazakhstani regions. The overall prevalence of endometriosis among Kazakhstani female population was 0.12%, with 50.1% of them suffering from endometriosis of the uterus, 34.5% with ovarian endometriosis, and 9.5% with endometriosis of pelvic peritoneum. The most affected group was reproductive-age women (25–44 years old). Endometriosis rates were higher among women of 35–39, 40–44, and 45–49 years old age groups – 0.4 per 1000 women of corresponding age. The most common procedures performed for surgical management were laparoscopic cystectomy and closed biopsy of the uterus, 16.4 and 13.5%, respectively.

**Conclusion:** Among all registered cases of endometriosis, ovarian endometriosis is the most prevalent condition. However, the analysis of the UNEHS records on endometriosis reveals incomplete and inconsistent registration of the disease, which results in the underestimation of the disease's real burden. Clinical specialist and health authorities in Kazakhstan must work to ensure the endometriosis proper diagnosis and registration to improve the disease management and outcomes.

#### KEYWORDS

endometriosis, infertility, epidemiology, prevalence, Kazakhstan

## 1 Background

Endometriosis is a chronic benign gynecological disease affecting reproductive-age women and associated with dysmenorrhea, pelvic organs' dysfunction, pelvic pain, and infertility (1–6). Although visual identification is often used for clinical verification of endometriosis, the definitive diagnosis requires histological confirmation of the ectopic endometrial glands and stroma presence outside of the uterine cavity (3, 6). Adenomyosis is characterized by the invasion of endometrial glands and stroma within the myometrium (7).

There are different classifications systematizing endometriosis nomenclature: based on localization, extension, and depth of the ectopic endometrial glands (1–3). The typical localization of endometriosis is pelvic organs. The most common types of pelvic endometriosis are ovarian endometriotic cysts and superficial peritoneal lesions (1, 3, 4). Deep infiltrating lesions are less common and defined as lesions with more than 5 mm depth of invasion into the organs' stromal tissues or beneath the peritoneum (1, 3).

According to the available statistical data, endometriosis affects 5–10% of reproductive-age women worldwide (3). However, due to the heterogeneity of endometriosis and multiple definitions used to describe the disease, due to the difference in the disease reporting and registration, the prevalence of endometriosis remains underestimated (6, 8). Moreover, according to different reports, the prevalence of endometriosis is even higher among infertile women and varies from 25 to 60% (1, 6, 9–11). Among women suffering from chronic pelvic pain, the prevalence of ovarian cysts and deep endometriosis were reported in over 25 and 1–5% of cases, respectively (6). Moreover, according to a recent study subtle endometriosis was reported in 40% of asymptomatic women (6). These data make it evident that the estimation of the epidemiology of endometriosis is important for female health care.

Endometriosis and adenomyosis interfere with fertility and pregnancy course via different mechanisms: disruption of pelvic anatomy, and affect oocyte release, uptake, or transport through the fallopian tubes (12, 13). Furthermore, chronic pelvic

inflammation with prostaglandins and various inflammatory cytokines production could potentially impact the physiology of ovulation, conception, embryo migration, and implantation (13). The recent meta-analysis reported increased sporadic and recurrent pregnancy loss rates and reduced pregnancy and live birth rates in women with endometriosis and adenomyosis (13, 14). Thus, approximately 10–25% of women with endometriosis-associated infertility require treatment with assisted reproductive technology (ART) (1, 11).

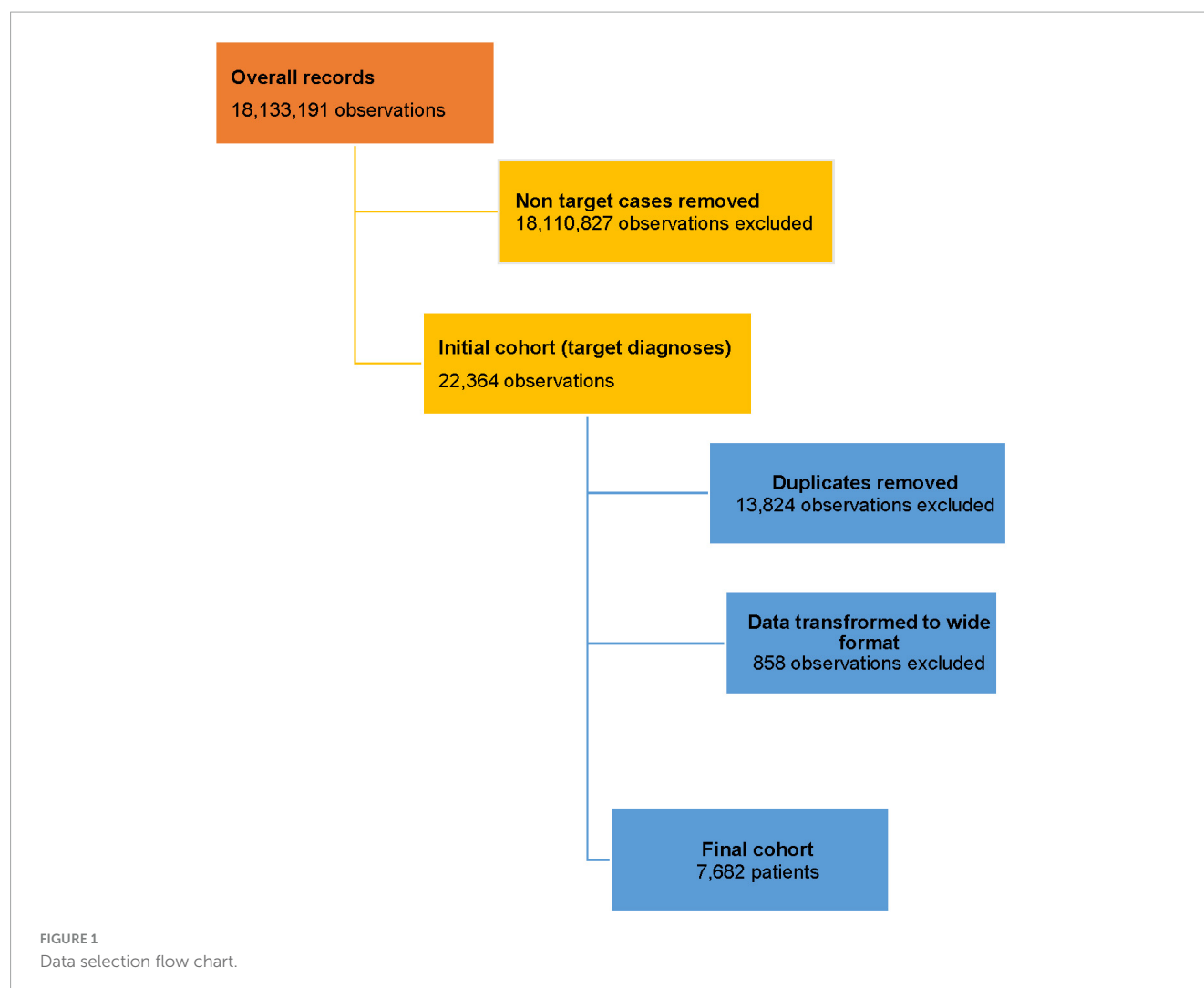
The Republic of Kazakhstan is a Central Asian country with a population of around 20 million (15–17). According to the Kazakhstani National Agency for Statistics, females account for 52% of the population with 51% belonging to the reproductive-age group (2, 17–20). To date no studies have been done on the epidemiology of endometriosis in Kazakhstan, thus, no data is available on the incidence, prevalence, and complications of the disease. At the same time, according to available resources, the prevalence of infertility is high in the Republic of Kazakhstan (19–22). Based on recent publications, the frequency of infertility varies between 12 and 15.5% (20–22). Assuming a contribution of endometriosis to female gynecological morbidity and the prevalence of endometriosis-associated infertility, it is important to estimate the disease epidemiology. Thus, considering the high prevalence of infertility and the absence of statistical data on endometriosis and its contribution to the pool of infertility, this study's aim was to investigate the epidemiology, complications, surgical management approach, and outcomes of endometriosis in Kazakhstan by analyzing large-scale Kazakhstani healthcare data from the Unified National Electronic Healthcare System (UNEHS).

## 2 Materials and methods

### 2.1 Study design and population

The study population consisted of patients who were hospitalized with endometriosis in any Kazakhstani clinical setting during the period of 2014–2019. The data was retrieved from UNEHS inpatient registry that was introduced at the end of 2013 to unify healthcare data storage through the country healthcare system (23). The International Classification of Diseases (ICD),

Abbreviations: ESHRE, European Society of Human Reproduction and Embryology; ICD, International Classification of Diseases; IQR, interquartile range; UNEHS, Unified National Electronic Healthcare System; USA, United States of America.



9th<sup>1</sup> and 10th (see text footnote 1) editions were used for coding surgeries and diagnoses (primary and complication), respectively.

## 2.2 Patient selection and definitions

The initial dataset consists of overall 22,364 medical records of women registered with endometriosis and infertility (ICD-10 codes “N80” and “N97”). ICD-9 codes through “65” and “66” were reviewed to identify the surgical procedures performed to manage patients with endometriosis. Data cleaning was performed using unique patient ID, which links data throughout the UNEHS database. The final dataset included 7,682 patients. The detailed patient selection process is depicted in Figure 1.

## 2.3 Statistical analysis

The study involved descriptive and bivariate analyses to explore the association of demographic characteristics and diagnosis

among the participants. Categorical variables were described by numbers and percentages, and their relationship with diagnosis was tested using Pearson’s chi-square test. Age was described by median and interquartile range (IQR), and the difference among groups was tested using Kruskal-Wallis test. Two-sided *p*-values less than 0.05 indicated statistical significance for all tests. Prevalence of endometriosis per 1000 women of specific age groups was calculated using population statistics according to the National Agency for Statistics and Strategic Planning of the Republic of Kazakhstan (18). Stata 16 MP2 software was used for data processing and statistical analysis (24). More details about the data and methodology were published previously (23).

## 2.4 Ethical approval

The study was conducted in compliance with the Declaration of Helsinki, and approved by the Nazarbayev University Institutional Review Ethics Committee (protocol reference NU-IREC 490/18112021). Exemption from informed consent has been granted due to the retrospective nature of the study and anonymized data analysis. No individual patients’ information was reported in this study.

<sup>1</sup> <https://www.icd10data.com/>



## 3 Results

### 3.1 Study subjects description

The study database included overall 30,221,986 with 18,133,191 female patients' records available. Out of total female patients, 22,364 (0.12%) had an endometriosis diagnosis recorded between 2014 and 2019. In total, for the 6-year period included in this investigation, 7,682 women were diagnosed with endometriosis of any localization and registered in the UNEHS (Figure 1). These records of patients with endometriosis were identified and analyzed in the national electronic database from all Kazakhstani regions (Figure 1).

The study subjects' social and demographic characteristics are presented in Table 1. The age of women registered with endometriosis ranged from 17 to 54 years, and the median age of the participants was 37.0 (IQR 30.0–45.0) years. The majority of women diagnosed with endometriosis were of reproductive age, between 25 and 44 years (66.4%).

The ethnic distribution of patients with endometriosis includes 53.2% of women of the Kazakh ethnic group, 14.2% of the Russian ethnic group, and 32.1% of other ethnicities living in Kazakhstan. For 0.5% of the study subjects ethnicity was not reported in the UNEHS.

The distribution of endometriosis cases reported in the UNEHS was very unequal in different regions (Supplementary Table 1). The largest number of endometriosis cases was reported from the North-Kazakhstan region (23.8%, 1,826 cases), Astana city (17.7%, 1,361 cases), Almaty city (14.2%, 1,092), and East-Kazakhstan region (13.0%, 1,000). However, only 0.2% (19) of cases were found in Shymkent, one of the large cities in the country. Low numbers were also reported from the Turkestan region (2.5%, 191), (Supplementary Table 1).

The number of patients registered with endometriosis from urban areas was much higher than that of the rural ones – 77.9 and 22.1%, respectively (Table 1).

### 3.2 Incidence and rates of endometriosis (2014–2019)

Figure 2 and Table 1 report the incidence of endometriosis among women in Kazakhstan in 2014–2019 years. The incidence was equally distributed among women of the following age groups: 25–29 years old (16.4%), 30–34 years old (17.5%), 35–39 years old (16.9%), and 40–44 years old (15.6%). There was a gradual decrease in incidence after 45 years. A low number of cases reported in adolescent patients group (15–19 years old) – 0.8%, early reproductive age group (20–24 years old) – 6.8%, and premenopausal age women (45–49 years old) – only 7.6% and 14.9% of participants, respectively (Table 1). Figure 2 shows the incidence of endometriosis and its dynamics for the period of 6 years (2014–2019). Endometriosis of the uterus (ICD-10 code “N80.0”) was one of the most reported types of endometriosis – 50.1% (Figures 2, 3 and Table 1). Its incidence reporting increased in 2017 to 873 cases and dropped almost twice (486 cases) in 2019 (Figure 3). The second and third most prevalent types of endometriosis were endometriosis of ovaries (ICD-10 code

“N80.1”) and endometriosis of pelvic peritoneum (ICD-10 code “N80.3”), 34.5 and 9.5%, respectively (Figure 2 and Table 1). However, the trends in the incidence of endometriosis of ovaries and peritoneal endometriosis were different: ovarian endometriosis cases are decreasing by 2019, while there was a slight increase in the incidence of peritoneal endometriosis through 2017–2019.

The endometriosis rates per 1000 women of corresponding age are shown in Table 1. While endometriosis rates were higher among women of 35–39, 40–44, and 45–49 years old age groups – 0.4 per 1000 women of corresponding age (Table 1). However, this indicator was low among women of early reproductive age groups (15–19 and 20–24 years old) – 0.02 and 0.2 per 1000 women of corresponding age, respectively.

### 3.3 Surgical procedures and invasive diagnostic manipulations performed for patients with endometriosis. Outcomes of disease management

Diagnostic manipulations and surgical procedures performed for patients with endometriosis are shown in Figure 4. The most common surgical treatment procedure was laparoscopic cystectomy (ICD-9 code “65.1”) performed for 1,713 cases (16.4%). Other laparoscopic local excision or destruction of ovary (ICD-9 code “65.25”) and laparoscopic lysis of adhesions of ovary and fallopian tube (ICD-9 code “65.81”) were done for 662 (6.3%) and 706 (6.8%) patients, respectively (Figure 4). Some patients had two or more procedures performed simultaneously. The most common combined procedures are laparoscopic cystectomy with laparoscopic lysis of adhesions of ovary and fallopian tube and other laparoscopic local excision or destruction of ovary; hysteroscopy with closed biopsy of the uterus (hysteroscopy with biopsy).

As the most common diagnostic manipulation, closed biopsy of the uterus (ICD-9 code “68.16” – hysteroscopy with biopsy) in 1,412 cases (13.5%) and hysteroscopy (ICD-9 code “68.12”) – 1,291 cases (12.4%) were documented in the UNEHS database (Figure 4).

Out of all analyzed records, 69.2% of patients with endometriosis had a planned admission for surgical treatment, while 30.8% of patients were admitted via emergency route due to torsion or rupture of endometriotic cyst, bleeding due to endometriosis of the uterus (Table 2). The vast majority of patients with endometriosis were discharged after treatment (99.9%) with recovery or improvement (75.2 and 24.8%, respectively). There were no cases of mortality due to endometriosis registered in the UNEHS for the analyzed period (2014–2019).

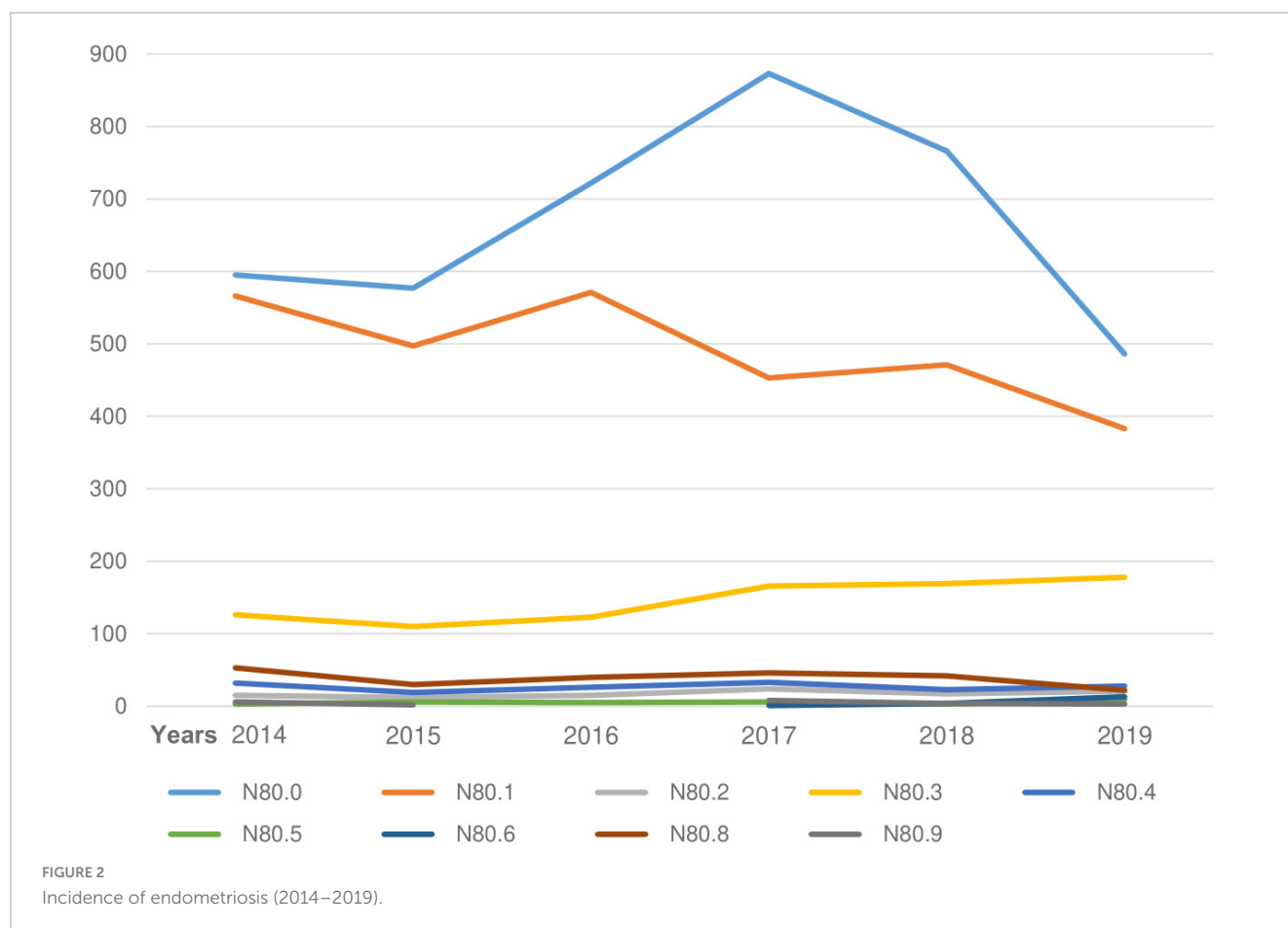
### 3.4 Endometriosis association with infertility

Figure 5 shows endometriosis cases associated with infertility among the studied population. The most common type of infertility associated with cases of endometriosis was female infertility of tubal origin (ICD-10 code “N97.1”) – 67.7% of all infertility cases associated with endometriosis (Figure 5A). Other reported but less common cases were female infertility of another origin (ICD-10

TABLE 1 Socio-demographic characteristics of the study subjects (2014–2019).

| Variable          | Overall, N (%)      | Rate per 1000 women of corresponding age | Diagnosis (ICD-10), N (%) |                      |                   |                   |                   |                   |                   |                   |                   | p-value  |
|-------------------|---------------------|------------------------------------------|---------------------------|----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|----------|
|                   |                     |                                          | N80.0                     | N80.1                | N80.2             | N80.3             | N80.4             | N80.5             | N80.6             | N80.8             | N80.9             |          |
| Age, median (IQR) | 37.0 (30.0, 45.0)   | –                                        | 43.0 (36.0, 48.0)         | 31.0 (27.0, 38.0)    | 34.0 (28.0, 41.0) | 31.0 (27.0, 36.0) | 32.0 (27.0, 38.0) | 37.0 (29.0, 44.0) | 35.0 (31.0, 37.0) | 36.0 (30.0, 42.0) | 34.0 (29.0, 40.0) | <0.001*  |
| <b>Age groups</b> |                     |                                          |                           |                      |                   |                   |                   |                   |                   |                   |                   | <0.001** |
| 15–19             | 65 (0.8%)           | 0.02                                     | 5 (0.1%)                  | 44 (1.7%)            | 1 (1.2%)          | 12 (1.7%)         | 0 (0.0%)          | 0 (0.0%)          | 0 (0.0%)          | 3 (1.5%)          | 0 (0.0%)          |          |
| 20–24             | 519 (6.8%)          | 0.2                                      | 68 (1.8%)                 | 345 (13.0%)          | 9 (11.1%)         | 74 (10.2%)        | 8 (6.6%)          | 0 (0.0%)          | 1 (7.7%)          | 11 (5.4%)         | 3 (13.6%)         |          |
| 25–29             | 1,262 (16.4%)       | 0.3                                      | 267 (6.9%)                | 702 (26.5%)          | 18 (22.2%)        | 198 (27.3%)       | 35 (28.7%)        | 5 (33.3%)         | 1 (7.7%)          | 34 (16.7%)        | 2 (9.1%)          |          |
| 30–34             | 1,348 (17.5%)       | 0.3                                      | 455 (11.8%)               | 578 (21.8%)          | 18 (22.2%)        | 206 (28.4%)       | 33 (27.0%)        | 3 (20.0%)         | 5 (38.5%)         | 44 (21.7%)        | 6 (27.3%)         |          |
| 35–39             | 1,299 (16.9%)       | 0.4                                      | 595 (15.5%)               | 466 (17.6%)          | 12 (14.8%)        | 144 (19.8%)       | 24 (19.7%)        | 2 (13.3%)         | 4 (30.8%)         | 47 (23.2%)        | 5 (22.7%)         |          |
| 40–44             | 1,200 (15.6%)       | 0.4                                      | 800 (20.8%)               | 278 (10.5%)          | 16 (19.8%)        | 58 (8.0%)         | 16 (13.1%)        | 2 (13.3%)         | 1 (7.7%)          | 25 (12.3%)        | 4 (18.2%)         |          |
| 45–49             | 1,141 (14.9%)       | 0.4                                      | 902 (23.4%)               | 175 (6.6%)           | 5 (6.2%)          | 26 (3.6%)         | 4 (3.3%)          | 3 (20.0%)         | 1 (7.7%)          | 23 (11.3%)        | 2 (9.1%)          |          |
| ≥50               | 847 (11.0%)         | 0.3                                      | 759 (19.7%)               | 60 (2.3%)            | 2 (2.5%)          | 8 (1.1%)          | 2 (1.6%)          | 0 (0.0%)          | 0 (0.0%)          | 16 (7.9%)         | 0 (0.0%)          |          |
| <b>Ethnicity</b>  |                     |                                          |                           |                      |                   |                   |                   |                   |                   |                   |                   | <0.001** |
| Kazakh            | 4,085 (53.2%)       | 0.3                                      | 1,705 (44.3%)             | 1,642 (62.0%)        | 40 (49.4%)        | 451 (62.1%)       | 81 (66.4%)        | 10 (66.7%)        | 10 (76.9%)        | 133 (65.5%)       | 13 (59.1%)        |          |
| Russian           | 1,092 (14.2%)       | 0.3                                      | 572 (14.9%)               | 372 (14.0%)          | 16 (19.8%)        | 95 (13.1%)        | 15 (12.3%)        | 1 (6.7%)          | 2 (15.4%)         | 19 (9.4%)         | 0 (0.0%)          |          |
| Other             | 2,468 (32.1%)       | 0.3                                      | 1,562 (40.6%)             | 614 (23.2%)          | 25 (30.9%)        | 178 (24.5%)       | 26 (21.3%)        | 4 (26.7%)         | 1 (7.7%)          | 49 (24.1%)        | 9 (40.9%)         |          |
| Missing           | 37 (0.5%)           | –                                        | 12 (0.3%)                 | 21 (0.8%)            | 0 (0.0%)          | 2 (0.3%)          | 0 (0.0%)          | 0 (0.0%)          | 0 (0.0%)          | 2 (1.0%)          | 0 (0.0%)          |          |
| <b>Residence</b>  |                     |                                          |                           |                      |                   |                   |                   |                   |                   |                   |                   | <0.001** |
| Rural             | 1,699 (22.1%)       | 0.3                                      | 923 (24.0%)               | 533 (20.1%)          | 19 (23.5%)        | 145 (20.0%)       | 16 (13.1%)        | 2 (13.3%)         | 2 (15.4%)         | 58 (28.6%)        | 1 (4.5%)          |          |
| Urban             | 5,983 (77.9%)       | 0.3                                      | 2,928 (76.0%)             | 2,116 (79.9%)        | 62 (76.5%)        | 581 (80.0%)       | 106 (86.9%)       | 13 (86.7%)        | 11 (84.6%)        | 145 (71.4%)       | 21 (95.5%)        |          |
| <b>Total</b>      | <b>7,682 (100%)</b> | <b>0.3</b>                               | <b>3,851 (50.1%)</b>      | <b>2,649 (34.5%)</b> | <b>81 (1%)</b>    | <b>726 (9.5%)</b> | <b>122 (1.6%)</b> | <b>15 (0.2%)</b>  | <b>13 (0.2%)</b>  | <b>203 (2.6%)</b> | <b>22 (0.3%)</b>  |          |

\*Kruskal-Wallis. \*\*Pearson's chi-squared. ICD-10 codes: N80.0, Endometriosis of uterus; N80.1, Endometriosis of ovary; N80.2, Endometriosis of fallopian tube; N80.3, Endometriosis of pelvic peritoneum; N80.4, Endometriosis of rectovaginal septum and vagina; N80.5, Endometriosis of intestine; N80.6, Endometriosis in cutaneous scar; N80.8, Other endometriosis; N80.9, Endometriosis, unspecified.



code “N97.8”) and female infertility associated with anovulation (ICD-10 code “N97.0”).

The yearly distribution of endometriosis associated with infertility registered in the UNEHS is illustrated in Figure 5B.

### 3.5 Complications

Complications in patients with endometriosis registered in the UNEHS are presented in Figure 6. The most common condition complicating endometriosis was female pelvic peritoneal adhesions (ICD-10 code “N73.6”). The other reported complications were unspecified ovarian cysts (ICD-10 “N83.2”) and acute posthaemorrhagic anemia (ICD-10 “D62”). Such complications as acute salpingitis and oophoritis (ICD-10 code “N70.0”), acute pelvic peritonitis (ICD-10 “N73.3”), acute posthaemorrhagic anemia (ICD-10 “D62”), and unspecified ovarian cysts (ICD-10 “N83.2”) registered after surgical treatment were seen with the same rate. However, the overall reported number of complications was very low.

## 4 Discussion

Estimation of the endometriosis epidemiology is essential, as this gynecological condition is a major cause of infertility, chronic pelvic pain, and dysmenorrhea in many women (6, 13, 25). It

is a significant health issue for reproductive-age women due to the necessity of specific medical and surgical management and associated fertility problems (6, 25). The Kazakhstani government prioritizes support of reproductive healthcare aiming to improve the birth rate and decrease maternal mortality (16, 17, 19, 26). For that, the State Program for the Development of Healthcare 2020–2025 has been approved with a budget of USD 7.5 billion (26). However, proper budget distribution and execution require a clear understanding of diseases’ prevalence and related health burdens. To date, no reliable statistics are available for endometriosis epidemiology in Kazakhstan. Therefore, this study’s aim was to investigate the epidemiology, complications, surgical management approaches, and outcomes of endometriosis in Kazakhstan.

### 4.1 Main findings and comparison with existing literature

In this study, the age of patients registered in the national electronic database with endometriosis ranged between 17 and 54 years, with an average age of 37 years. These data are comparable with the most recent studies on the epidemiology of endometriosis from Türkiye (27) and the United States of America (USA) (28) where the average age of women with endometriosis was 30 years old (range 18–50) and 37 years old (range 18–45), respectively. In the current study, the majority of women diagnosed with endometriosis were of reproductive age, between 25 and 44 years

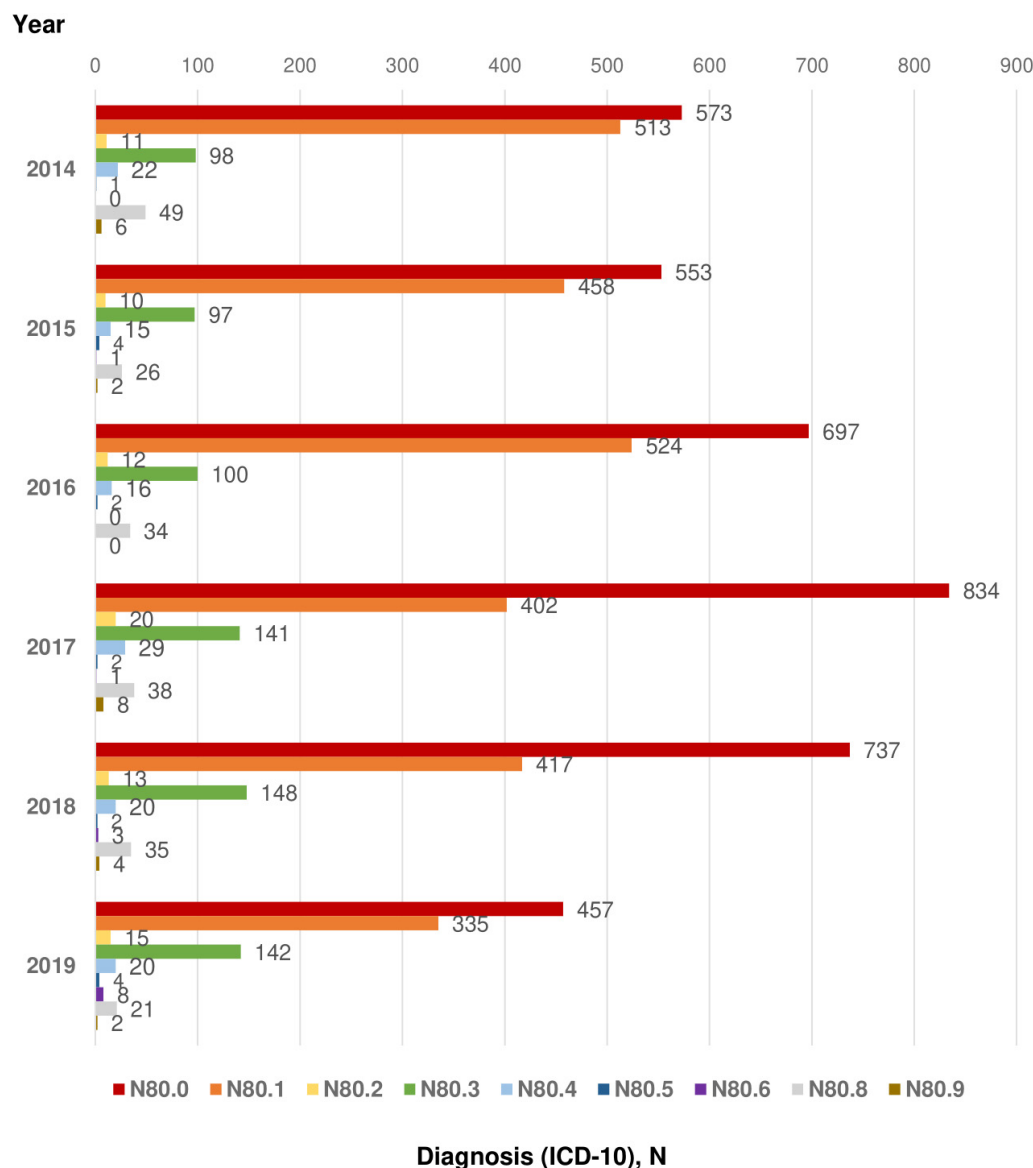


FIGURE 3

Main diagnosis, ICD-10. ICD-10 codes: N80.0 - Endometriosis of uterus; N80.1 - Endometriosis of ovary; N80.2 - Endometriosis of fallopian tube; N80.3 - Endometriosis of pelvic peritoneum; N80.4 - Endometriosis of rectovaginal septum and vagina; N80.5 - Endometriosis of intestine; N80.6 - Endometriosis in cutaneous scar; N80.8 - Other endometriosis; N80.9 - Endometriosis, unspecified.

with the highest incidence in the 20–24, 25–29, and 30–34 years old age groups. These findings are in line with the results of the Australian study by Rowlands et al. (29), however, are in contradiction to the findings of the study from the USA by Christ et al. the incidence was highest among women aged 36–45 years (28).

This study revealed a low rate of endometriosis among adolescent patients and young women of in the 20–24 years old age group — 0.02 and 0.2 per 1,000 women of the corresponding age, that reflects to 0.8 and 6.8% incidence, respectively. This finding of our study is comparable and in agreement with the Australian study on the epidemiology of endometriosis where the rate of the disease was 0.2 per 1,000 persons of the same age (29) and with the findings of the Spanish study (30). However, low rates of endometriosis in adolescent patients and young women

found in our study contradicts findings of the recent research by Zannoni et al. where the prevalence of endometriosis and adenomyosis in young women was 25.0 and 46.0%, respectively (31). Moreover, the prevalence of endometriosis in young women with dysmenorrhea is even higher (ranged between 25 and 73%) (32). On the other hand, similar to our findings, Zannoni et al. reported higher incidence of endometriosis among young women (20–24 years old group) than among adolescents (14–19 years old group). Thus, since endometriosis represents the main cause of secondary dysmenorrhea among adolescent and young women, the condition should be carefully managed as dysmenorrhea has a great impact on adolescents' lives and future reproductive function (32).

In the study by Rowlands et al. the authors found a sharp increase (30-fold) in endometriosis incidence at age 30–34 years (6 compared to 0.2 per 1,000 women of the corresponding age), while

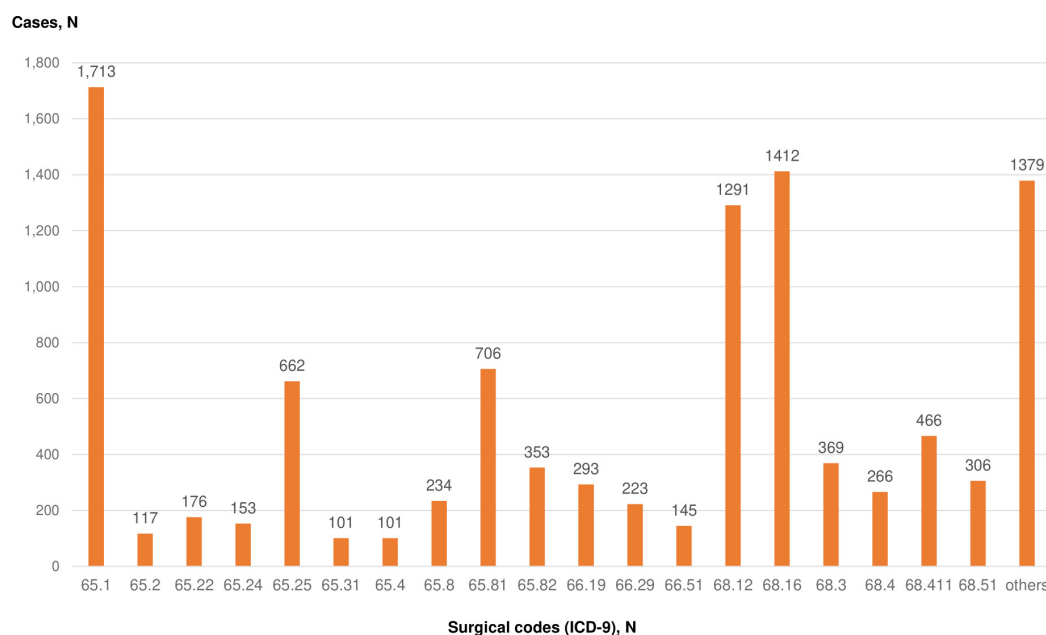


FIGURE 4

Diagnostic and surgical procedures performed for patients with endometriosis. ICD-9 codes: 65.1 – Laparoscopic cystectomy; 65.2 – Local excision or destruction of ovarian lesion or tissue; 65.22 – Ovarian wedge resection; 65.24 – Laparoscopic wedge resection of ovary; 65.25 – Other laparoscopic local excision or destruction of ovary; 65.31 – Laparoscopic unilateral oophorectomy; 65.4 – Unilateral salpingo-oophorectomy; 65.8 – Lysis of adhesions of ovary and fallopian tube; 65.81 – Laparoscopic lysis of adhesions of ovary and fallopian tube; 65.82 – Laparoscopic lysis of adhesions of ovary and fallopian tube; 66.19 – Other diagnostic procedures on fallopian tubes; 66.29 – Other bilateral endoscopic destruction or occlusion of fallopian tubes; 66.51 – removal of both tubes; 68.12 – Hysteroscopy; 68.16 – Closed biopsy of uterus (hysteroscopy with biopsy); 68.3 – subtotal abdominal hysterectomy; 68.4 – total abdominal hysterectomy; 68.411 – Laparoscopic total hysterectomy; 68.51 – Laparoscopically assisted vaginal hysterectomy.

in our study these findings are in contradiction to the compared study as the disease increased only 2-fold (0.4 compared to 0.2 per 1,000 women of corresponding age) among women of 35–39, 40–44, and 45–49 years old age groups. Rates of endometriosis reported by the study from the USA (29) are also higher than the results of our study (17.4–30.2 per 10,000 women in the USA vs. 0.2–0.4 per 1,000 women in Kazakhstan) (29). This can be explained by the underestimation of the endometriosis cases in Kazakhstan and the existing inaccuracy of the data registration in the Kazakhstani healthcare electronic system due to the recently introduced electronic healthcare system. The efforts on precise registration of healthcare data have to be reinforced in Kazakhstan.

Unfortunately, no studies on the epidemiology of endometriosis are available from Central Asian countries and/or post-Soviet countries with similar population and healthcare systems to compare the epidemiological indicators of the disease.

In this study, unequal incidence of endometriosis was found in the different regions of the country, which has no underlying objective background. Moreover, regions with a larger population were found to have lower incidence of endometriosis. The distribution of endometriosis cases analyzed in the nationwide database reveals a huge difference in the number of cases registered in the different regions. This may be a result of improper registration. Thus, the accuracy of disease registration and reporting has to be improved to ensure proper management.

The number of patients registered with endometriosis from urban areas was much higher than that of the rural regions. This finding is in apparent agreement with the compared Spanish study

on epidemiology of endometriosis, where incidence rates of the disease in women from rural areas were lower (30). There are two possible explanations for this fact. On one side, residents of rural areas are less exposed to the environmental toxins and pollutants playing role in the pathogenesis of endometriosis (33), thus have less risk factors. On the other side as studies report, population of rural and remote areas may have unequal access to medical care including gynecology specialists (16, 27).

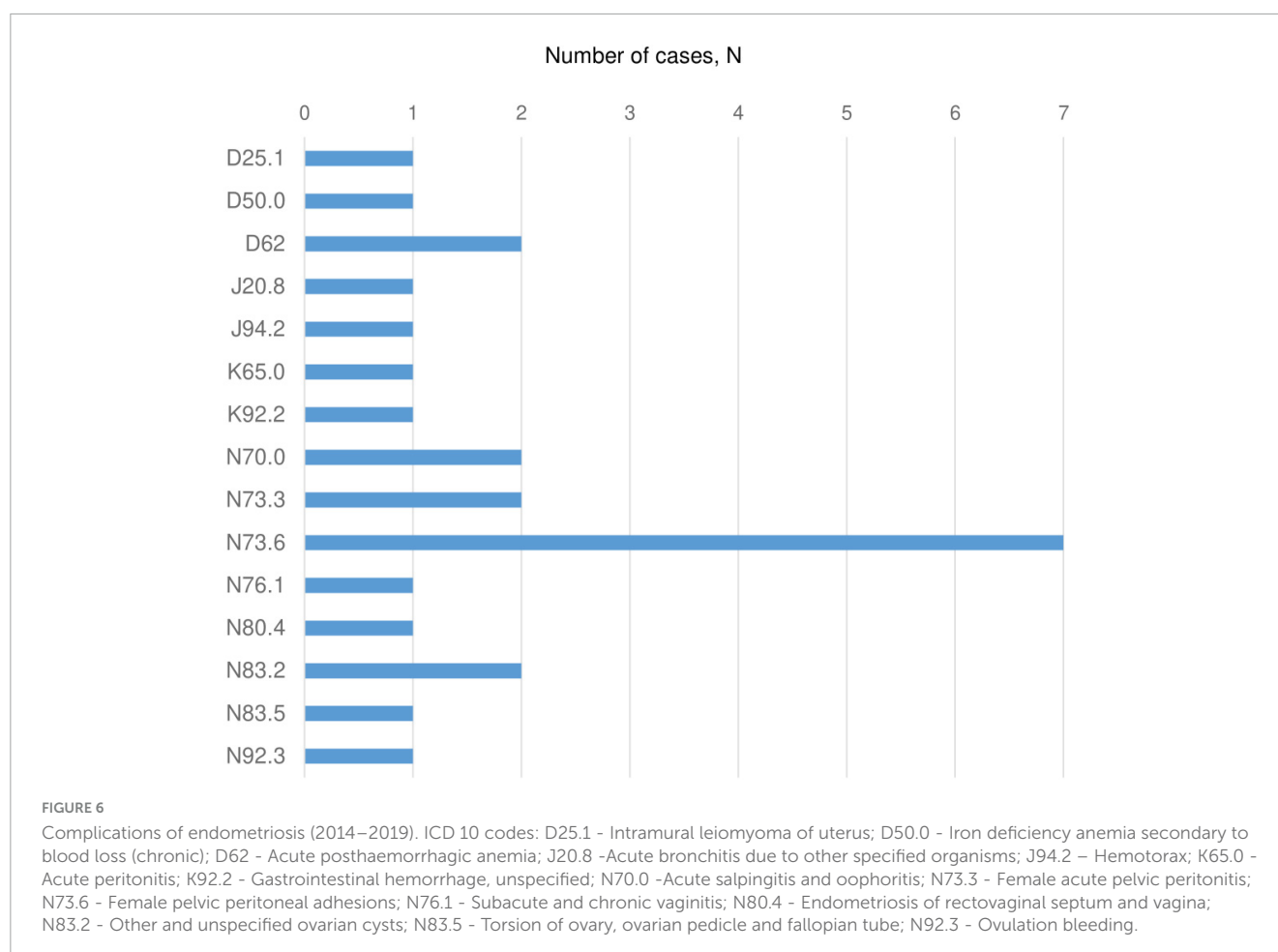
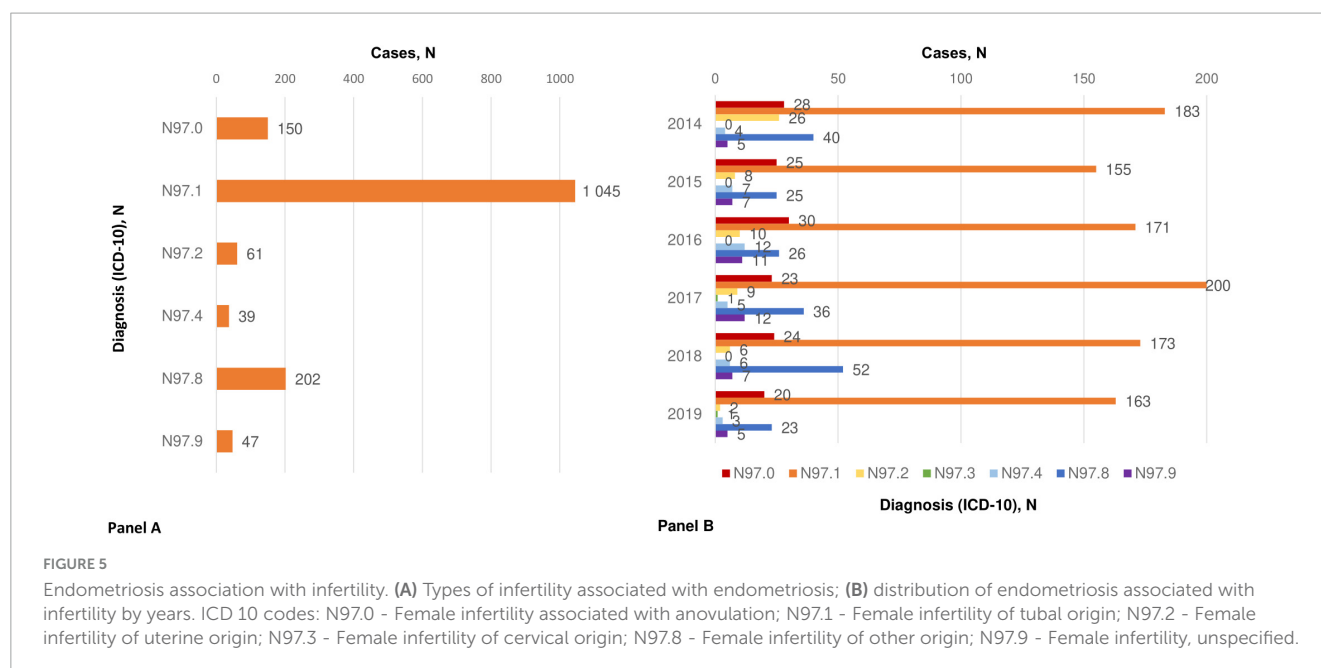
Although the previous research on endometriosis outlined the estimated prevalence as around 10% (3, 8), a low prevalence of endometriosis was reported in the Kazakhstani UNEHS (0.12%). Despite the fact that this finding contradicts the overall trend on endometriosis prevalence, it is comparable with previous studies among the Spanish female population (30) where the prevalence of the disease was reported at the level of 0.7% or the USA population (28) with the reported low prevalence of endometriosis at the level of 1.9%. However, if compared with the recent study among women in Turkiye (27), our study population had significantly lower endometriosis prevalence (0.12%) than Turkish women where the disease is reported among 18.3% of the study subjects. These reported variations in prevalence between studies with low prevalence from the USA, Spain, and with high prevalence could be explained by differences in the studies' design: the results of our study, study from the USA (28) and study from Spain (30) are based on the national electronic information systems reports, while the study from Turkiye (27) was based on self-reported surveys. The low prevalence of endometriosis reported in the Kazakhstani UNEHS clearly shows that the ICD-10 code utilization and overall



TABLE 2 Outcomes of endometriosis treatment (2014–2019).

| Variable                    | Overall, <i>N</i> (%) | Diagnosis (ICD-10), <i>N</i> (%) |               |            |             |             |            |            |             |            | <i>p</i> -value |
|-----------------------------|-----------------------|----------------------------------|---------------|------------|-------------|-------------|------------|------------|-------------|------------|-----------------|
|                             |                       | N80.0                            | N80.1         | N80.2      | N80.3       | N80.4       | N80.5      | N80.6      | N80.8       | N80.9      |                 |
| <b>Admission</b>            |                       |                                  |               |            |             |             |            |            |             |            | <0.001*         |
| Emergency                   | 2,368 (30.8%)         | 1,386 (36.0%)                    | 762 (28.8%)   | 29 (35.8%) | 134 (18.5%) | 14 (11.5%)  | 3 (20.0%)  | 3 (23.1%)  | 34 (16.7%)  | 3 (13.6%)  |                 |
| Planned                     | 5,314 (69.2%)         | 2,465 (64.0%)                    | 1,887 (71.2%) | 52 (64.2%) | 592 (81.5%) | 108 (88.5%) | 12 (80.0%) | 10 (76.9%) | 169 (83.3%) | 19 (86.4%) |                 |
| <b>Outcome of stay</b>      |                       |                                  |               |            |             |             |            |            |             |            |                 |
| Discharge                   | 7,672 (99.9%)         | 3,846 (99.9%)                    | 2,644 (99.8%) | 81 (100%)  | 726 (100%)  | 122 (100%)  | 15 (100%)  | 13 (100%)  | 203 (100%)  | 22 (100%)  |                 |
| Transfer                    | 8 (0.1%)              | 5 (0.1%)                         | 3 (0.1%)      | 0 (0.0%)   | 0 (0.0%)    | 0 (0.0%)    | 0 (0.0%)   | 0 (0.0%)   | 0 (0.0%)    | 0 (0.0%)   |                 |
| Voluntary discharge         | 2 (< 1%)              | 0 (0.0%)                         | 2 (0.1%)      | 0 (0.0%)   | 0 (0.0%)    | 0 (0.0%)    | 0 (0.0%)   | 0 (0.0%)   | 0 (0.0%)    | 0 (0.0%)   |                 |
| <b>Outcome of treatment</b> |                       |                                  |               |            |             |             |            |            |             |            |                 |
| Deterioration               | 1 (< 1%)              | 0 (0.0%)                         | 1 (< 1%)      | 0 (0.0%)   | 0 (0.0%)    | 0 (0.0%)    | 0 (0.0%)   | 0 (0.0%)   | 0 (0.0%)    | 0 (0.0%)   |                 |
| Improvement                 | 1,904 (24.8%)         | 761 (19.8%)                      | 839 (31.7%)   | 12 (14.8%) | 150 (20.7%) | 48 (39.3%)  | 2 (13.3%)  | 0 (0.0%)   | 88 (43.3%)  | 4 (18.2%)  |                 |
| Recovery                    | 5,775 (75.2%)         | 3,088 (80.2%)                    | 1,809 (68.3%) | 69 (85.2%) | 576 (79.3%) | 74 (60.7%)  | 13 (86.7%) | 13 (100%)  | 115 (56.7%) | 18 (81.8%) |                 |
| Without changes             | 2 (<1%)               | 2 (0.1%)                         | 0 (0.0%)      | 0 (0.0%)   | 0 (0.0%)    | 0 (0.0%)    | 0 (0.0%)   | 0 (0.0%)   | 0 (0.0%)    | 0 (0.0%)   |                 |
| <b>Total</b>                | <b>7,682</b>          | <b>3,851</b>                     | <b>2,649</b>  | <b>81</b>  | <b>726</b>  | <b>122</b>  | <b>15</b>  | <b>13</b>  | <b>203</b>  | <b>22</b>  |                 |

\*Pearson's chi-squared. ICD-10 codes: N80.0, Endometriosis of uterus; N80.1, Endometriosis of ovary; N80.2, Endometriosis of fallopian tube; N80.3, Endometriosis of pelvic peritoneum; N80.4, Endometriosis of rectovaginal septum and vagina; N80.5, Endometriosis of intestine; N80.6, Endometriosis in cutaneous scar; N80.8, Other endometriosis; N80.9, Endometriosis, unspecified. Outcome of stay terminology description: Discharge – patient went home after treatment; Transfer – patient was transferred to another hospital; Voluntary discharge – patient left a hospital before treatment completed due to personal demand; Death – patient death associated with treatment/surgery. Outcome of treatment terminology description: Without changes – patient was discharged without improvement; Recovery – patient was discharged with recovery; Improvement – patient was discharged with improvement; Deterioration – patient was discharged/transferred to another hospital with deterioration.



disease-reporting arrangement should be improved in the frame of the local healthcare system. This finding is in line with assumption stating that endometriosis reporting and registration appears to be inaccurate, which leads to underestimation of the disease (6, 8).

In the current study endometriosis of the uterus, ovarian endometriosis, and endometriosis of pelvic peritoneum were the most prevalent types of endometriosis. These findings are in agreement with the investigation from the USA

population-based study reporting data for the period of 2006–2015 (28, 29).

While researching the management of endometriosis, in this study laparoscopic cystectomy was found to be the most common procedure associated with ovarian endometriosis. This approach with laparoscopic management is in line with the most recent European Society of Human Reproduction and Embryology (ESHRE) guideline on endometriosis management (34). The other common procedures were hysteroscopy and hysteroscopy with biopsy performed for women with uterine endometriosis. This frequency of these surgical procedures is logical considering that endometriosis of the uterus and ovarian endometriosis were the most prevalent types of the disease. This is in line with the available sources, where researchers recommend operative hysteroscopy as a suitable option for cases of superficial adenomyosis as a treatment modality (35–37).

Data from UNEHS shows that female infertility is strongly associated with endometriosis of any localization, which is in agreement with previous reports (6, 13, 26–30).

Interestingly, in this study inflammatory complications of endometriosis appeared with the same rate as acute posthaemorrhagic anemia, which highlights the importance of proper infection prevention and application of hemostatic techniques during surgery. Furthermore, a recent research reported a strong interconnection between presence of endometriosis and recurrence of pelvic inflammatory diseases (38). Thus, early detection of inflammatory complications is essential to administer adequate treatment and facilitate the healing processes (39).

Generally, a very small number of complications are reported in the UNEHS among women with endometriosis and in association with the disease treatment. This could be related to the “punishment culture” that was present in the Kazakhstani national healthcare system, when any complication that happened with a patient was considered as a physician’s mistake even if it is a statistically prevalent and expected type of complication. This led to the development of specific “complication-hiding” culture when healthcare professionals did not report complications concerning negative impacts on their careers.

## 4.2 Study strengths and limitations

The main strength is novelty of this study, as this is the first one providing epidemiological data on the incidence, prevalence, complications, surgical management approach, and outcomes of endometriosis in Kazakhstan. In this investigation a large cohort of patients’ data are analyzed and covered the female population of Kazakhstan for the period of 6 years (2014–2019). Since the health-related records in the UNEHS were associated with the available socio-demographic information, this enabled to reduce missing data. This study also has some limitations mostly related to the electronic healthcare system design. UNEHS was established and introduced into the clinical practice in 2014, and is still under continuous development due to existing drawback requiring improvements (17, 23). Namely, the electronic system in its current form is not ideal as it does not have information on important socio-demographic data like education and income. Moreover, it does not save a woman’s marital status, gynecological

anamnesis, parity history, symptoms and staging of endometriosis. It should be noted as another limitation that, compared to surgical management options, which are documented in the UNEHS and could be retrieved via ICD-9 codes, information on medical management of patients with endometriosis are not available in the national healthcare information system. Moreover, improvements in accuracy of coding from the healthcare professionals should be improved to adequately report different types of endometriosis and cases of deep endometriosis. For the further analysis of the data from 2023 and onward, the UNEHS is expected to be improved to provide these missing variables, which could facilitate the healthcare data analysis’ results and conclusions.

## 5 Conclusion

Endometriosis is a chronic, underestimated disease that according to the UNEHS affects 0.12% of Kazakhstani reproductive-age women. A huge proportion of women with endometriosis in Kazakhstan suffer from infertility. Analysis of the UNEHS reveals that there is an inconsistent and incomplete reporting and registration of endometriosis and its treatment, which affect the overall statistics on epidemiology and outcomes of the disease. Therefore, the data from the national healthcare electronic system does not reflect the endometriosis real burden. Gynecology specialists should be aware that the proper diagnosis of the disease would ensure provision of an adequate management. Establishing incidence and prevalence of endometriosis is an important initial step toward building a strong background for future research, which would improve knowledge on the disease etiology, pathogenesis, and progression, thus contribute to better management. Governmental health authorities and gynecology clinical specialists must work together to ensure the endometriosis proper diagnosis and registration. New treatment options recently approved for endometriosis should be applied.

## Data availability statement

The original contributions presented in this study are included in this article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving humans were approved by Nazarbayev University Institutional Review Ethics Committee (protocol reference NU-IREC 490/18112021). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants’ legal guardians/next of kin because Exemption from informed consent has been granted due to the retrospective nature of the study and anonymized data analysis. No individual patients’ information was reported in this study.

## Author contributions

GA: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review and editing. YS: Data curation, Methodology, Software, Visualization, Writing – original draft, Writing – review and editing. TU: Data curation, Visualization, Writing – original draft, Writing – review and editing. MT: Conceptualization, Writing – original draft, Writing – review and editing. AM: Visualization, Writing – original draft, Writing – review and editing. NK: Data curation, Visualization, Writing – original draft. AG: Data curation, Formal analysis, Writing – original draft, Writing – review and editing. GZ: Methodology, Visualization, Writing – original draft. SY: Methodology, Supervision, Visualization, Writing – original draft, Writing – review and editing. KM: Data curation, Software, Writing – original draft. DS: Data curation, Software, Visualization, Writing – original draft. AbG: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review and editing.

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## References

1. Terzic M, Aimagambetova G, Garzon S, Bapayeva G, Ukybassova T, Terzic S, et al. Ovulation induction in infertile women with endometriotic ovarian cysts: current evidence and potential pitfalls. *Minerva Med.* (2020) 111:50–61. doi: 10.23736/S0026-4806.19.06346-8
2. Aimagambetova G, Terzic S, Bapayeva G, Micic J, Kongrtay K, Laganà AS, et al. Endometriotic cyst and infertility. In: Duncan LT editor. *Advances in Health and Disease*. Vol. 38, New York, NY: Nova Science Publisher (2021). p. 31–67.
3. Taylor HS, Kotlyar AM, Flores VA. Endometriosis is a chronic systemic disease: clinical challenges and novel innovations. *Lancet.* (2021) 397:839–52. doi: 10.1016/S0140-6736(21)00389-5
4. Terzic M, Aimagambetova G, Kunz J, Bapayeva G, Aitbayeva B, Terzic S, et al. Molecular basis of endometriosis and endometrial cancer: current knowledge and future perspectives. *Int J Mol Sci.* (2021) 22:9274. doi: 10.3390/ijms22179274
5. Bulun SE, Yildiz S, Adli M, Chakravarti D, Parker JB, Milad M, et al. Endometriosis and adenomyosis: shared pathophysiology. *Fertil Steril.* (2023) 119:746–50. doi: 10.1016/j.fertnstert.2023.03.006
6. Koninckx PR, Ussia A, Adamyan L, Tahlak M, Keckstein J, Wattiez A, et al. The epidemiology of endometriosis is poorly known as the pathophysiology and diagnosis are unclear. Best practice & research. *Clin Obstet Gynaecol.* (2021) 71:14–26. doi: 10.1016/j.bpobgyn.2020.08.005
7. Bourdon M, Santulli P, Marcellin L, Maignien C, Maitrot-Mantelet L, Bordonne C, et al. Adenomyosis: an update regarding its diagnosis and clinical features. *J Gynecol Obstetr Hum Reprod.* (2021) 50:102228. doi: 10.1016/j.jogoh.2021.102228
8. Sarria-Santamera A, Orazumbekova B, Terzic M, Issanov A, Chaowen C, Asúnolo-Del-Barco A. Systematic review and meta-analysis of incidence and prevalence of endometriosis. *Healthcare (Basel, Switzerland).* (2020) 9:29. doi: 10.3390/healthcare9010029
9. Parazzini F, Esposito G, Tozzi L, Noli S, Bianchi S. Epidemiology of endometriosis and its comorbidities. *Eur J Obstet Gynecol Reprod Biol.* (2017) 209:3–7.
10. Nowak-Psiorz I, Ciećwież SM, Brodowska A, Starczewski A. Treatment of ovarian endometrial cysts in the context of recurrence and fertility. *Adv Clin Exp Med.* (2019) 28:407–13.
11. Somigliana E, Viganò P, Benaglia L, Busnelli A, Berlanda N, Vercellini P. Management of endometriosis in the infertile patient. *Semin Reprod Med.* (2017) 35:31–7.
12. Coccia ME, Nardone L, Rizzello F. Endometriosis and infertility: a long-life approach to preserve reproductive integrity. *Int J Environ Res Public Health.* (2022) 19:6162.
13. Vercellini P, Viganò P, Bandini V, Buggio L, Berlanda N, Somigliana E. Association of endometriosis and adenomyosis with pregnancy and infertility. *Fertil Steril.* (2023) 119:727–40. doi: 10.1016/j.fertnstert.2023.03.018
14. Vidali A, Riccio LGC, Abrao MS. Endometriosis and recurrent pregnancy loss: two manifestations of the same underlying dysfunction? *Fertil Steril.* (2023) 119:836–7. doi: 10.1016/j.fertnstert.2023.03.012
15. World Population Review Kazakhstan. (2023). Available at <https://worldpopulationreview.com/countries/kazakhstan-population> (accessed July 24, 2023).
16. Sakko Y, Aimagambetova G, Terzic M, Ukybassova T, Bapayeva G, Gusmanov A, et al. The prevalence, indications, outcomes of the most common major gynecological surgeries in Kazakhstan and recommendations for potential improvements into public health and clinical practice: analysis of the national electronic healthcare system (2014–2019). *Int J Environ Res Public Health.* (2022) 19:14679. doi: 10.3390/ijerph192214679

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2024.1436458/full#supplementary-material>

17. Sakko Y, Turesheva A, Gaipov A, Aimagambetova G, Ukybassova T, Marat A, et al. Epidemiology of spontaneous pregnancy loss in Kazakhstan: a national population-based cohort analysis during 2014–2019 using the national electronic healthcare system. *AOGS*. (2023) 102:1682–93.
18. National Agency for Statistics and Strategic Planning of the Republic of Kazakhstan. *Population count*. (2023). Available online at: <https://stat.gov.kz/ru/> (accessed July 24, 2023).
19. Issanov A, Aimagambetova G, Terzic S, Bapayeva G, Ukybassova T, BAikoshkarova S, et al. Impact of governmental support to the IVF clinical pregnancy rates: differences between public and private clinical settings in Kazakhstan—a prospective cohort study. *BMJ Open*. (2022) 12:e049388. doi: 10.1136/bmjopen-2021-049388
20. Bapayeva G, Aimagambetova G, Issanov A, Terzic S, Ukybassova T, Aldiyarova A, et al. The effect of stress, anxiety and depression on in vitro fertilization outcome in Kazakhstani public clinical setting: a cross-sectional study. *J Clin Med*. (2021) 10:937. doi: 10.3390/jcm10050937
21. Lokshin VN, Khoroshilova IG, Kuandykov EU. Personified approach to genetic screening of infertility couples in ART programs. *Report of the National Academy of Sciences of the Republic of Kazakhstan*. Vol. 1, (2018). p. 37–41.
22. Aimagambetova G, Issanov A, Terzic S, Bapayeva G, Ukybassova T, Baikoshkarova S, et al. The effect of psychological distress on IVF outcomes: reality or speculations? *PLoS One*. (2020) 15:e0242024. doi: 10.1371/journal.pone.0242024
23. Gusmanov A, Zhakhina G, Yerdessov S, Sakko Y, Mussina K, Alimbayev A, et al. Review of the research databases on population-based registries of unified electronic healthcare system of Kazakhstan (UNEHS): possibilities and limitations for epidemiological research and real-world evidence [published online ahead of print, 2022 Dec 7]. *Int J Med Inform*. (2022) 170:104950. doi: 10.1016/j.ijmedinf.2022.104950
24. StataCorp LLC. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC (2019).
25. Guo SW. Various types of adenomyosis and endometriosis: in search of optimal management. *Fertil Steril*. (2023) 119:711–26. doi: 10.1016/j.fertnstert.2023.03.021
26. International Trade Administration Kazakhstan - Country Commercial Guide. (2023). Available online at: <https://www.trade.gov/country-commercial-guides/kazakhstan-healthcare> (accessed August 02, 2023).
27. Yuksel Ozgor B, Azamat S, Berkay EG, Türeli D, Ozdemir I, Topaloğlu S, et al. Epidemiology of endometriosis awareness in Turkey. *Cureus*. (2023) 15:e37536. doi: 10.7759/cureus.37536
28. Christ JP, Yu O, Schulze-Rath R, Grafton J, Hansen K, Reed SD. Incidence, prevalence, and trends in endometriosis diagnosis: a United States population-based study from 2006 to 2015. *Am J Obstet Gynecol*. (2021) 225:e1–500. doi: 10.1016/j.ajog.2021.06.067
29. Rowlands I, Abbott J, Montgomery G, Hockey R, Rogers P, Mishra G. Prevalence and incidence of endometriosis in Australian women: a data linkage cohort study. *BJOG*. (2020) 128:657–65. doi: 10.1111/1471-0528.16447
30. Medina-Perucha L, Pistillo A, Raventós B, Jacques-Aviñó C, Munros-Feliu J, Martínez-Bueno C, et al. Endometriosis prevalence and incidence trends in a large population-based study in Catalonia (Spain) from 2009 to 2018. *Womens Health (Lond)*. (2022) 18:17455057221130566. doi: 10.1177/17455057221130566
31. Zannoni L, Del Forno S, Raimondo D, Arena A, Giaquinto I, Paradisi R, et al. Adenomyosis and endometriosis in adolescents and young women with pelvic pain: prevalence and risk factors. *Minerva Pediatr*. (2024) 76:57–63. doi: 10.23736/S2724-5276.20.05842-9
32. Martire FG, Piccione E, Exacoustos C, Zupi E. Endometriosis and adolescence: the impact of dysmenorrhea. *J Clin Med*. (2023) 12:5624. doi: 10.3390/jcm12175624
33. Terzic S, Kongrtay K, Aimagambetova G, Bapayeva G, Micic J, Laganà AS, et al. Environmental and occupational pollutants exposure and infertility. In: Duncan LT editor. *Advances in Health and Disease*. Vol. 38, New York, NY: Nova Science Publisher (2021). p. 123–46.
34. Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, Kiesel L, et al. ESHRE endometriosis guideline group. ESHRE guideline: endometriosis. *Hum Reprod Open*. (2022) 2022:hoac009. doi: 10.1093/hropen/hoac009
35. Sardo ADS, Calagna G, Santangelo F, Zizolfi B, Tanos V, Perino A, et al. The role of hysteroscopy in the diagnosis and treatment of adenomyosis. *BioMed Res. Int*. (2017) 2017:1–7.
36. Gordts S, Grimbizis G, Campo R. Symptoms and classification of uterine adenomyosis, including the place of hysteroscopy in diagnosis. *Fertil Steril*. (2018) 109:380–8.e1. doi: 10.1016/j.fertnstert.2018.01.006
37. Gkrozou F, Vatopoulou A, Skentou C, Paschopoulos M. Diagnosis and treatment of adenomyosis with office hysteroscopy—a narrative review of literature. *Diagnostics (Basel, Switzerland)*. (2023) 13:2182. doi: 10.3390/diagnostics13132182
38. Zografou Themeli M, Nirgianakis K, Neumann S, Imboden S, Mueller MD. Endometriosis is a risk factor for recurrent pelvic inflammatory disease after tubo-ovarian abscess surgery. *Arch Gynecol Obstetr*. (2023) 307:139–48. doi: 10.1007/s00404-022-06743-6
39. Montanari E, Reh LM, Dauser B, Birsan T, Hudelist G. Serial assessment of inflammatory parameters for prediction of septic complications following surgery for colorectal endometriosis: a descriptive, retrospective study. *Wiener Klin Wochenschrift*. (2022) 134:118–24. doi: 10.1007/s00508-021-01916-w





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# NOD1, NOD2, PYDC1, and PYDC2 gene polymorphisms in ovarian endometriosis

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**Background:** Endometriosis, a prevalent chronic gynecologic disorder, significantly impacts women's health, with both genetic and environmental factors contributing to its heritability. Within the adaptive immune system, the NOD-like receptors (NLR) pathway plays pivotal roles in various autoinflammatory diseases, regulating interleukins, proinflammatory cytokines, and NF- $\kappa$ B activity. However, the potential association between single nucleotide polymorphisms (SNPs) of the NOD1, NOD2, PYDC1, and PYDC2 genes and the predisposition to endometriosis risk remains unexplored.

**Methods:** In this cross-sectional study, 54 patients diagnosed with ovarian endometriosis and 54 control subjects were included. The genetic SNPs of NOD1 (rs2075820 and rs2075818) and NOD2 (rs104895461) were assessed using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method. Additionally, the polymorphisms of PYDC1 and PYDC2 were evaluated using Sanger sequencing. After conducting polymorphism analysis, the genetic profiles were assessed with the clinical manifestations and the size of ovarian endometriomas, categorized as either small (<4 cm) or large ( $\geq$ 4 cm).

**Results:** Significant differences in the NOD1 rs2075820 (G: A) genotypes were found. The GG genotype was more prevalent in endometriosis patients ( $p = 0.04$ ), while the GA genotype was less common ( $p = 0.029$ ). The AA genotype was associated with higher rates of perimenstrual gastrointestinal symptoms ( $p = 0.005$ ) and infertility ( $p = 0.037$ ). The PYDC2 rs293833 variant was detected in 22.2% of patients. Carriers of this variant exhibited higher rates of perimenstrual gastrointestinal symptoms ( $p = 0.004$ ), infertility ( $p = 0.001$ ) and larger endometriomas ( $\geq$ 4 cm) ( $p < 0.001$ ). No significant differences were found in NOD1 rs2075818 genotypes ( $p = 0.89$ ) and no polymorphisms were detected in NOD2 or PYDC1 genes.

**Conclusion:** These findings emphasize the influence of genetic polymorphisms on the clinical manifestations of endometriosis. Specifically, gene polymorphisms in NLRs have been found to significantly impact infertility and increase endometrioma size.

## KEYWORDS

endometriosis, infertility, pain, gene polymorphism, NOD, PYDC

## Introduction

Endometriosis is characterized by an estrogen-dependent chronic inflammatory pathology that affects reproductive-aged women with pelvic pain and infertility (1). Understanding the mechanisms underlying endometriosis is crucial due to its clinical and therapeutic relevance. While numerous theories have been proposed, none fully explain the disease's progression and diverse clinical manifestations. Sampson's retrograde menstruation theory remains the most widely cited explanation (2). However, this theory does not adequately explain why only 10% of women with retrograde menstrual flow develop endometriosis.

A common element in all theories is the dysregulation of hormonal signaling and an inflammatory microenvironment, which, together with genetic and epigenetic factors, drive the disease's initiation, persistence, and progression (3). Genetic predisposition is significant, as daughters of affected mothers have double the risk of developing endometriosis, and monozygotic twins show a 51% increased risk (4, 5). Ovarian endometriomas are a significant and prominent component of endometriosis. About 17–44% of patients with endometriosis have endometriomas, with bilateral endometriomas occurring in 19–28% of these patients (6). Endometriosis is a chronic pelvic inflammatory condition where local inflammation significantly contributes to pain and infertility. Excessive reactive oxygen species (ROS) production affects gene expression, with NF- $\kappa$ B involvement in the disease. Activated NF- $\kappa$ B in lesions and macrophages drives proinflammatory cytokine production, supporting lesion formation and persistence (7).

The innate immune system detects various danger and pathogen-associated molecular patterns through pattern-recognition receptors (PRRs), such as nod-like receptors (NLRs) (8). The NLR family comprises over 20 members, including nucleotide-binding oligomerization domain-containing proteins 1 and 2 (NOD1 and NOD2) (9). Engagement of NLRs triggers cooperative signaling between mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF- $\kappa$ B) pathways, leading to the transcription of pro-inflammatory cytokines, assembly of NLR inflammasomes, and cell death (10). Moreover, pyrin-only protein/pyrin domain (POP/PYDC) domain proteins also disrupt NF- $\kappa$ B signaling by forming an inflammasome complex by certain NLRs and interleukins (11). Studies highlight that mutations and dysregulation in NLRs, such as NOD2 and NLRP3, significantly impact these pathways, altering immune responses and contributing to diseases like Crohn's disease and cryopyrinopathies. Polymorphisms in the NOD1 and NOD2 genes can disrupt the balance between pro- and anti-inflammatory cytokines, fostering chronic inflammation and increasing the risk of cancer. These findings emphasize the critical role of structure–function relationships in understanding NLR-mediated immune regulation and their relevance to disease pathogenesis (12).

Polymorphisms play a crucial role in understanding the genetic underpinnings of complex diseases, including endometriosis. Given the multifactorial nature of endometriosis, the identification of genetic variants that contribute to disease susceptibility has significant implications for advancing diagnostic and therapeutic strategies. However, there remains a substantial research gap in understanding the precise contribution of genetic polymorphisms to endometriosis, with many studies producing inconsistent results across populations and ethnic groups. This variability underscores the complexity of

genetic influence on endometriosis, suggesting that multiple, potentially interacting loci may contribute to its pathology (13, 14).

In this study, we aim to investigate inflammasome regulators PYDC1 and PYDC2 and genetic variations in the NOD1 and NOD2 genes in patients with ovarian endometriosis. Additionally, we will evaluate the genetic profile of these patients with the size of the endometriomas and their clinical symptoms.

## Method

### Subjects

All subjects provided written informed consent for inclusion before participating in the study. The study was conducted by the Declaration of Helsinki of 1975 (as revised in 2013), and the protocol was reviewed and approved by the Health Research Ethics Committee of Dokuz Eylül University (7511-GOA). Blood samples were collected from a total of 108 patients who had either undergone laparoscopic surgery or exploratory laparotomy between March 2022 and November 2023. The study population comprised 54 patients diagnosed with ovarian endometriosis (endometriosis group) and 54 control subjects without endometriosis (control group).

### Endometriosis group

Diagnosis of endometriosis was confirmed and classified based on visual and histopathological examinations according to the American Association of Gynecologic Laparoscopists (AAGL) and the revised American Society for Reproductive Medicine (rASRM) Endometriosis Classification Systems (15, 16). According to AAGL Classification, 16 (29.6%) were in stage II, 34 (63%) in stage III, and 4 (7.4%) in stage IV. When classified with rASRM, 44 patients (81.5%) were classified as stage 3, and 10 patients (18.5%) as stage 4. To explore potential genetic differences related to endometrioma size, a subgroup analysis was performed, categorizing endometriomas as larger ( $\geq 4$  cm) or smaller ( $< 4$  cm).

The control group consisted of patients who underwent surgery for fibroids, menorrhagia, benign adnexal masses, and pelvic organ prolapse. Endometriosis was ruled out in these patients through histopathological evaluation. Patients with additional autoimmune diseases, pelvic inflammatory disease, or gynecological malignancies were excluded from both the endometriosis and control groups.

### Genotyping polymorphisms

DNA was collected in 5 mL peripheral blood, followed by ficol separation (Sigma Histopaque-1077, cat no: 10771). DNA isolation was then performed using Trizol (Invitrogen TM, cat no: 15596018). Conventional polymerase chain reaction (PCR) was conducted using Taq DNA Polymerase (A.B.T., cat no: E02-01-50) for the target genes, with the following protocol: 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 65°C for 95 min. Primer sequences for the target genes previously created before (17). Genotypes rs2075818 and rs104895461 were determined using the PCR-restriction fragment length polymorphism (PCR-RFLP) method. PCR products were incubated overnight at 37°C with specific restriction enzymes for

the restriction enzyme process. Samples were loaded onto a 2% agarose gel to determine allele separation and visualized (Figure 1).

The PCR primers used for Sanger sequencing of the PYDC1 and PYDC2 genes are listed in [Supplementary material 1](#). After PCR amplification, the products were purified, and sequencing reactions were performed (Macrogen Europe). After completing the electrophoresis process, the samples were analyzed using the “Sequence Analysis” program. Sequence comparisons and analyses were conducted using the MutationSurveyor 1.2 program.

## Statistical analysis

The sample size was calculated using a power analysis, achieving 95% confidence level. Hardy–Weinberg equilibrium was assessed for

each evaluated SNP. The student's t-test was employed to compare means of continuous variables. The Chi-square test was used to compare mutations and allele frequencies among groups and clinical features within subgroups categorized by endometrioma size. Statistical analyses were conducted using IBM SPSS version 26.0, with a *p*-value of <0.05 accepted as statistically significant.

## Results

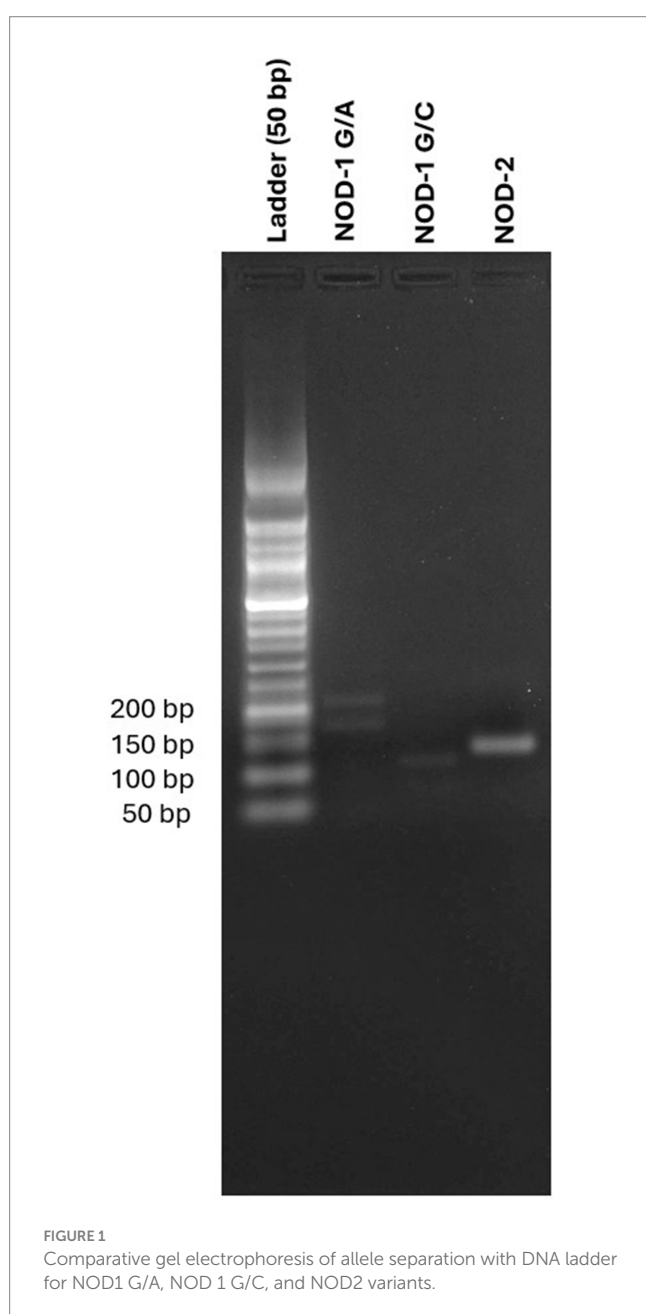
There were no significant differences between endometriosis patients and control subjects regarding age ( $39.28 \pm 8.22$  vs.  $39.31 \pm 7.86$ ), BMI ( $23.1 \pm 1.4$  vs.  $22.8 \pm 1.5$ ), and age at menarche ( $12.4 \pm 1.6$  vs.  $12.1 \pm 1.8$ ) ( $p > 0.05$ ). Out of the patients studied, 54 had ovarian endometriosis. Of these, 42 patients (77.8%) had unilateral ovarian endometriosis, while 12 patients (22.2%) had bilateral involvement. In the endometriosis group, 48 patients (88.9%) underwent first-time surgery, and 6 patients (11.1%) had recurrent endometriomas.

The symptoms reported by patients with endometriosis included dysmenorrhea 38 (70.4%), dyspareunia 28 (51.9%), perimenstrual gastrointestinal system (GIS) complaints 23 (42.6%), ovulatory pain 18 (33.3%), menorrhagia 18 (33.3%), perimenstrual genitourinary system (GUS) complaints 10 (18.5%). Infertility was present in 13 patients (24.1%), with 9 patients (16.7%) experiencing primary infertility and 4 patients (7.4%) experiencing secondary infertility (Table 1).

There were no significant differences in allele frequencies between endometriosis and control subjects for NOD1 rs2075820 (G vs. A) ( $p = 0.89$ ) and rs2075818 (G vs. C) ( $p = 0.89$ ). A statistically significant difference in the distribution of the rs2075820 (NOD1 G/A) genotypes was observed between endometriosis patients and control subjects. The GG wild-type genotype was found to be significantly more prevalent in the endometriosis group 17 (31.5%) compared to the control group 11 (20.3%) ( $p = 0.04$ ). Conversely, the GA genotype was significantly less common among endometriosis patients 28 (51.9%) than in controls 39 (72.2%) ( $p = 0.029$ ). Although the AA genotype was more frequent in endometriosis patients 9 (16.6%) than in control subjects 4 (7.5%), this difference did not reach statistical significance ( $p = 0.13$ ) (Table 2).

No significant differences were detected when evaluating the NOD1 (rs2075818) genotypes between endometriosis patients and control subjects. The frequencies of the GG genotype were identical in both groups (13% vs. 13%;  $p = 0.54$ ). Similarly, the distribution of the GC genotype (68.5% in endometriosis patients vs. 72.2% in controls;  $p = 0.67$ ) and the CC genotype (18.5% in endometriosis patients vs. 14.8% in controls;  $p = 0.6$ ) showed no significant differences (Table 2). No polymorphisms were detected at the NOD2 (rs104895461) and PYDC1 genes. PYDC2 rs293833 (c.242A>G) variant was detected in 12 endometriosis patients (22.2%).

We also evaluated the association of three polymorphisms in the NOD1, NOD2, and PYDC2 genes with the clinical manifestations of endometriosis. The NOD1 rs2075820 AA genotype was associated with significantly higher rates of perimenstrual GIS symptoms 8 (88.9%) compared to other NOD1 rs2075820 genotypes 17 (37.8%) ( $p = 0.005$ ). Additionally, infertility was significantly more common in patients with the AA genotype 5 (55.5%) compared to those with other genotypes 8 (17.8%) ( $p = 0.037$ ) (Table 3).



**FIGURE 1**  
Comparative gel electrophoresis of allele separation with DNA ladder for NOD1 G/A, NOD 1 G/C, and NOD2 variants.

TABLE 1 Characteristics of ovarian endometriosis patients.

| Characteristics of ovarian endometriosis group | n (%)     |
|------------------------------------------------|-----------|
| Unilateral Endometrioma                        | 42 (77.8) |
| Bilateral Endometrioma                         | 12 (22.2) |
| AAGL                                           |           |
| Stage 2                                        | 16 (29.6) |
| Stage 3                                        | 34 (63)   |
| Stage 4                                        | 4 (7.4)   |
| rASRM                                          |           |
| Stage 3                                        | 44 (81.5) |
| Stage 4                                        | 10 (18.5) |
| First-time endometriosis surgery               | 48 (88.9) |
| Recurrent endometriosis surgery                | 6 (11.1)  |
| Medical treatment                              |           |
| NSAIDs                                         | 39 (72.2) |
| OCPs                                           | 20 (37.0) |
| Oral Progestins                                | 16 (29.6) |
| GnRH Agonists                                  | 2 (3.7)   |
| LNG-IUD                                        | 4 (7.4)   |
| Clinical complaints                            |           |
| Dysmenorrhea                                   | 38 (70.4) |
| Ovulatory pain                                 | 18 (33.3) |
| Menorrhagia                                    | 18 (33.3) |
| Dyspareunia                                    | 28 (51.9) |
| Perimenstrual GIS complaints                   | 23 (42.6) |
| Perimenstrual GUS complaints                   | 10 (18.5) |
| Infertility                                    | 13 (24.1) |
| Primary infertility                            | 9 (16.7)  |
| Secondary infertility                          | 4 (7.4)   |

AAGL, the American Association of Gynecologic Laparoscopists; rASRM, the revised American Society for Reproductive Medicine; NSAIDs, Nonsteroidal Anti-inflammatory Drugs; OCPs, Combined Oral Contraceptive Pills; LNG-IUD, Levonorgestrel-releasing intrauterine system; GIS, Gastrointestinal; GUS, Genitourinary. \*This table provides a comprehensive overview of the characteristics observed in patients with ovarian endometriosis, including the distribution of unilateral and bilateral endometriomas, surgical stages, treatment history, and clinical complaints.

PYDC2 rs293833 (c.242A>G) positive patients exhibited a lower incidence of dysmenorrhea compared to negative patients (41.7% vs. 78.6%;  $p = 0.014$ ). Moreover, perimenstrual gastrointestinal symptoms were significantly more prevalent in positive patients (83.3% vs. 35.7%;  $p = 0.004$ ). Additionally, PYDC2-positive patients had significant differences in infertility and the presence of larger endometriomas. Infertility rates were markedly higher in positive patients (66.6% vs. 11.9%;  $p = 0.001$ ), and large endometriomas were more frequently observed in positive patients (90.9% vs. 62%;  $p < 0.001$ ) (Table 4).

## Discussion

Genome-wide association studies (GWAS) have described that ovarian endometriosis partly contributes to the larger effect sizes

TABLE 2 NOD1 (rs2075820 and rs2075818) allele frequencies and genotypes.

|                      | Endometriosis | Control      | <i>p</i>     |
|----------------------|---------------|--------------|--------------|
|                      | <i>n</i> (%)  | <i>n</i> (%) |              |
| NOD 1 (rs2075820)    |               |              |              |
| Allele frequencies   |               |              |              |
| G                    | 62 (57.4)     | 61 (56.4)    | 0.89         |
| A                    | 46 (42.6)     | 47 (43.6)    |              |
| Genotype frequencies |               |              |              |
| GG                   | 17 (31.5)     | 11 (20.3)    | <b>0.04</b>  |
| GA                   | 28 (51.9)     | 39 (72.2)    | <b>0.029</b> |
| AA                   | 9 (16.6)      | 4 (7.5)      | 0.13         |
| NOD 1 (rs2075818)    |               |              |              |
| Allele frequencies   |               |              |              |
| G                    | 51 (47.2)     | 53 (49)      | 0.89         |
| C                    | 57 (52.8)     | 55 (51)      |              |
| Genotype frequencies |               |              |              |
| GG                   | 7 (13)        | 7 (13)       | 0.54         |
| GC                   | 37 (68.5)     | 39 (72.2)    | 0.67         |
| CC                   | 10 (18.5)     | 8 (14.8)     | 0.60         |

A, Adenine; G, Guanine; C, Cytosine. \*The Chi-square test. \*\*An allele refers to a variant form of a gene. In this context, each individual has two alleles for each gene—one inherited from each parent. A genotype refers to the combination of alleles an individual possesses for a particular gene. For a gene with two possible alleles (like G and A or G and C), the possible genotypes are: Homozygous for one allele (GG, AA, or CC). Heterozygous (GA and GC). Bold values represent statistically significant results ( $p < 0.05$ ).

observed in ASRM Stage 3-4, indicating a genetic basis distinct from other disease manifestations (18). In this study, we hypothesized that genetic factors may play a role in the pathophysiology of ovarian endometriosis. This study aimed to assess the genetic predisposition to the development and characteristics of this disease, focusing on the presence of four specific inflammasome-related polymorphisms: NOD1 (rs2075820 and rs2075818), NOD2 (rs104895461), PYDC1, and PYDC2 gene polymorphisms. This is the first report to detail the analysis of gene polymorphisms for these genes in endometriosis.

Previously, NOD1 and NOD2 genes were assessed for their potential predisposition to endometrial cancer; however, no associations were observed (19). Our study revealed that the NOD1 rs2075820 had lower (G>A) genotypes in endometriosis patients when compared with the control group. A pro-apoptotic protein NOD1 can trigger apoptosis through interactions with the caspase pathway whereas NF- $\kappa$ B serves to suppress the apoptotic process (20). NOD proteins can initiate signaling pathways involving both NF- $\kappa$ B and caspase in endometriosis. On the other hand, the allele frequencies of G and A in NOD1 rs2075820 did not differ significantly. Other studies revealed that the presence of the A allele of rs2075820 correlated with decreased expression and activation of NF- $\kappa$ B when intracellular *Propionibacterium acnes* (*P. acnes*) infection present in the Japanese population (21).

A few studies investigated the expression of NODs in the female reproductive tract. NOD1 and NOD2 are differentially expressed and regulated in the human endometrium, playing roles in the innate immune response and potentially in the inflammatory events



**TABLE 3 NOD1 rs2075820 gene polymorphism analysis according to the recessive model.**

|                            | GG + GA       | AA            | <i>p</i>     |
|----------------------------|---------------|---------------|--------------|
|                            | <i>n</i><br>% | <i>n</i><br>% |              |
| Unilateral endometrioma    | 35 (77.8)     | 7 (77.8)      | 0.99         |
| Bilateral endometrioma     | 10 (22.2)     | 2 (22.2)      | 0.99         |
| Dysmenorrhea               | 34 (75.6)     | 4 (44.4)      | 0.06         |
| Ovulatory Pain             | 17 (37.8)     | 1 (11.1)      | 0.12         |
| Menorrhagia                | 16 (35.6)     | 2 (22.2)      | 0.43         |
| Dyspareunia                | 24 (53.3)     | 4 (44.4)      | 0.62         |
| Perimenstrual GIS symptoms | 17 (37.8)     | 8 (88.9)      | <b>0.005</b> |
| Perimenstrual GUS symptoms | 9 (20.0)      | 1 (11.1)      | 0.53         |
| Infertility                | 8 (17.8)      | 5 (55.5)      | <b>0.037</b> |
| <b>Endometrioma size</b>   |               |               |              |
| Small endometrioma (<4 cm) | 12 (26.7)     | 5 (55.5)      | 0.08         |
| Large endometrioma (≥4 cm) | 33 (73.3)     | 4 (44.5)      |              |

GIS, Gastrointestinal; GUS, Genitourinary. \*The Chi-square tests. \*\*This table provides a comparison of clinical characteristics and endometrioma sizes in patients with different genotypes (GG + GA vs. AA) for a specific polymorphism, with *p*-values indicating statistical significance for each comparison. Additionally, analysis for the G and C polymorphism (GG + GC vs. CC) was not included due to the absence of significant results. Bold values represent statistically significant results (*p* < 0.05).

**TABLE 4 PYDC2 gene polymorphism analysis for endometriosis patients.**

|                            | PYDC2 rs293833 (c.242A > G) |              |                  |
|----------------------------|-----------------------------|--------------|------------------|
|                            | Positive                    | Negative     | <i>p</i>         |
|                            | <i>n</i> (%)                | <i>n</i> (%) |                  |
| Unilateral endometrioma    | 9 (75)                      | 33 (78.6)    | 0.79             |
| Bilateral endometrioma     | 3 (25)                      | 9 (21.4)     | 0.79             |
| Dysmenorrhea               | 5 (41.7)                    | 33 (78.6)    | <b>0.014</b>     |
| Ovulatory Pain             | 6 (50)                      | 12 (28.6)    | 0.16             |
| Menorrhagia                | 2 (16.7)                    | 16 (38.1)    | 0.16             |
| Dyspareunia                | 7 (58.3)                    | 21 (50)      | 0.61             |
| Perimenstrual GIS symptoms | 10 (83.3)                   | 15 (35.7)    | <b>0.004</b>     |
| Perimenstrual GUS symptoms | 2 (16.7)                    | 8 (19)       | 0.85             |
| Infertility                | 8 (66.6)                    | 5 (11.9)     | <b>0.001</b>     |
| <b>Endometrioma size</b>   |                             |              |                  |
| Small endometrioma (<4 cm) | 1 (9.1)                     | 16 (38)      |                  |
| Large endometrioma (≥4 cm) | 11 (90.9)                   | 26 (62)      | <b>&lt;0.001</b> |

GIS, Gastrointestinal; GUS, Genitourinary. \*The Chi-square test. \*\*This table compares clinical characteristics and endometrioma sizes between patients with positive and negative PYDC2 rs293833 (c.242A > G) polymorphism, with associated *p*-values indicating statistical significance. Bold values represent statistically significant results (*p* < 0.05).

associated with menstruation with interleukins (22). In another study, ectopic endometrial stromal cells showed increased levels of NOD1 expression and interleukin-8, while the NOD1 inhibitor ML-130 suppressed proliferation, clonal expansion, invasion, and migration of these cells without impacting apoptosis (23). The pathophysiological mechanism behind diminished ovarian reserve in endometriosis

remains unclear. It is debated whether endometriomas reduce functional tissue through mechanical stretching (space-occupying effect) or direct inflammatory impact. Ovarian endometriomas contain immune components like reactive oxygen species (ROS), metalloproteinases, and cytokines, which may progressively damage the ovarian stroma and reduce the primordial follicular reserve over time (24).

Ovarian endometriosis poses a challenge to ovarian reserve, though the extent of its uniform impact on reserve remains debated. A retrospective study on women with ovarian endometriomas (mean diameter 26 ± 8 mm) undergoing multiple ovarian stimulation cycles found consistent oocyte retrieval rates from affected ovaries across cycles, at 44% for both initial and subsequent cycles. Another study reported a statistically significant 26% decrease in anti-müllerian hormone (AMH) levels over six months in 40 women with endometriomas (mean diameter 46 ± 17 mm), indicating a progressive decline in ovarian reserve (25).

Ovarian endometrioma size has been studied in relation to ovarian stimulation, with a 4 cm diameter threshold commonly used to indicate potential impact on ovarian response. Generally, small cysts have minimal effects, while larger cysts can significantly affect ovarian function. Our findings reveal that the NOD1 rs2075820 AA phenotype and PYDC2 rs293833 (c.242A > G) polymorphism are strongly associated with female infertility. Additionally, PYDC2 rs293833 (c.242A > G) correlates with larger endometriomas (≥4 cm). Subgroup analysis supports GWAS recommendations for assessing genetic variations, particularly in cases with larger ovarian cysts and severe endometriosis, to improve reproductive outcomes.

The primary treatments for endometriosis include surgery and pharmacological options like hormone therapy and NSAIDs for pain management. Surgical excision can improve symptoms and fertility; however, recent reviews show recurrence rates of 21.5% at 2 years and 40–50% at 5 years, indicating that recurrences and repeat surgeries may exacerbate pain and further reduce fertility (26).

Therefore, regular and long-term medication use is recommended to prevent postoperative recurrence of endometriosis. However, hormone therapies, due to estrogen's role in endometriosis development, may suppress follicular development and ovulation, making treatment challenging for women seeking pregnancy. NLRs are hypothesized as promising therapeutic targets for addressing inflammation-associated endometriosis via their pivotal role in innate immunity (10). NLR family pyrin domain containing 3 (NLRP3) and NLR family CARD domain containing 5 (NLRC5) have prominent improving effects on endometriosis with altering fibrosis and inflammation in previous studies (27, 28).

The NLRP3/IL-1β pathway plays a role in endometriosis development, and NLRP3 inhibitors may help reduce ovarian endometrioma size and improve ovarian function (29). In a study, increased NOD1 expression and inflammatory cytokines in ectopic endometrial cells in peritoneal fluid, with the NOD1 inhibitor ML130 significantly reducing cell viability and cytokine production (30). Furthermore, mifepristone has been shown to exhibit protective effects against NLRP1 inflammasome activation and to minimize damage to hippocampal neurons caused by dexamethasone (31). Thus, strategies targeting the inflammasome axis may serve as potential therapeutic options for treating endometriosis.

Women with pelvic endometriosis often experience pain due to pelvic visceral hypersensitivity, along with abdominal and



pelvic discomfort. Studies show that the inflammatory microenvironment within ectopic lesions activates sensory nerve endings through inflammatory mediators, amplifying pain signal transmission (32). This hypothesis is reinforced by fluctuations in cyclic inflammatory markers during the menstrual cycle, which correlate with heightened gastrointestinal symptoms. The overlap between endometriosis and irritable bowel syndrome (IBS)—more commonly diagnosed in women with pelvic endometriosis—adds complexity to interpreting gastrointestinal symptoms. Additionally, endometriosis patients show lower pain thresholds in response to bowel distension and other gastrointestinal triggers (33, 34). In another study, NOD1 rs2075820 was not associated with inflammatory bowel disease in the Turkish population (35).

Our findings suggest that NOD1 rs2075820 AA phenotype and PYDC2 rs293833 (c.242A>G) polymorphism is strongly associated with increased gastrointestinal complaints in ovarian endometriosis patients. The localization of ovarian endometriosis in areas closely related to the terminal parts of the colon, along with its inflammatory characteristics and local factors such as prostaglandin release, may explain the increased incidence of gastrointestinal complaints in endometriosis patients. However, painful symptoms associated with deep infiltrative endometriosis (DIE) may also cause pain characteristics, often specific to precise anatomical locations or affected organs, such as severe deep dyspareunia or painful defecation. A limitation of the study includes the potential for more robust results if the sample size for subgroup analysis is increased, even though the sample size was previously calculated specifically for ovarian endometriosis. On the other hand, to the best of our knowledge, this is the first study to evaluate endometriomas with their sizes and genetic profiles together. Obtaining significant differences between these groups may provide valuable insights for further studies.

## Conclusion

Our study shows a correlation between genetic predispositions, inflammatory pathways, and the clinical manifestations of ovarian endometriosis. By investigating specific inflammasome-related polymorphisms, NOD1, and PYDC2 gene variants, we have uncovered potential associations with infertility and gastrointestinal complaints in affected individuals. These findings imply that the inflammatory microenvironment substantially influences infertility, particularly through pathways associated with the inflammasome complexes. The importance of considering genetic variations is shown in the evaluation and management of endometriosis, especially in subgroups characterized by severe disease phenotypes. Moreover, our results highlight the complex nature of endometriosis pathophysiology, implicating not only mechanical and inflammatory processes but also genetic factors in disease progression and symptomatology.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material. Further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving humans were approved by Health Research Ethics Committee of Dokuz Eylul University (7511-GOA). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

## Author contributions

HK: Conceptualization, Data curation, Funding acquisition, Methodology, Visualization, Writing – original draft. BB: Investigation, Methodology, Software, Writing – review & editing. TT: Data curation, Validation, Writing – original draft. PY: Data curation, Writing – original draft. OY: Data curation, Writing – original draft. SK: Methodology, Project administration, Writing – review & editing. EU: Conceptualization, Validation, Writing – original draft. CP: Formal analysis, Funding acquisition, Investigation, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2024.1495002/full#supplementary-material>

## References

- Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol.* (2014) 10:261–75. doi: 10.1038/nrendo.2013.255
- Sampson JA. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol.* (1927) 14:422–69. doi: 10.1016/S0002-9378(15)30003-X
- Bonavina G, Taylor HS. Endometriosis-associated infertility: from pathophysiology to tailored treatment. *Front Endocrinol.* (2022) 13:1020827. doi: 10.3389/fendo.2022.1020827
- Dalsgaard T, Hjordt Hansen MV, Hartwell D, Lidegaard O. Reproductive prognosis in daughters of women with and without endometriosis. *Hum Reprod.* (2013) 28:2284–8. doi: 10.1093/humrep/det231
- Hadfield RM, Mardon HJ, Barlow DH, Kennedy SH. Endometriosis in monozygotic twins. *Fertil Steril.* (1997) 68:941–2. doi: 10.1016/S0015-0282(97)00359-2
- Somigliana E, Li Piani L, Paffoni A, Salmeri N, Orsi M, Benaglia L, et al. Endometriosis and IVF treatment outcomes: unpacking the process. *Reprod Biol Endocrinol.* (2023) 21:107. doi: 10.1186/s12958-023-01157-8
- Defrère S, González-Ramos R, Lousse JC, Colette S, Donnez O, Donnez J, et al. Insights into iron and nuclear factor-kappa B (NF-kappaB) involvement in chronic inflammatory processes in peritoneal endometriosis. *Histol Histopathol.* (2011) 26:1083–92. doi: 10.14670/HH-26.1083
- Wilmansky JM, Petnicki-Ocwieja T, Kobayashi KS. NLR proteins: integral members of innate immunity and mediators of inflammatory diseases. *J Leukoc Biol.* (2008) 83:13–30. doi: 10.1189/jlb.0607402
- Strober W, Murray PJ, Kitani A, Watanabe T. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat Rev Immunol.* (2006) 6:9–20. doi: 10.1038/nri1747
- Guo B, Chen J, Zhang JH, Fang Y, Liu XJ, Zhang J, et al. Pattern-recognition receptors in endometriosis: a narrative review. *Front Immunol.* (2023) 14:14. doi: 10.3389/fimmu.2023.1161606
- Porter KA, Duffy EB, Nyland P, Atianand MK, Sharifi H, Harton JA. The CLRX.1/NOD24 (NLRP2P) pseudogene codes a functional negative regulator of NF-κB, pyrin-only protein 4. *Genes Immun.* (2014) 15:392–403. doi: 10.1038/gene.2014.30
- Zerrad C, Lkhider M, Bouqdayr M, Belkouchi A, Badre W, Tahiri M, et al. NOD1 and NOD2 genetic variants: impact on hepatocellular carcinoma susceptibility and progression in Moroccan population. *Gene.* (2024) 931:148847. doi: 10.1016/j.gene.2024.148847
- Falconer H, D'Hooghe T, Fried G. Endometriosis and genetic polymorphisms. *Obstet Gynecol Surv.* (2007) 62:616–28. doi: 10.1097/01.ogx.0000279293.60436.60
- Tempfer CB, Simoni M, Destenaves B, Fauser BCJM. Functional genetic polymorphisms and female reproductive disorders: part II—endometriosis. *Hum Reprod Update.* (2008) 15:97–118. doi: 10.1093/humupd/dmn040
- American Society. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril.* (1997) 67:817–21. doi: 10.1016/S0015-0282(97)81391-X
- Abrao MS, Andres MP, Miller CE, Gingold JA, Rius M, Neto JS, et al. AAGL 2021 endometriosis classification: an anatomy-based surgical complexity score. *J Minim Invasive Gynecol.* (2021) 28:1941–1950.e1. doi: 10.1016/j.jmig.2021.09.709
- Kocaaga A, Cakmak Genc G, Karakas Celik S, Koca R, Dursun A. Association of NOD1, NOD2, PYDC1 and PYDC2 genes with Behcet's disease susceptibility and clinical manifestations. *Ophthalmic Genet.* (2021) 42:691–7. doi: 10.1080/13816810.2021.1955273
- Rahmioglu N, Nyholt DR, Morris AP, Missmer SA, Montgomery GW, Zondervan KT. Genetic variants underlying risk of endometriosis: insights from meta-analysis of eight genome-wide association and replication datasets. *Hum Reprod Update.* (2014) 20:702–16. doi: 10.1093/humupd/dmu015
- Ashton KA, Proietto A, Otton G, Symonds I, McEvoy M, Attia J, et al. Toll-like receptor (TLR) and nucleosome-binding oligomerization domain (NOD) gene polymorphisms and endometrial cancer risk. *BMC Cancer.* (2010) 10:382. doi: 10.1186/1471-2407-10-382
- Kasimsetty SG, Hawkes A, Barekatin K, Soo E, Welch AK, McKay DB. TLR2 and NODs1 and 2 cooperate in inflammatory responses associated with renal ischemia reperfusion injury. *Transpl Immunol.* (2020) 58:101260. doi: 10.1016/j.trim.2019.101260
- Tanabe T, Ishige I, Suzuki Y, Aita Y, Furukawa A, Ishige Y, et al. Sarcoidosis and NOD1 variation with impaired recognition of intracellular *Propionibacterium acnes*. *Biochim Biophys Acta.* (2006) 1762:794–801. doi: 10.1016/j.bbdis.2006.07.006
- King AE, Horne AW, Hombach-Klonisch S, Mason JI, Critchley HOD. Differential expression and regulation of nuclear oligomerization domain proteins NOD1 and NOD2 in human endometrium: a potential role in innate immune protection and menstruation. *Mol Hum Reprod.* (2009) 15:311–9. doi: 10.1093/molehr/gap020
- Li C, Luo S, Guo A, Su Y, Zhang Y, Song Y, et al. Human endometrium derived mesenchymal stem cells with aberrant NOD1 expression are associated with ectopic endometrial lesion formation. *Int J Stem Cells.* (2024) 17:309–18. doi: 10.15283/ijsc22200
- Sanchez AM, Viganò P, Somigliana E, Panina-Bordignon P, Vercellini P, Candiani M. The distinguishing cellular and molecular features of the endometriotic ovarian cyst: from pathophysiology to the potential endometrioma-mediated damage to the ovary. *Hum Reprod Update.* (2014) 20:217–30. doi: 10.1093/humupd/dmt053
- Kasapoglu I, Ata B, Uyaniklar O, Seyhan A, Orhan A, Yildiz Oguz S, et al. Endometrioma-related reduction in ovarian reserve (ERROR): a prospective longitudinal study. *Fertil Steril.* (2018) 110:122–7. doi: 10.1016/j.fertnstert.2018.03.015
- Koga K, Takamura M, Fujii T, Osuga Y. Prevention of the recurrence of symptom and lesions after conservative surgery for endometriosis. *Fertil Steril.* (2015) 104:793–801. doi: 10.1016/j.fertnstert.2015.08.026
- Guo C, Fu R, Wang S, Huang Y, Li X, Zhou M, et al. NLRP3 inflammasome activation contributes to the pathogenesis of rheumatoid arthritis. *Clin Exp Immunol.* (2018) 194:231–43. doi: 10.1111/cei.13167
- Wang Y, Huang C, Bian E, Lei T, Lv X, Li J. NLRP5 negatively regulates inflammatory responses in LPS-induced acute lung injury through NF-κB and p38 MAPK signal pathways. *Toxicol Appl Pharmacol.* (2020) 403:115150. doi: 10.1016/j.taap.2020.115150
- Murakami M, Osuka S, Muraoka A, Hayashi S, Bayasula X, Kasahara Y, et al. Effectiveness of NLRP3 inhibitor as a non-hormonal treatment for ovarian endometriosis. *Reprod Biol Endocrinol.* (2022) 20:924. doi: 10.1186/s12958-022-00924-3
- Wei X, Liu Y, Li W, Shao X. Nucleotide-binding oligomerization domain-containing protein 1 regulates inflammatory response in endometriosis. *Curr Protein Pept Sci.* (2022) 23:31. doi: 10.2174/1389203723666220322125031
- Zhang B, Zhang Y, Xu T, Yin Y, Huang R, Wang Y, et al. Chronic dexamethasone treatment results in hippocampal neurons injury due to activate NLRP1 inflammasome in vitro. *Int Immunopharmacol.* (2017) 49:222–30. doi: 10.1016/j.intimp.2017.05.039
- Patel BG, Lenk EE, Lebovic DI, Shu Y, Yu J, Taylor RN. Pathogenesis of endometriosis: interaction between endocrine and inflammatory pathways. *Best Pract Res Clin Obstet Gynaecol.* (2018) 50:50–60. doi: 10.1016/j.bpobgyn.2018.01.006
- Seaman HE, Ballard KD, Wright JT, de Vries CS. Endometriosis and its coexistence with irritable bowel syndrome and pelvic inflammatory disease: findings from a national case-control study—part 2. *BJOG.* (2008) 115:1392–6. doi: 10.1111/j.1471-0528.2008.01879.x
- Issa B, Onon TS, Agrawal A, Shekhar C, Morris J, Hamdy S, et al. Visceral hypersensitivity in endometriosis: a new target for treatment? *Gut.* (2012) 61:367–72. doi: 10.1136/gutjnl-2011-300306
- Ozen SC, Dagli U, Kiliç MY, Törün M, Celik Y, Ozkan M, et al. NOD2/CARD15, NOD1/CARD4, and ICAM-1 gene polymorphisms in Turkish patients with inflammatory bowel disease. *J Gastroenterol.* (2006) 41:304–10. doi: 10.1007/s00535-005-1780-z



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# Scientific landscape and visualization analysis of the link between adenomyosis and infertility from 2000 to 2024

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**Objectives:** Adenomyosis (AM) is a chronic disorder that significantly impacts women's health and quality of life worldwide, particularly by causing progressive impairment in fertility. This study aimed to summarize and visualize the literature concerning AM-associated infertility using scientometric analysis.

**Methods:** We conducted a literature search in the Web of Science™ Core Collection (WoSCC) database for “adenomyosis” and “infertility” as topics from 2000 to 2024. The collected data were organized in Microsoft Office Excel for further analysis. Bibliometric analyses and visualizations were performed using Origin, CiteSpace, VOSviewer, and the Bibliometrix package.

**Results:** A total of 456 articles were published across 153 journals, reflecting a growing trend in both published and cited articles. The scholars with the highest output were Petraglia F., Chapron C., and Pellicer A., while the Fertility and Sterility were the most publications' journal. China, the United States, and Italy ranked as the top three countries globally regarding relevant publications worldwide. The 190 keywords in the literature were divided into eight clusters primarily related to pathogenesis, adverse factors affecting pregnancy, treatment methods, diagnostic methods, disease progression, *in vitro* fertilization (IVF) management, infertility in women, and fertility management. Current hotspots in this field include investigating potential mechanisms of pathogenesis, diagnostic strategies, and improving pregnancy outcomes for patients with AM-associated infertility.

**Conclusion:** This study highlights that infertility is the most significant and complex issue associated with AM. Although chronic disease management strategies, pharmacological treatments, and assisted reproductive technologies (ART) have improved fertility outcomes in women with AM, further clinical translational research is still warranted.

## KEYWORDS

adenomyosis, infertility, association, bibliometric analysis, CiteSpace, research trends

# 1 Introduction

Adenomyosis (AM) is a prevalent and chronic condition affecting reproductive-aged women. Pathologically, it is similar to endometriosis and is characterized by the benignly infiltrate of endometrial glands and stroma into the underlying myometrium, leading to progressive uterine enlargement (1, 2). The most common clinical manifestations of AM are abnormal uterine bleeding associated with anemia, chronic pelvic pain (such as dysmenorrhea and dyspareunia), infertility, and an increased risk of adverse pregnancy outcomes, all of which seriously affect the quality of women's lives in their reproductive age (3, 4).

In recent years, the incidence of AM has risen, with a notable trend of the younger women being affected, and an increasing number of AM patients are of childbearing age with seeking fertility needs. Alarming, 19.5% of AM patients experience infertility (5), with over 80% of infertile patients attributed to AM and more than 30% of these individuals having previously failed assisted reproductive technology (ART) treatments (6). Moreover, female infertility and subfertility present complex challenges, accompanied by substantial economic burden and profound psychosocial effects (7), including elevated levels of anxiety and depression (8). Therefore, understanding the mechanisms through which AM impacts fertility has garnered significant scholarly attention, elucidating these pathways is critical for developing accurately targeted treatment strategies.

Despite extensive research on AM-associated infertility, there remains a scarcity articles that offer preliminary insight into its pathogenesis. The exact pathogenesis underlying AM's impact on fertility have yet to be fully elucidated, hindering the development of targeted therapies and presenting an enormous scientific challenge for researchers. Consequently, a comprehensive big data analysis of the pathogenesis, research progress, trends, and focal points concerning AM-associated infertility is essential. This effort not only facilitates the generation of innovative research ideas but also fosters collaborative global initiatives aimed at overcoming the identified challenges (9).

Bibliometrics, a field that qualitatively and quantitatively analyzes academic publishing, employs mathematical and statistical method to assess published works within specific disciplines (10). Recently, scientometric analysis and data visualization have emerged as valuable methodologies, extensively applied across various biomedical sciences and public health disciplines (11, 12). Compared to the traditional literature reviews, scientometrics with its visual capabilities offers advantages in quickly identifying research hotspots, critical issues, and guiding future exploration within exciting fields (13–15). For instance, Jin et al. (16) employed bibliometrics techniques to reveal gaps, traditional focal points, and potential prospects in menopausal syndrome research, clarifying future research directions for investigators. Despite the emergence of several literature reviews and meta-analyses on AM-associated infertility in the last two decades, there has been a notable lacking in scientometric studies exploring the link between AM and infertility.

To fill the apparent gap in knowledge, our study conducted bibliometrics analysis for drawing scientific knowledge maps and generating data visualization to reveal the relationship between AM and infertility by using multiple software tools. The statistical results of the keyword analysis were analyzed and summarized, which included publication year, countries and regions, institutions, authors,

journals, relevant references, timeline view, and keyword co-occurrence and citation burst analysis from 2000 to 2024. This study aims to elucidate research trends and core challenges in AM-associated infertility, ultimately providing new perspectives and ideas for future investigations and attracting increased attention from scientific community.

# 2 Materials and methods

## 2.1 Data retrieval and extraction

We utilized the Web of Science Core Collection (WoSCC) of the Science Citation Index Expanded (SCIE) to retrieve and download the citation data on May 29th, 2024. The WoS database is recognized as one of the most authoritative and comprehensive citation databases, frequently employed for bibliometric studies due to its inclusion of nearly all impactful and high-quality journals, as well as its extensive data sources (17–19). Furthermore, previous studies have shown that the WoS for is more accurate than other databases literature-type labeling (13, 20). We chosen “Adenomyosis” and “Infertility” as our search terms. The retrieval formula used was as follows: [#1 was “Adenomyosis” OR “Adenomyomectomy” OR “Adenomyosis uteri” OR “Cystic Adenomyosis” OR “Diffuse Adenomyosis” OR “Focal Adenomyosis” OR “Uterine Adenomyosis.” #2 was “Infertility” OR “Impaired fecundity” OR “Diminished semen quality” OR “Reproductive failure” OR “Fertility impairment” “Barrenness” OR “Sterility.” Final dataset was constructed as follows: #1 AND #2]. The topical terms were restricted to the title, abstract, or keywords. The retrieval time range was from January 1st, 2000, to May 29th, 2024, with the search limited to the English languages and document types restricted to articles and reviews. A total of 456 pieces of literature were retrieved. The matching citation data were output as “Full Record and Cited References” and saved in “Plain Text” format.

## 2.2 Analysis method

Microsoft Office Excel 2019 was utilized to store and manage the relevant data. Subsequently, the pertinent data were subjected to further visualization analysis using OriginPro 2023, CiteSpace (version 6.1.R6), VOSviewer (version 1.6.20), and the Bibliometrix package.<sup>1</sup>

Origin software was employed to analyze and map the number of annual publications, providing an intuitive understanding of the trends in the volume of research papers (21). CiteSpace was initially utilized for bibliometric analysis, encompassing country/regions, organization, category, cited journal, keyword, and reference (22). CiteSpace is a robust visualization tool that aids in identifying trends and hotspots within research fields by analyzing citation networks and exhibiting relationships between publications, including collaboration networks and keyword co-occurrence. Its broad user community, regular feature updates, cross-platform compatibility, and free accessibility make it the preferred software for bibliometric analysis

<sup>1</sup> [www.bibliometrix.org/home/](http://www.bibliometrix.org/home/)



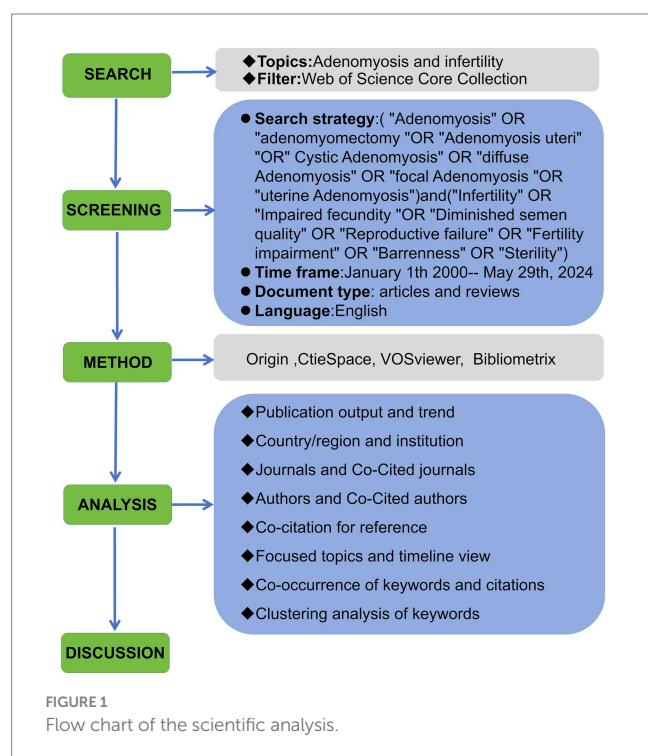
(23). VOSviewer was used to optimize and visualize the scientific knowledge graph (24). Known for its versatility and user-friendly interface, VOSviewer excels in producing high-quality visualizations and offers extensive customization options. It efficiently processes large datasets, integrates seamlessly with major bibliometric databases, and includes text mining capabilities. Additionally, the software benefits from strong community support and comprehensive documentation, making it an invaluable tool for researchers (25). Bibliometrix provides a comprehensive analysis features for conducting the mapping of the co-occurrence network and clustering of keywords, enabling researchers to explore various aspects of scholarly communication (26). In all visualization networks, the size of the node represents the number of publications, the color of the node indicates different periods or clusters, and the thickness of the lines reflects the correlation's strength.

The impact factor (IF) and *H*-index were included in the data table to help objectively assess the reliability and value of the journal and article research. The IF serves as a critical indicator for measuring the influence and prestige of academic journals (27), while the *H*-index evaluates scholarly contributions and predict future research accomplishments (28). To avoid bias, given that the database is updated daily, both authors individually conducted a comprehensive online search and analysis within a single day. The strategy of literature retrieval and scientometric analysis is shown in Figure 1.

## 3 Results

### 3.1 Annual publication output and trend

A total of 456 articles on AM-associated infertility from 2000 to 2024 were identified. The annual publication count is exhibited in Figure 2A. Despite some fluctuations in annual publications, the



overall trend has been upward. Before 2011, published documents were primarily in the single digits, from 2012 onward, publications consistently remained in double digits. The peak occurred in 2023, with 71 publications. Numerous publishers contributed literature across various subject categories, with the top 10 publishers and categories listed in Table 1. The largest publishers are Elsevier (130); the most common research category is obstetrics and gynecology (265). Figure 2B shows the annual citation counts, totaling 13,426 citations across retrieved articles, resulting in an average number of 29.44 citations per paper. The *H*-index for screened publications was 62, indicating a steady upward trend in annual citations. In 2023, citations peaked at 2,375. Notably, the most substantial research output and citation frequency increase occurred between 2019 to 2023.

### 3.2 Distribution of countries/regions and institutions

The publications involved 51 countries/regions and 123 institutions. The top 10 countries/regions by total published papers are shown in Table 2. China led with 25.44% (116 articles), followed by the USA (17.54%, 80 articles), Italy (14.69%, 67 articles), France (8.77%, 40 articles), and Japan (7.46%, 34 articles). Figures 3A,B depict the top-ranking countries regarding published articles and corresponding authors, revealing China's significant influence in AM-associated infertility research. The *H*-index for the top 10 most productive countries/regions indicates that the USA (3,051), England (1,928), Germany (1,690), France (1,514), Australia (1,377), and China (1,333) have made notable contributions. High-yield institutions mainly originate from Europe. The cooperation network analysis among countries is illustrated in Figures 3C,D, showing that China, Belgium, and France collaborated closely. The top 10 productive institutions and their cooperation network are displayed in Figure 4. Leading organizations include the Assisting the Paris Public Hospital (4.83%, 22 papers), University Paris (3.73%, 17 papers), Cochin University Hospital (3.29%, 15 papers), National Institute of Health and Medical Research (3.07%, 14 papers) and Katholieke Universiteit Leuven (3.07%, 14 papers). Additionally, institutions with prominent cooperation networks include Siena University, Shanghai Jiao Tong University, Ku Leuven Catholic University, Fudan University, and the University of Milan.

### 3.3 Journals and co-cited journals

This analysis include 153 journals and 401 co-cited journals. The top 20 most productive and co-cited journals are summarized in Table 3. Fertility and Sterility (10.53%, 48 papers) published the most papers in this field, followed by Human Reproduction (5.48%, 25 papers), Reproductive Biomedicine online (5.26%, 25 papers), Reproductive Sciences (3.29%, 15 papers), American Journal of Obstetrics and Gynecology (2.85%, 13 papers) and Journal of Minimally Invasive Gynecology (2.41%, 11 papers). Co-citation network analysis is displayed in Figure 5, revealing that Fertility and Sterility was the most frequently co-cited journal, with 1,578 total citations, followed by Human Reproduction (1,477 citations), Reproductive Biomedicine Online (954 citations); Human Reproduction Update (865 citations) and Best Practice & Research Clinical Obstetrics & Gynecology (441



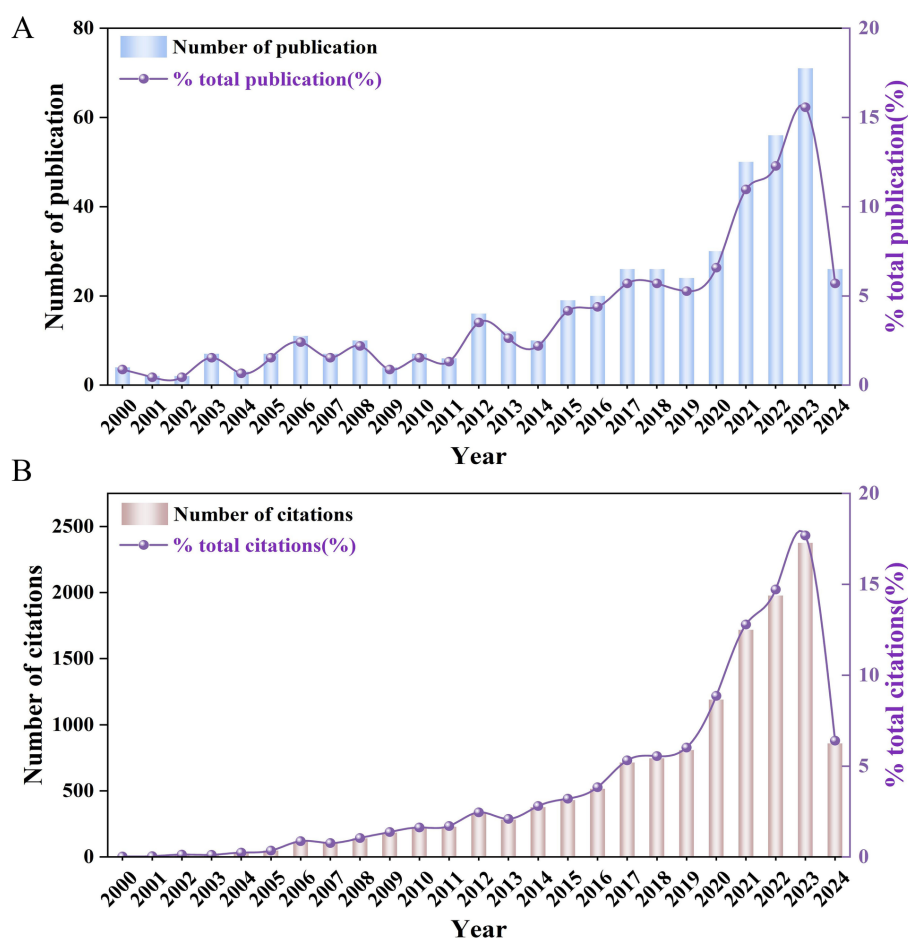


FIGURE 2

Annual publication and citation trends related to AM-associated infertility in the past 24 years. (A) The blue bars represent the yearly publications per year, the purple line represents the trend of annual publications in the total number of publications, and the purple solid dots represent the specific percentage (%) of yearly publications to total publications. (B) The brown bars represent the annual citations per year, the purple line represents the trend of yearly citations in the total number of citations, and the purple solid dots represent the specific percentage (%) of annual citations to total citations.

citations). Among the top 20 journals, Human Reproduction Update had the highest IF of 13.3 in 2024, while Human Reproduction boasted the highest *H*-index of 209 in 2024.

### 3.4 Authors and co-cited authors

The analysis identified 98 authors (with more than two articles were published) and 179 co-cited authors (with over 30 citations). Figures 6A,B feature the top most productive authors and contributors, while Figures 6C,D illustrate the top cited authors and the cooperation network between different authors. Detailed information on the top 10 authors and co-cited authors is presented in Table 4. Petraglia F., Santulli P., and Pellicer A. were the most published authors, each contributing 13 papers in the field of AM-associated infertility. Following them, Santulli P. and Bourdon M. each published 10 papers. The centrality of the top 10 published authors ranged from 0.018 to 0.029, with Petraglia F., Santulli P., and Pellicer A. achieving the highest centrality of 0.029. In terms of total co-cited frequency, the leading authors were Vercellini P. (314 citations), Leyendecker G. (199 citations), Kunz G. (178

citations). The close collaboration among different authors and co-cited authors indicate their crucial role in advancing the field.

### 3.5 Co-citation analysis for reference, focused topics, and timeline views

Table 5 lists the 30 most highly cited literature in the field of AM-associated infertility, highlighting six studies that have been co-cited over 200 times. The most co-cited article by Chen C. et al. (2017), published in Nature Communications, with 458 citations. This is followed by Koninckx P. R. et al. (2012) in Fertility and Sterility, with 325 citations, Kunz G. et al. (2005) and Vercellini P. et al. (2014) in Human Reproduction, with 276 and 242 citations, respectively. Co-citation analysis of the research topics was performed using CiteSpace, the results of which are presented in Figure 7. This analysis categorized all included papers into 10 clusters based on their primary research topics, endometrial receptivity (#0), AM (#1), preterm birth (#2), tobacco consumption (#3), junctional zone (#4), endometrium (#5), infertility (#6), fallopian tubes (#7), endometriosis (#8), and

TABLE 1 Top 10 publishers and categories related to adenomyosis associated infertility in the past 24 years.

| Rank | Publishers                    | Counts | Counts | Rank | Category                                   | Counts | Counts |
|------|-------------------------------|--------|--------|------|--------------------------------------------|--------|--------|
| 1    | Elsevier                      | 130    | 28.51% | 1    | Obstetrics Gynecology                      | 265    | 58.11% |
| 2    | Springer Nature               | 57     | 12.50% | 2    | Reproductive Biology                       | 177    | 38.82% |
| 3    | Oxford University Press       | 45     | 9.87%  | 3    | Medicine General Internal                  | 43     | 9.43%  |
| 4    | Wiley                         | 37     | 8.11%  | 4    | Radiology Nuclear Medicine Medical Imaging | 41     | 8.99%  |
| 5    | MDPI                          | 32     | 7.02%  | 5    | Endocrinology Metabolism                   | 26     | 5.70%  |
| 6    | Taylor & Francis              | 25     | 5.48%  | 6    | Medicine Research Experimental             | 18     | 3.95%  |
| 7    | Frontiers Media SA            | 17     | 3.73%  | 7    | Multidisciplinary Sciences                 | 10     | 2.19%  |
| 8    | Lippincott Williams & Wilkins | 16     | 3.51%  | 8    | Biochemistry Molecular Biology             | 9      | 1.97%  |
| 9    | Thieme Medical Publishers     | 11     | 2.41%  | 9    | Oncology                                   | 9      | 1.97%  |
| 10   | Hindawi Publishing Group      | 6      | 1.32%  | 10   | Public Environmental Occupational Health   | 9      | 1.97%  |

TABLE 2 Top 10 countries and organizations related to adenomyosis associated infertility in the past 24 years.

| Rank | Country   | Counts | Counts | H-index | Rank | Organizations                                                    | Counts | Counts |
|------|-----------|--------|--------|---------|------|------------------------------------------------------------------|--------|--------|
| 1    | China     | 116    | 25.44% | 1,333   | 1    | Assistance Publique Hopitaux Paris APHP                          | 22     | 4.83%  |
| 2    | USA       | 80     | 17.54% | 3,051   | 2    | Universite Paris Cite                                            | 17     | 3.73%  |
| 3    | Italy     | 67     | 14.69% | 1,333   | 3    | Hopital Universitaire Cochin APHP                                | 15     | 3.29%  |
| 4    | France    | 40     | 8.77%  | 1,514   | 4    | Institut National de la Sante et de la Recherche Medicale INSERM | 14     | 3.07%  |
| 5    | Japan     | 34     | 7.46%  | 1,301   | 5    | KU Leuven                                                        | 14     | 3.07%  |
| 6    | Belgium   | 31     | 6.80%  | 1,067   | 6    | University of Siena                                              | 14     | 3.07%  |
| 7    | Germany   | 31     | 6.80%  | 1,690   | 7    | Sapienza University Rome                                         | 11     | 2.41%  |
| 8    | England   | 29     | 6.36%  | 1,928   | 8    | Shanghai Jiao Tong University                                    | 11     | 2.41%  |
| 9    | Spain     | 22     | 4.83%  | 1,215   | 9    | Fudan University                                                 | 10     | 2.19%  |
| 10   | Australia | 17     | 3.73%  | 1,377   | 10   | IRCCS CA Granda Ospedale Maggiore Policlinico                    | 10     | 2.19%  |

adenomyoma (# 9). Timeline view analysis indicates that the most popular research topics are endometrial receptivity (#0), AM (#1), endometrium (#5), infertility (#6), and adenomyoma (# 9). The earliest papers with citation bursts emerged between 2010 and 2015.

### 3.6 Analysis of co-occurrence of keywords and citations

This analysis included a total of 190 keywords with a frequency exceeding five occurrences. We performed a keyword co-occurrence analysis to further explore hot topics using the Bibliometrix package. Figure 8A display the 10 most common keywords with the strongest associations within the keyword network. The most frequently occurring keywords was AM ( $n = 320$ ; total link strength = 2,263), followed by endometriosis ( $n = 205$ ; total link strength = 1,563), infertility ( $n = 193$ ; total link strength = 1,469), and women ( $n = 127$ ; a total link strength = 993), diagnosis ( $n = 105$ ; a total link strength = 865). High-frequency keywords are valuable for aiding researchers in effectively identifying current hot topics in the field. A network diagram illustrating the most frequently used keywords is shown in Figure 8B. This study identified 190 keywords classified into eight clusters: pathology and mechanisms, adverse pregnancy-associated, surgery treatment, diagnosis, ART, infertility factors,

quality of life, and medical treatment (Figures 8C–J). Additionally, we analyzed citation bursts using CiteSpace and displayed the top 25 keywords exhibiting the most significant citation bursts in Figure 9. This figure shows the period during which keyword citation bursts occurred, particularly relating to the disease concepts. For example, disease, rapid sperm transport, and hormone agonists were among the earliest to exhibit citation bursts.

Keywords related to AM treatment and clinical research, including magnetic resonance imaging, junctional zone, pregnancy, bowel resection, infertile women, and transvaginal ultrasound, typically experienced citation burst between 2006 and 2009, with moderate intensity. Notably, our analysis identified keywords that continue to exhibit significant citation burst projected through 2024, including pathogenesis (strength = 5.91; period = 2022–2024), uterine volume (strength = 2.63, period = 2022–2024), cancer (strength = 2.49, period = 2022–2024). These keywords may represent key focal points and objectives in current AM-associated infertility research.

### 3.7 Clustering analysis of keywords

We manually classified the keywords from network data into eight clusters to elucidated the current research trends related to

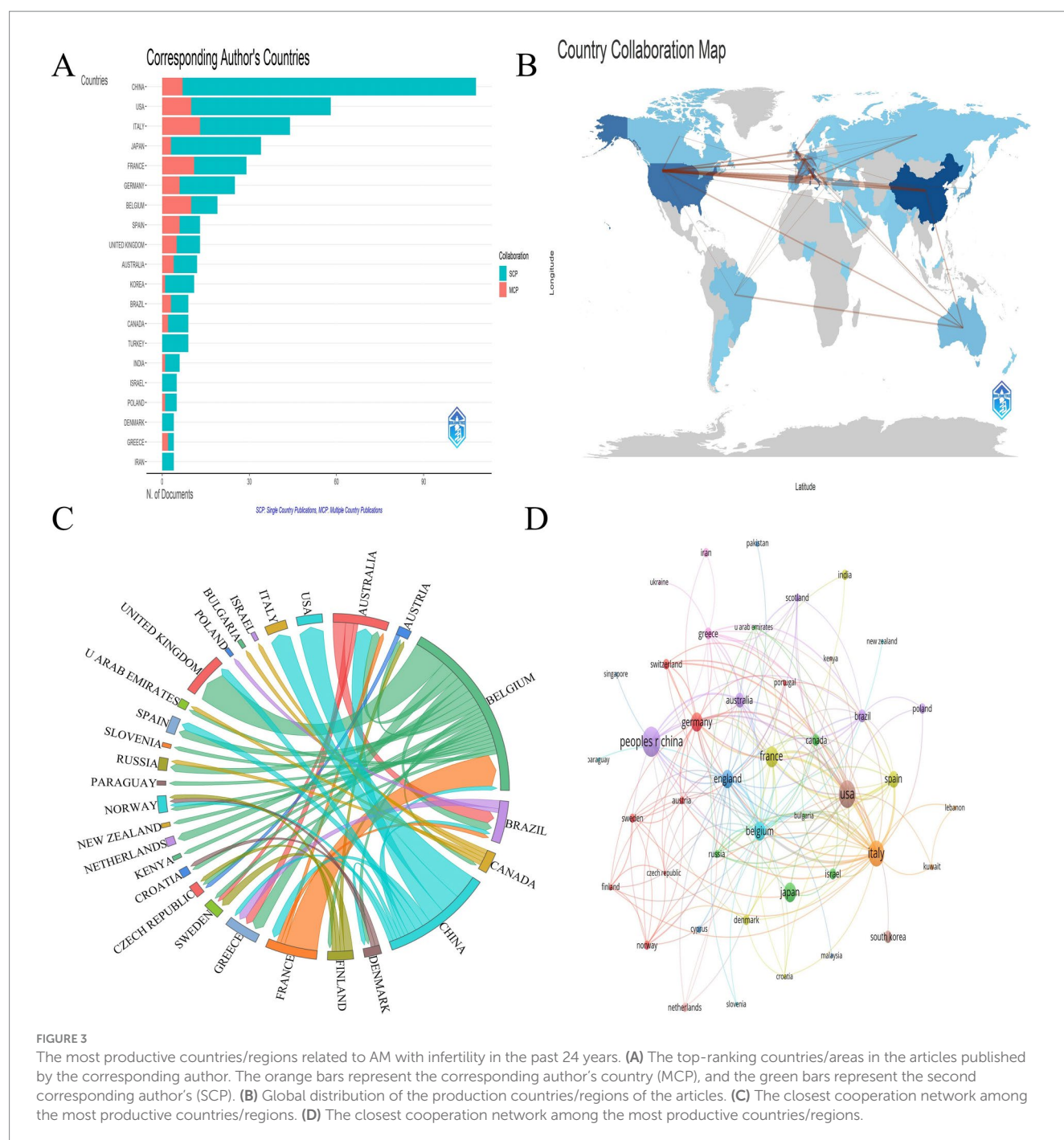


FIGURE 3

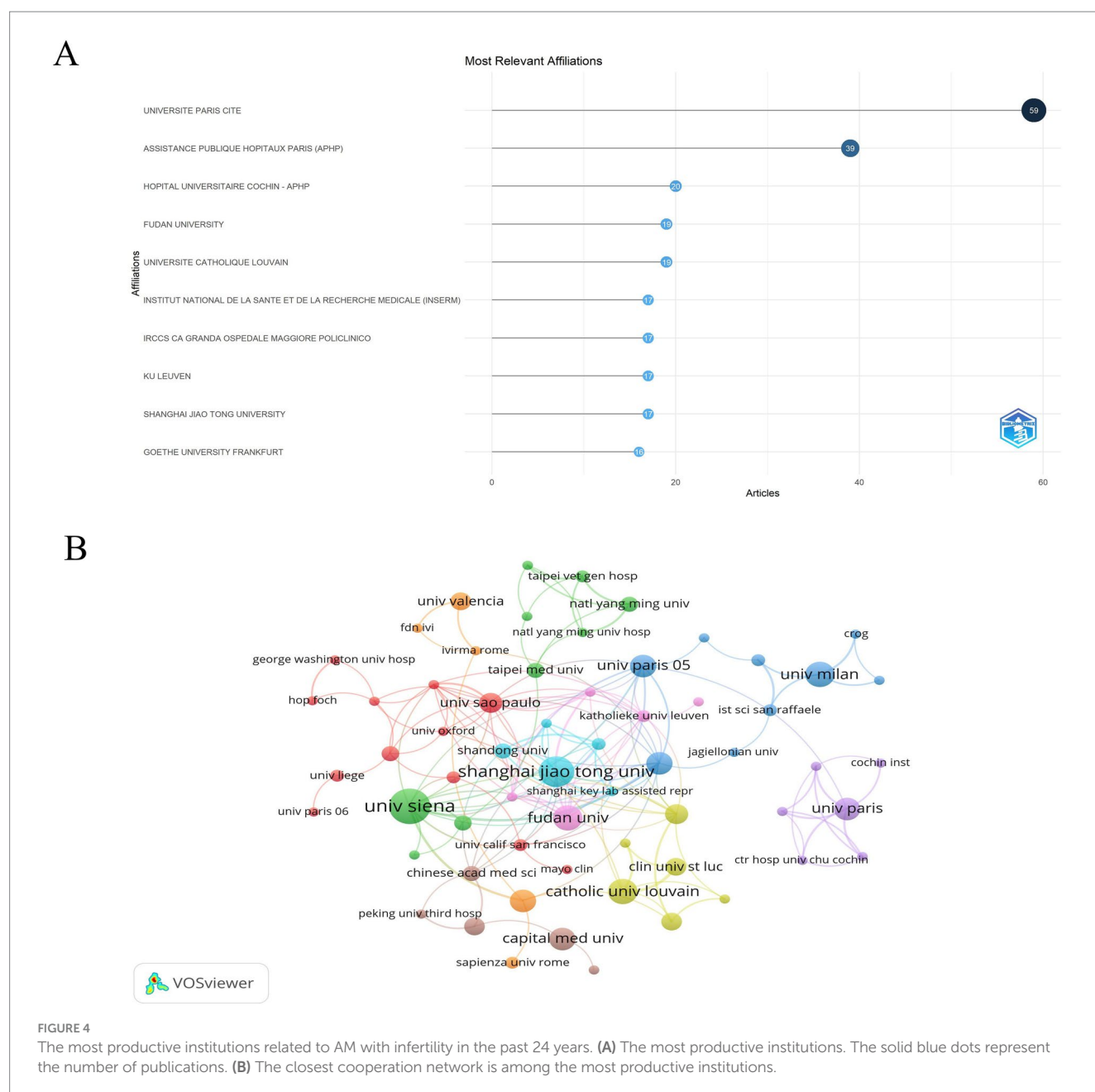
The most productive countries/regions related to AM with infertility in the past 24 years. **(A)** The top-ranking countries/areas in the articles published by the corresponding author. The orange bars represent the corresponding author's country (MCP), and the green bars represent the second corresponding author's (SCP). **(B)** Global distribution of the production countries/regions of the articles. **(C)** The closest cooperation network among the most productive countries/regions. **(D)** The closest cooperation network among the most productive countries/regions.

AM-associated infertility. These clusters encompass pathogenesis, adverse factors affecting pregnancy, treatment and diagnostic methods, disease progression, IVF, infertility women, and fertility management.

Cluster 1 indicates that AM encompass a spectrum of diseases influenced by epithelial-mesenchymal transition, eutopic endometrium, and inflammation, which collectively impair endometrial receptivity and may cause infertility. Notably, research hotspots in this field predominantly focus on gene expression (Figure 10A). Cluster 2 highlights that AM poses significant risks for pregnancy, resulting in increased complications such as placenta previa, preterm birth, and preeclampsia (Figure 10B). In cluster 3, the primary treatment means for AM have been divided into surgical

interventions, hormone therapy, and uterine artery embolization, all of which can adversely affect quality of life of AM patients (Figure 10C). Cluster 4 reveals that the diagnosis of AM primarily relies on ultrasound and magnetic resonance imaging (MRI), but variability in diagnostic methods impacts the accuracy of the results (Figure 10D). From cluster 5, we infer that AM significant impacts the implantation success rates for patients attempting to conceive, as disease progression can lead to myometrial fibrosis and alterations in uterine volume (Figure 10E).

Furthermore, according to cluster 6, AM may increase the difficulty and risk of miscarriage among patients undergoing IVF, possibly lead to damage to the junctional zone. Thus, enhanced pretreatment strategies



and vigilant monitoring are essential (Figure 10F). Cluster 7 reinforces that AM is a significant risk factor for infertile women, emphasizing gene-associated pathogenesis, which holds the potential to be expected to address the current challenges of AM-associated infertility effectively (Figure 10G). Lastly, cluster 8 reveals that ensuring fertility preservation in AM patients represents a crucial and challenging long-term objective, necessitating individualized treatment choices such as dienogest, the levonorgestrel-releasing intrauterine system, high-intensity focused ultrasound, or laparoscopic myomectomy (Figure 10H).

## 4 Discussion

This study represents the first analysis of the global research landscape surrounding AM-associated infertility utilizing

bibliometrics methodologies. Both AM and infertility are common gynecologic diseases that not only pose significant challenges for individuals but also impose substantial economic burdens on the national healthcare systems, society, and families (29, 30). The reported prevalence of AM can be as high as 70% (31), and it affects approximately 24.4% of infertile women (32). AM is widely recognized for its detrimental affects on fertility, contributing to infertility among women of childbearing age (33–35). Moreover, the incidence of AM associated with infertility is increasing annually, correlating with the tendency of delayed childbearing among women (5, 31). Cozzolino et al. (36) confirmed that women with AM have reduced live birth rates (LBR) (OR 0.59, 95% CI 0.37–0.92,  $p = 0.02$ ), clinical pregnancy rate (OR 0.66, 95% CI 0.48–0.90), and ongoing pregnancy rate (OR 0.43, 95% CI 0.21–0.88), alongside an increased miscarriage rate (OR 2.11, 95% CI 1.33–3.33). Younes et al. (37) reported a 41%

TABLE 3 Top 20 output and most co-cited journals related to adenomyosis associated infertility in the past 24 years.

| Rank | Journal                                                              | Counts | Counts | Rank | Co-cited journals                                                    | Citation counts | IF (2024) | H-index (2024) |
|------|----------------------------------------------------------------------|--------|--------|------|----------------------------------------------------------------------|-----------------|-----------|----------------|
| 1    | Fertility and Serility                                               | 48     | 10.53% | 1    | Fertility and Sterility                                              | 3,080           | 6.9       | 190            |
| 2    | Human Reproduction                                                   | 25     | 5.48%  | 2    | Human Reproduction                                                   | 1,477           | 6.1       | 209            |
| 3    | Reproductive Biomedicine Online                                      | 24     | 5.26%  | 3    | Reproductive Biomedicine online                                      | 954             | 4         | 100            |
| 4    | Reproductive Sciences                                                | 15     | 3.29%  | 4    | Human Reproduction update                                            | 865             | 13.3      | 158            |
| 5    | Journal of Clinical Medicine                                         | 13     | 2.85%  | 5    | Best Practice & Research Clinical Obstetrics & Gynaecology           | 441             | 5.5       | 72             |
| 6    | Journal of Minimally Invasive Gynecology                             | 11     | 2.41%  | 6    | Radiographics                                                        | 297             | 5.5       | 151            |
| 7    | European Journal of Obstetrics & Gynecology and Reproductive Biology | 9      | 1.97%  | 7    | Journal of Minimally Invasive Gynecology                             | 267             | 4.1       | 70             |
| 8    | Archives of Obstetrics and Gynecology                                | 8      | 1.75%  | 8    | Reproductive Sciences                                                | 265             | 2.9       | 70             |
| 9    | Journal of Obstetrics and Gynaecology Research                       | 8      | 1.75%  | 9    | European Journal of Obstetrics & Gynecology and Reproductive Biology | 240             | 2.6       | 90             |
| 10   | Seminars in Reproductive Medicine                                    | 8      | 1.75%  | 10   | Seminars in Reproductive Medicine                                    | 219             | 2.7       | 69             |
| 11   | Frontiers in Endocrinology                                           | 7      | 1.54%  | 11   | American Journal of Obstetrics and Gynecology                        | 178             | 9.1       | 203            |
| 12   | Gynecological Endocrinology                                          | 7      | 1.54%  | 12   | Taiwanese Journal of Obstetrics & Gynecology                         | 164             | 2.1       | 29             |
| 13   | Reproductive Biology and Endocrinology                               | 7      | 1.54%  | 13   | Ultrasound in Obstetrics & Gynecology                                | 162             | 7.1       | 128            |
| 14   | Taiwanese Journal of Obstetrics & Gynecology                         | 7      | 1.54%  | 14   | Reproductive Biology and Endocrinology                               | 158             | 4.4       | 79             |
| 15   | Acta Obstetrica et Gynecologica Scandinavica                         | 6      | 1.32%  | 15   | Gynecological Endocrinology                                          | 155             | 2         | 53             |
| 16   | Best Practice & Research Clinical Obstetrics & Gynaecology           | 6      | 1.32%  | 16   | Journal of Obstetrics and Gynaecology research                       | 151             | 1.6       | 44             |
| 17   | Current Opinion in Obstetrics Gynecology                             | 6      | 1.32%  | 17   | Acta Obstetrica et Gynecologica Scandinavica                         | 136             | 4.3       | 93             |
| 18   | Frontiers in Medicine                                                | 6      | 1.32%  | 18   | Biomed Research International                                        | 128             | 0         | 94             |
| 19   | Human Reproduction Open                                              | 6      | 1.32%  | 19   | Current Opinion in Obstetrics & Gynecology                           | 118             | 2.1       | 66             |
| 20   | Human Reproduction Update                                            | 6      | 1.32%  | 20   | Cells                                                                | 111             | 6         | 14             |

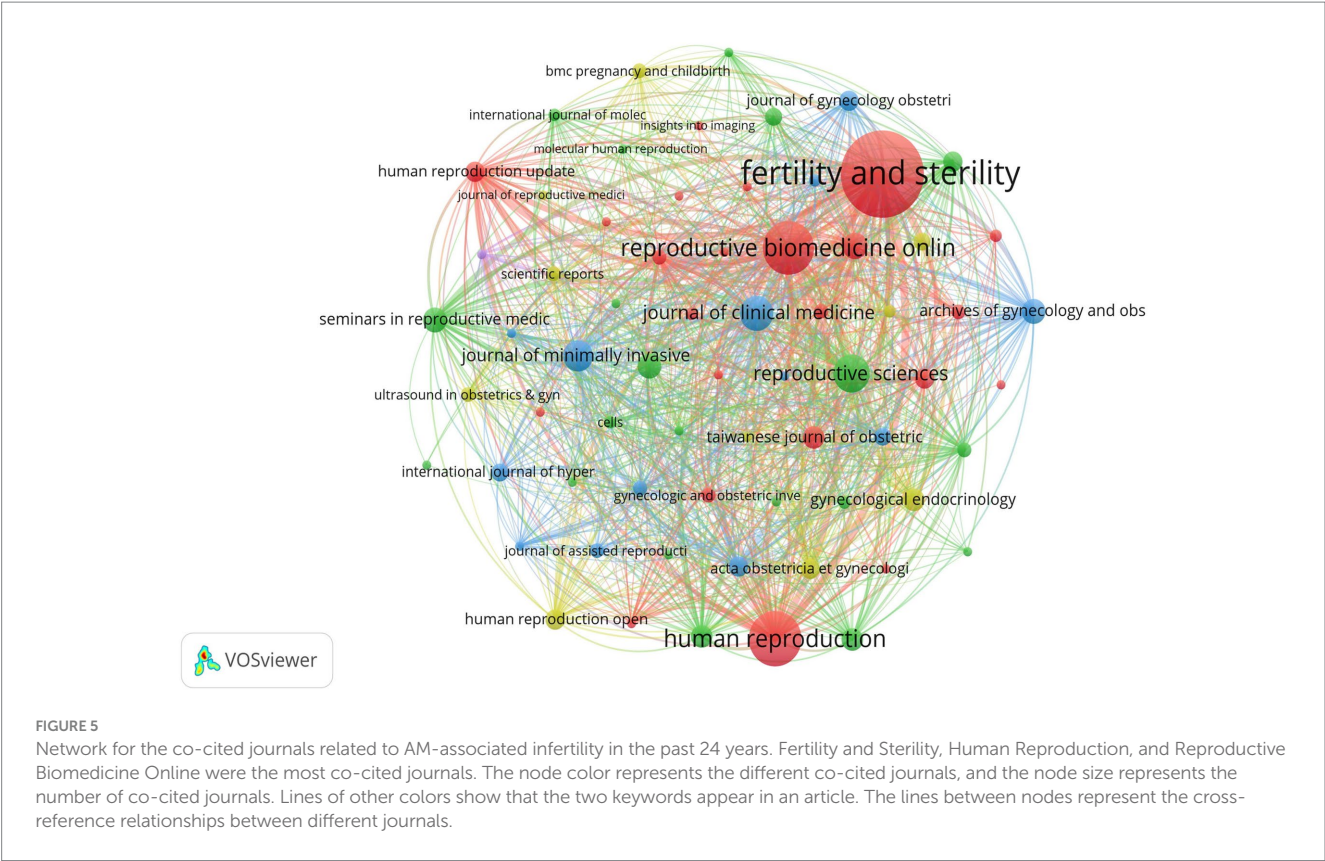
reduction in LBR among patients with AM. Vercellini et al. (38) elaborated a 28% decrease in the likelihood of clinical pregnancy via ART compared to women without AM. Additionally, Marvelous et al. (39) indicated a decline in clinical pregnancy among AM patients, ranging from 42.7% with an AM score of zero to 13% with a score of seven.

Consequently, a growing number of scholars are focusing on the relationship between AM and infertility, resulting in a large aggregation of articles and reviews exploring the complex mechanisms underlying this association. Despite this, a systematic method for analyzing and identifying key areas of interest in this research domain has been lacking. Bibliometric analysis, similar to epidemiological

approaches, offers a robust means of highlighting potential future research directions by examining authorship, institutional contributions, journal impact, and keyword usage in existing literature. This approach provides valuable insights that can inform and deepen future investigations in the field (10, 40, 41).

In this study, we performed a scientometric analysis to grasp the current research hotspots, keywords, focal points, challenges, and trends pertaining to AM-associated infertility. Our analysis encompassed 456 articles and reviews published across 153 journals by 123 institutions in 51 countries/regions, yielding a total of 13,426 citations and 62 *H*-indexes. We established that AM remains a primary concern and a significant challenge within infertility research globally,





**TABLE 4** Top 10 authors and co-cited authors related to adenomyosis associated infertility in the past 24 years.

| Rank | Published author | Counts | Centrality | RANK | Co-cited author | Citation |
|------|------------------|--------|------------|------|-----------------|----------|
| 1    | Petraglia F.     | 13     | 0.029      | 1    | Vercellini P.   | 314      |
| 2    | Chapron C.       | 13     | 0.029      | 2    | Leyendecker G.  | 199      |
| 3    | Pellicer A.      | 13     | 0.029      | 3    | Kunz G.         | 178      |
| 4    | Santulli P.      | 10     | 0.022      | 4    | Bazot M.        | 165      |
| 5    | Bourdon M.       | 10     | 0.022      | 5    | Chapron C.      | 151      |
| 6    | Vannuccini S.    | 9      | 0.020      | 6    | Dueholm M.      | 125      |
| 7    | Maignien C.      | 9      | 0.020      | 7    | Exacoustos C.   | 123      |
| 8    | Marcellin L.     | 9      | 0.020      | 8    | Benagiano G.    | 115      |
| 9    | Ayoubi J.        | 8      | 0.018      | 9    | Khan K.         | 105      |
| 10   | Benagiano G.     | 8      | 0.018      | 10   | Reinhold C.     | 104      |

with a continuous growth in the quantity of published articles since 2006. The significance of studying the association between AM and infertility is gradually gaining recognition within both academic and clinical circles. Major contributions were identified from China, the USA, Italy, France, and Japan, collectively ranking as the top five countries in terms of publications. This trend may be attributed to the high prevalence of AM and the relatively advanced status of infertility and biomedical study in these nations. We divided 190 keywords, which appeared more than five times, into eight clusters, mainly focused on pathogenesis, adverse factors affecting pregnancy, treatment methods, diagnostic modalities, disease progression, IVF management, infertility, and fertility management. These clusters indicate significant interest in AM-related infertility research over the past 24 years.

Additionally, by analyzing the citation burst of keywords, we discovered emerging research hotspots in the potential mechanisms of pathogenesis, diagnostic methods, and strategies for improving pregnancy success in AM-associated infertility. Recent investigations have increasingly aimed at decoding the intricate mechanism involved in the application of targeted therapies for AM patients with infertility. Although an unambiguous understanding of the pathogenesis is still pending, several hypotheses have gained traction. Altered endometrial function and receptivity in AM patients may give rise to a pro-inflammatory environment and heightened oxidative stress, negatively affecting embryo implantation and survival (42, 43). Other proposed mechanisms include abnormal uterotubal transport caused by adenomyomas with obstruction, which may block sperm transport

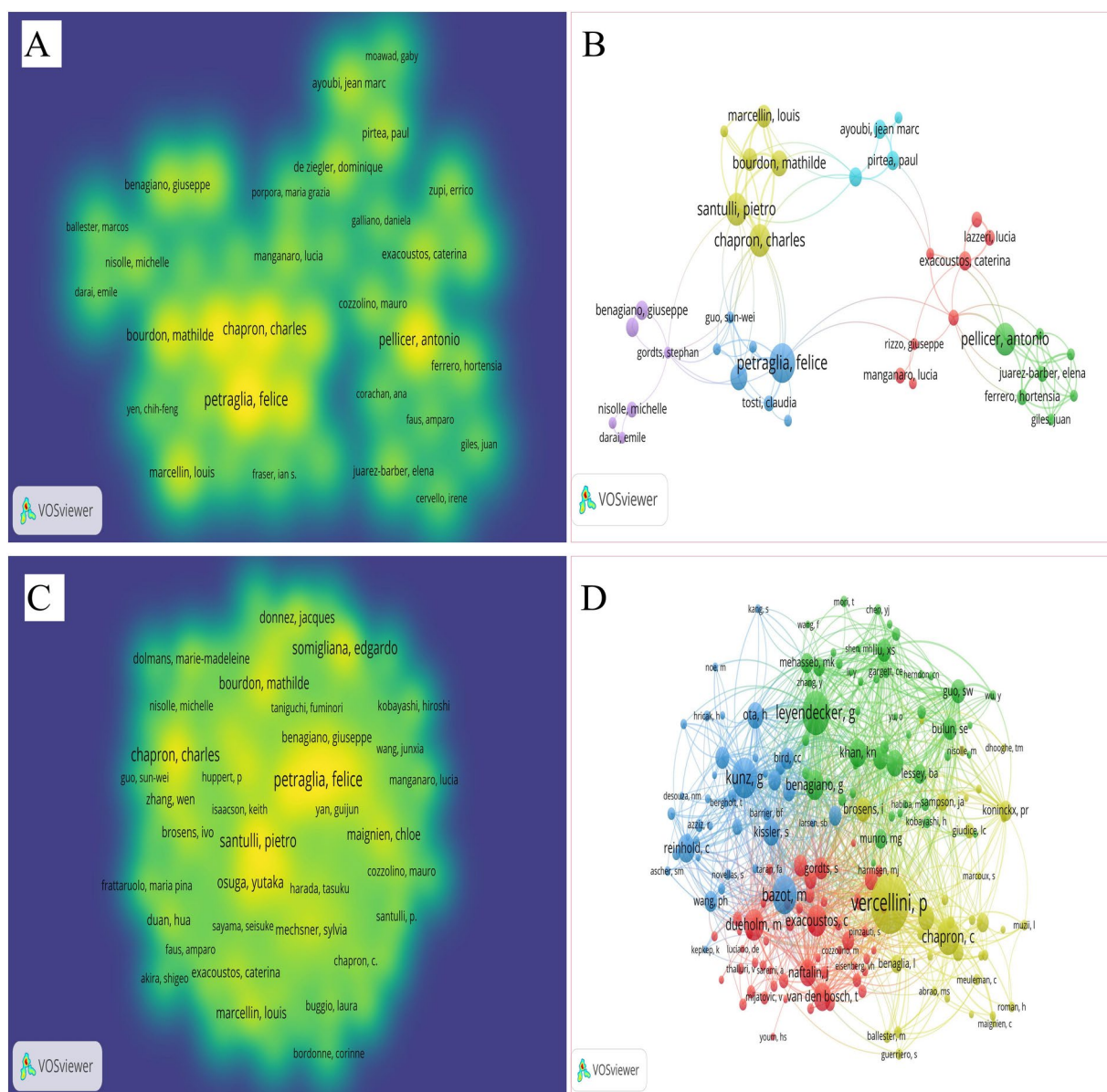


FIGURE 6

Map of authors and co-cited authors related to AM-associated infertility in the past 24 years. (A) Spectrum density diagram of the most productive authors. (B) The most productive contributing authors of the network diagram. (C) The spectrum density map of the co-cited authors. The authors' closest relationship is allocated to one cluster with the same color in this cluster density map. (D) The cooperation of different authors with co-cited authors in the network diagram.

by distorting the uterine cavity and disrupting normal myometrium structure and function (31, 33). Additionally, irregular uterine contractions during the follicular phase and disturbance in the uterine junctional zone have been implicated as potential contributors to AM-associated infertility (44–46). Recent findings show that increased amplitude and decreased contraction coordination in AM patients could significantly lead to infertility, particularly during the luteal phase when implantation occurs (47). The importance of uterine peristalsis during the peri-implantation phase is notably highlighted in the context of IVF implantation failures and adverse pregnancy outcomes in AM (48). These findings indicate quantifying abnormal patterns and measures of uterine contractility offers a potential new tool for explaining infertility associated with AM (47).

Moreover, evidence of AM with infertility, involving various biomarkers such as matrix metalloproteinases (MMP), interleukins (IL-6, IL-10), HOXA10, leukemia inhibitory factor (LIF), cytochrome P450, and RCAS1 (49). Previous studies suggest that the downregulation of HOXA10, NR4A receptor, and FOXO1A appears to impaired implantation in women with AM (50, 51), and dysregulation of LIF has a similar effect (52). Additionally, molecules like nitric oxide, which are expressed at abnormally high levels, adversely impact sperm transport, implantation, and decidualization, leading to AM-related infertility (53). Pro-oxidative and antioxidative cytokines, including copper (Cu), manganese superoxide dismutase (Mn-SOD), and zinc superoxide dismutase (Zn-SOD), are associated with increased inflammatory responses in

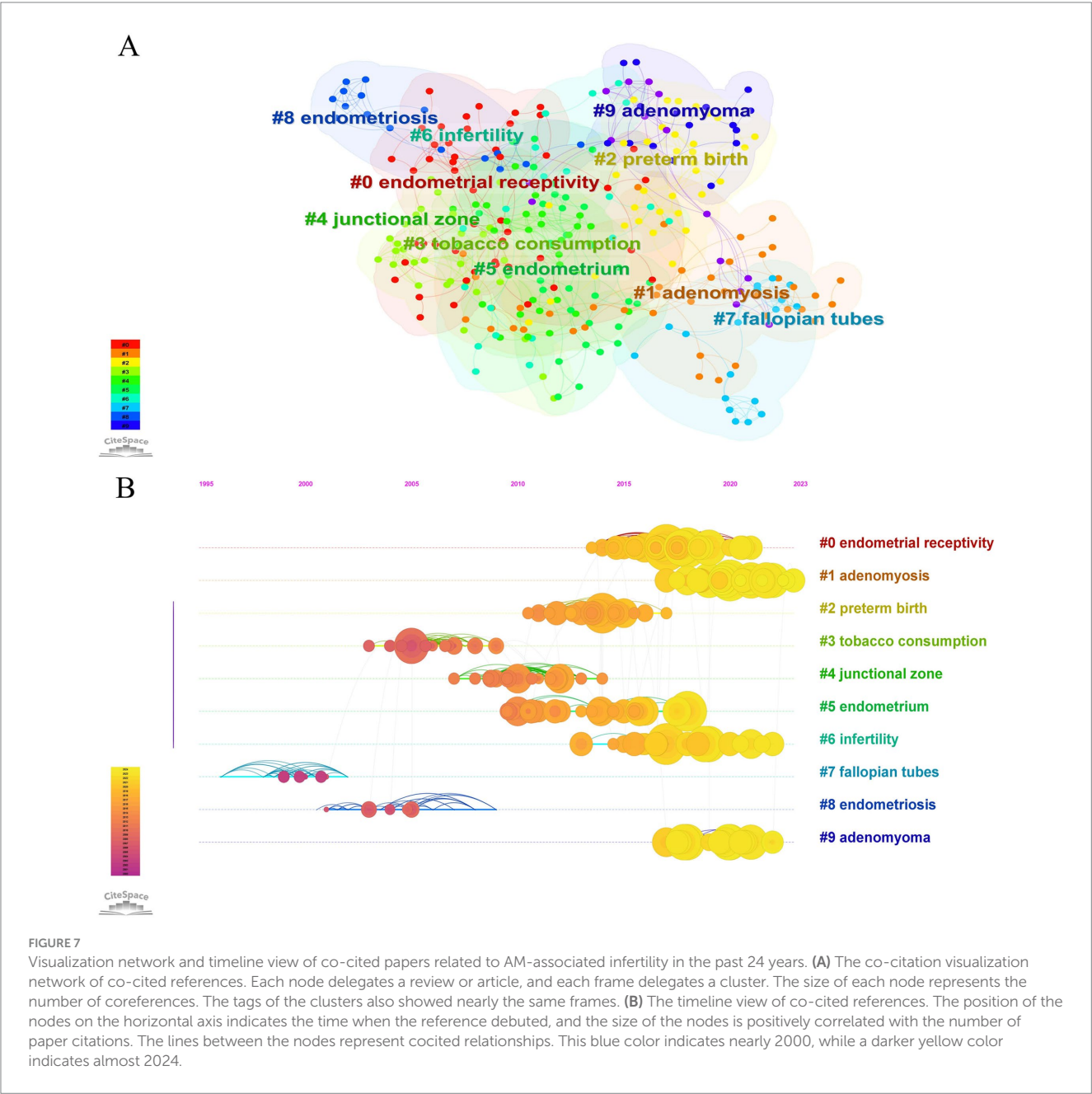
TABLE 5 Top 30 most highly cited literature related to adenomyosis associated infertility in the past 24 years.

| Rank | Title                                                                                                                                                    | Author          | Source title                                               | Publication year | Total citations | Average per year |
|------|----------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|------------------------------------------------------------|------------------|-----------------|------------------|
| 1    | The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases                                                | Jia H.          | Nature Communications                                      | 2017             | 458             | 57.25            |
| 2    | Deep endometriosis: definition, diagnosis, and treatment                                                                                                 | Donnez J.       | Fertility and Sterility                                    | 2012             | 325             | 25               |
| 3    | Adenomyosis in endometriosis—prevalence and impact on fertility. Evidence from magnetic resonance imaging                                                | Leyendecker G.  | Human Reproduction                                         | 2005             | 276             | 13.8             |
| 4    | Uterine adenomyosis and <i>in vitro</i> fertilization outcome: a systematic review and meta-analysis                                                     | Somigliana E.   | Human Reproduction                                         | 2014             | 242             | 22               |
| 5    | Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome                                              | Petraglia F.    | Human reproduction update                                  | 2016             | 216             | 24               |
| 6    | Pathogenesis of endometriosis: the genetic/epigenetic theory                                                                                             | Martin D. C.    | Fertility and Sterility                                    | 2019             | 209             | 34.83            |
| 7    | Adenomyosis: epidemiological factors                                                                                                                     | Fedele L.       | Best Practice & Research Clinical Obstetrics & Gynecology  | 2006             | 192             | 10.11            |
| 8    | Magnetic resonance imaging and transvaginal ultrasonography for the diagnosis of adenomyosis                                                             | Olesen F.       | Fertility and Sterility                                    | 2001             | 189             | 7.88             |
| 9    | Pathogenesis of uterine adenomyosis: invagination or metaplasia?                                                                                         | Dolmans M. M.   | Fertility and Sterility                                    | 2018             | 187             | 26.71            |
| 10   | Oxidative stress may be a piece in the endometriosis puzzle                                                                                              | Mikolajczyk M.  | Fertility and Sterility                                    | 2003             | 185             | 8.41             |
| 11   | Diagnosing adenomyosis: an integrated clinical and imaging approach                                                                                      | Petraglia F.    | Human Reproduction Update                                  | 2020             | 184             | 36.8             |
| 12   | Pathogenesis of adenomyosis: an update on molecular mechanisms                                                                                           | Petraglia F.    | Human Reproduction Online                                  | 2017             | 172             | 21.5             |
| 13   | Uterine adenomyosis in the infertility clinic                                                                                                            | Timmerman D.    | Human Reproduction Update                                  | 2003             | 148             | 6.73             |
| 14   | The impact of adenomyosis on women's fertility                                                                                                           | Taniguchi F.    | Obstetrical & Gynaecological Survey                        | 2016             | 147             | 16.33            |
| 15   | Effects of adenomyosis on <i>in vitro</i> fertilization treatment outcomes: a meta-analysis                                                              | Tulandi T.      | Fertility and Sterility                                    | 2017             | 146             | 18.25            |
| 16   | Recurrence of ovarian endometrioma after laparoscopic excision                                                                                           | Taketani Y.     | Human Reproduction                                         | 2006             | 146             | 7.68             |
| 17   | Adenomyosis and subfertility: a systematic review of prevalence, diagnosis, treatment and fertility outcomes                                             | Bhattacharya S. | Human Reproduction Update                                  | 2012             | 145             | 11.15            |
| 18   | Role of medical therapy in the management of uterine adenomyosis                                                                                         | Petraglia F.    | Fertility and Sterility                                    | 2018             | 142             | 20.29            |
| 19   | Medical and surgical management of adenomyosis                                                                                                           | Brosens I.      | Best practice & Research Clinical Obstetrics & Gynaecology | 2006             | 135             | 7.11             |
| 20   | The role of <i>HOX</i> genes in female reproductive tract development, adult function, and fertility                                                     | Taylor H. S.    | Cold Spring Harbor Perspectives in Medicine                | 2016             | 134             | 14.89            |
| 21   | The pathophysiology of uterine adenomyosis: an update                                                                                                    | Brosens I.      | Fertility and Sterility                                    | 2012             | 134             | 10.31            |
| 22   | Structural abnormalities of the uterine wall in women with endometriosis and infertility visualized by vaginal sonography and magnetic resonance imaging | Leyendecker G.  | Human Reproduction                                         | 2000             | 132             | 5.28             |
| 23   | MR Imaging of endometriosis: ten imaging pearls                                                                                                          | Edward R.       | Radiographics                                              | 2012             | 129             | 9.92             |
| 24   | Uterine adenomyosis: a need for uniform terminology and consensus classification                                                                         | Brosens I.      | Human Reproduction Online                                  | 2008             | 125             | 7.35             |
| 25   | Transvaginal sonographic features of diffuse adenomyosis in 18–30-year-old nulligravid women without endometriosis: association with symptoms            | Petraglia F.    | Ultrasound in Obstetrics & Gynecology                      | 2015             | 123             | 12.3             |
| 26   | Uterine polyps, adenomyosis, leiomyomas, and endometrial receptivity                                                                                     | Munro M. G.     | Fertility and Sterility                                    | 2019             | 122             | 20.33            |

(Continued)

TABLE 5 (Continued)

| Rank | Title                                                                                                                           | Author       | Source title                    | Publication year | Total citations | Average per year |
|------|---------------------------------------------------------------------------------------------------------------------------------|--------------|---------------------------------|------------------|-----------------|------------------|
| 27   | Uterine peristaltic activity and the development of endometriosis                                                               | Wildt L.     | Uterus human Reproduction       | 2004             | 120             | 5.71             |
| 28   | Adenomyosis and infertility                                                                                                     | Benagiano G. | Reproductive Biomedicine Online | 2012             | 115             | 8.85             |
| 29   | The motile and invasive capacity of human endometrial stromal cells: implications for normal and impaired reproductive function | Gellersen B. | Human Reproduction Update       | 2013             | 113             | 9.42             |
| 30   | Long-term pituitary downregulation before frozen embryo transfer could improve pregnancy outcomes in women with adenomyosis     | Feng Y.      | Gynaecological Endocrinology    | 2013             | 104             | 8.67             |





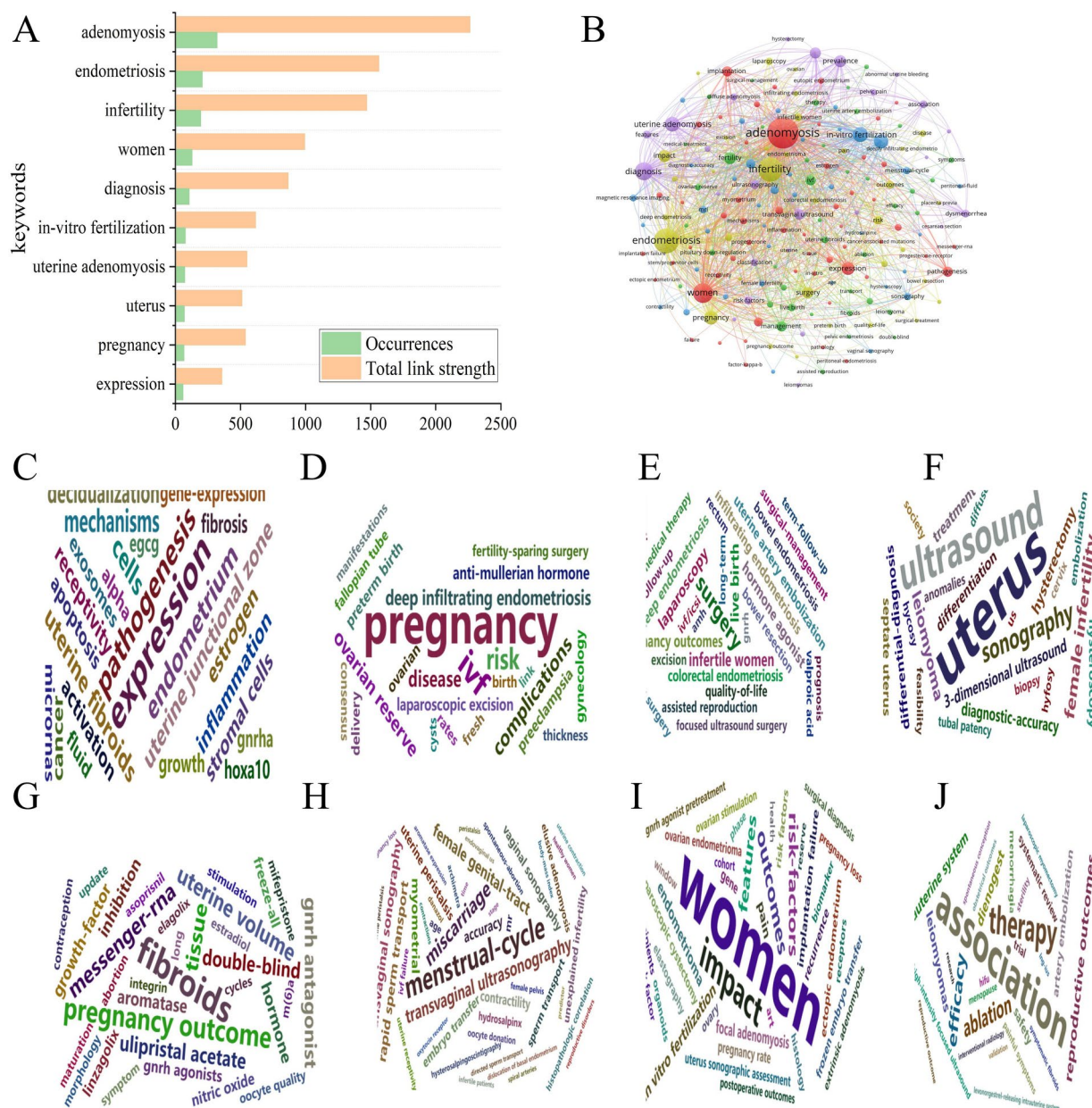


FIGURE 8

Keywords of the distribution, co-occurrence network diagram, and word cloud cluster map. (A) The distribution of keywords: the green histogram represents occurrences, and the orange histogram shows the total link strength. (B) The co-occurrence network of keywords; the minimum frequency of occurrences of keywords  $\geq 5$ . Node size and color represent the frequency of keywords and clusters, respectively. Lines of different colors show that the two keywords appear in an article. (C–J) The word cloud cluster map of pathophysiology, adverse pregnancy-associated factors, surgical treatment, diagnosis, assisted reproduction treatment, infertility factors, quality of life, and medical treatment.

the endometrium (54, 55). Consequently, prior research indicates significant opportunities for further investigations into the correlation between AM and infertility.

Although histopathological reports are considered the gold standard for diagnosing AM, they can lead to diagnostic delays of up to 12 years (56) and are not essential for treating patients with concurrent infertility. Instead, imaging techniques serve as the primary diagnostic tools (5, 29). Some studies utilize trans-vaginal ultrasound, while others employ MRI or a combination of both approaches, leading to potential inconsistencies in diagnostic effectiveness (57, 58). The incidence of infertility linked to

AM appears to be classification-dependent (59). Moreover, underdiagnosis by less experienced practitioners cannot be discounted, as this may lead to the erroneous inclusion of women with AM in control group, thereby potentially underestimating the actual effect of AM on reproductive outcomes (34). Therefore, the accuracy of diagnosing AM in the context of infertility remains contentious. Further research is imperative to establish uniform diagnostic criteria that clarify the definitive connection between AM and infertility.

Regarding treatment options to improve pregnancy outcomes, there are currently no harmonized international guidelines for



### Top 25 Keywords with the Strongest Citation Bursts

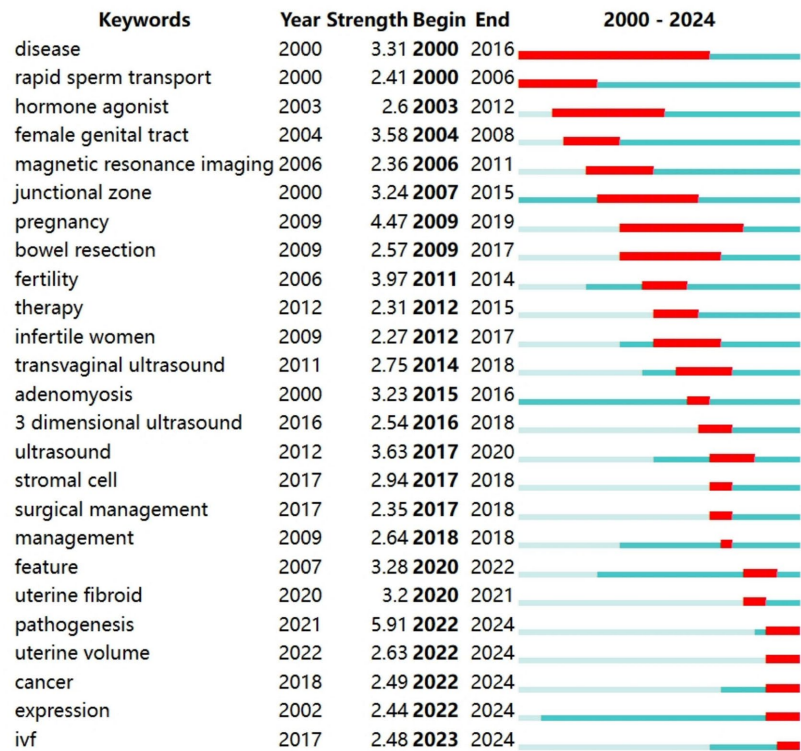


FIGURE 9  
Top 25 keywords with the most vigorous citation bursts. The blue line indicates the timeline, and the red sections indicate the burst duration, including the start and end years.

managing patients with AM who wish to preserve fertility (60, 61). Nonetheless, available evidence suggests that treatment can positively effect on fertility outcomes (33). For instance, surgical interventions have been shown to increase rates of natural conception (36). Additionally, the use of danazol-loaded devices yield a pregnancy rate of 41%, while GnRHa therapy results in a LBR of 36.2%. Uterine artery embolization has an even higher LBR of 83.3% (35). Other studies report pregnancy rates of 60.5% following complete excision and 46.9% after partial excision of AM (62). The odds ratio of clinical pregnancy post-surgery is reported as 6.22 (CI 2.34–16.54) (37). Furthermore, variations in AM types demonstrate different effects on fertility outcomes, focal AM is associated with a pregnancy rate of 49.1%, compared to 38.5% for the diffuse AM, and a miscarriage rate of 27.6% for focal AM versus 16.2% for the diffuse AM (63).

However, Mijatovic et al. (64), noted no significant increase in clinical pregnancy rates among infertile women with AM who had previously undergone GnRH treatment (36). The overall effectiveness of surgical treatment for AM affecting pregnancy rate remains inconclusive, with a reported risk of uterine rupture estimated at 6.0% (65). A systematic review further indicated that treatments involving oral contraceptives, antiprostaglandins, progestins, danazol, and GnRHa have not improved pregnancy rates for women with AM planning to conceive. However, high-intensity focused ultrasound and combination therapies before ART may benefit these patients (5). Although existing research confirms that pharmacological and surgical treatments for

AM positively impact reproductive outcomes, including pregnancy rates and LBR, the comparative effectiveness of different treatments and the optimal timing for delaying pregnancy remain unclear. Additionally, limited evidence on the correlation between infertility and the severity and classification of AM may affect pregnancy rate statistics (66). Therefore, developing standardized protocols to address AM-related infertility is crucial, and the efficacy of these therapeutic options must be validated through prospective randomized controlled trials.

### 5 Limitations and superiority

To the best of our knowledge, our study is the first in-depth scientometric analysis of AM-associated infertility. However, several limitations need attention. First, the data were sourced solely from the SCI-E database within the WoSCC, potentially omitting relevant literature and causing a bias in research conclusions. Second, the use of bibliometric software for author analysis does not currently allow for the differentiation of author name abbreviations, which may lead to inaccuracies. Additionally, bibliometric analysis based on machine algorithms does not permit an in-depth exploration of individual studies, possibly omitting some information. Moreover, as the review focuses exclusively on studies addressing infertility in AM, there may be a selection bias present. Finally, the lack of authoritative guidelines for bibliometric analyses in medical research is a significant challenge for academics who wish to gain a comprehensive and accessible

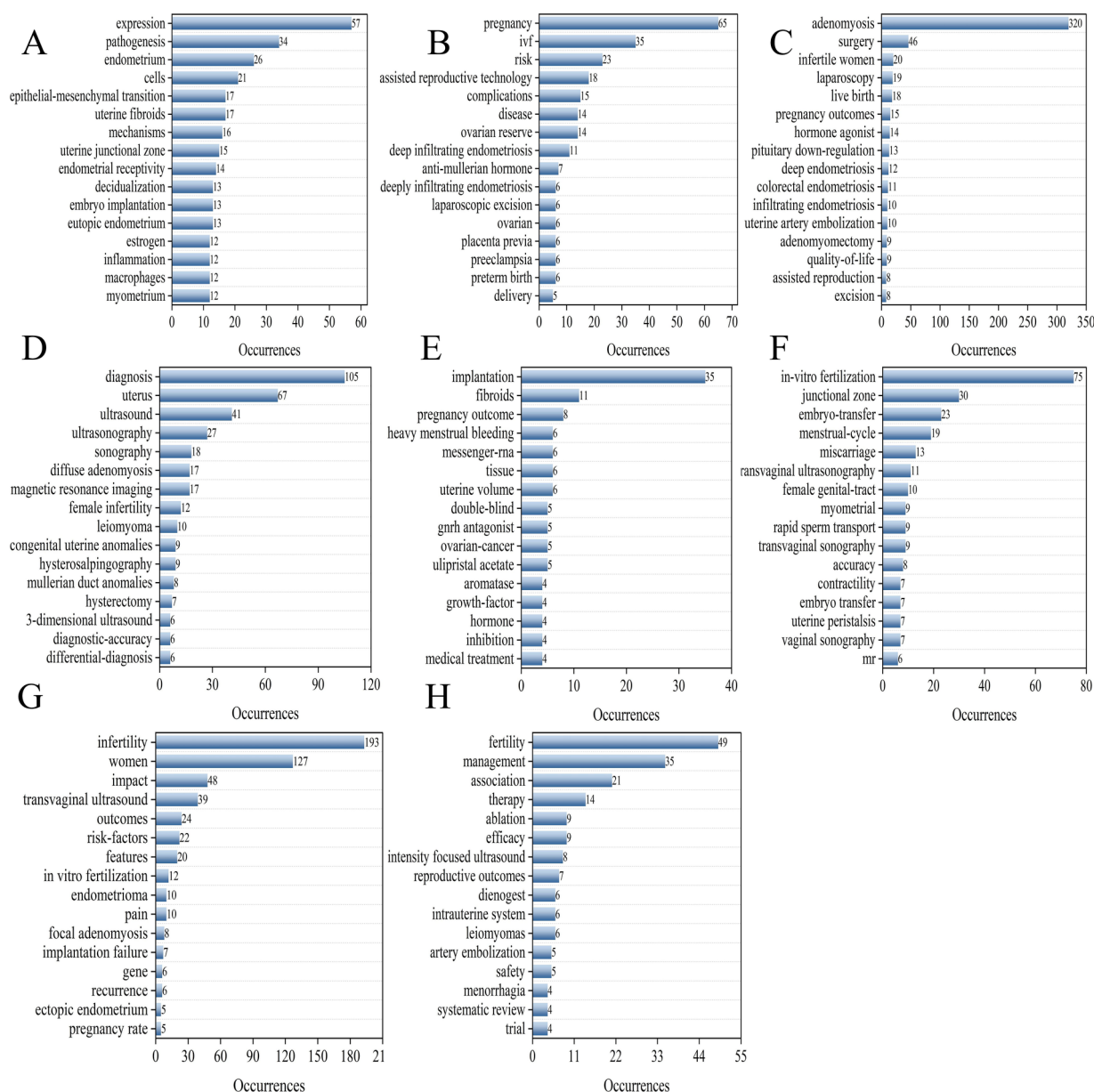


FIGURE 10

Manual analysis and clustering of keywords. The blue histograms represent the frequency of keyword occurrences. (A) Pathogenesis. (B) Adverse factors affecting pregnancy. (C) Treatment means. (D) Diagnosis methods. (E) Disease progressive progress. (F) *In-vitro* fertilization management. (G) Infertility women. (H) Fertility management, respectively.

understanding of bibliometric methods and their application in medical research.

However, the WoS is the most powerful search engine, and the WoSCC database contains extensive data on the theme of AM-associated infertility. In addition, the WoS is the premier research platform for biomedical and natural science, and the world's most trusted publisher with an independent global citation database. Therefore, based on an adequate amount of data and the correct scientometrics methods, the outcomes of this study are convincing and may help accurately identify knowledge gaps, research hotspots, and development trends in AM-associated infertility. The perspectives presented here can guide the generation of novel ideas for further in-depth investigations into AM-associated infertility. Specifically, research on improving uterine receptivity

during the peri-implantation period offers direction and encourages further exploration for focused collaboration between researchers and clinicians.

## 6 Conclusion

This study is the first to use bibliometric methods to detail global trends and the current status of AM-associated infertility over the past 20 years. The research highlights that international interest in this complex field remains strong. Key topics include pathogenesis, factors affecting pregnancy, treatment and diagnostic methods, disease progression, and IVF management. Although chronic disease management strategies, pharmacological treatments, and ART have

improved fertility outcomes in AM patients, further collaboration between researchers and clinicians is crucial to facilitate translational clinical research. This study aids in identifying research hotspots and fostering regional collaboration for a deeper understanding of the AM-associated infertility landscape and its evolution.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

## Author contributions

QY: Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. XZ: Writing – review & editing, Formal analysis, Software, Validation, Visualization, Writing – original draft. FZ: Data curation, Formal analysis, Validation, Visualization, Writing – review & editing. LC: Investigation, Validation, Writing – review & editing. JH: Formal analysis, Software, Supervision, Writing – review & editing. XL: Data curation, Investigation, Methodology, Writing – review & editing. JJ: Funding acquisition, Supervision, Writing – review & editing.

## References

- Wang L, Yang R. Comparing the therapeutic effects of Chinese herbal medicine with western medicine on adenomyosis: a systematic review and meta-analysis. *Asian J Surg.* (2024) 47:578–80. doi: 10.1016/j.asjsur.2023.09.117
- Salmeri N, Ottolina J, Bartiromo L, Schimberni M, Dolci C, Ferrari S, et al. 'Guess who?' An Italian multicentric study on pigmentation traits prevalence in endometriosis localizations. *Eur J Obstet Gynecol Reprod Biol.* (2022) 274:5–12. doi: 10.1016/j.ejogrb.2022.05.001
- Moawad G, Fruscalzo A, Youssef Y, Kheil M, Tawil T, Nehme J, et al. Adenomyosis: an updated review on diagnosis and classification. *J Clin Med.* (2023) 12:4828. doi: 10.3390/jcm12144828
- Chen Y, Lin S, Xie X, Yi J, Liu X, Guo SW. Systematic review and meta-analysis of reproductive outcomes after high-intensity focused ultrasound (HIFU) treatment of adenomyosis. *Best Pract Res Clin Obstet Gynaecol.* (2024) 92:102433. doi: 10.1016/j.bpobgyn.2023.102433
- Szuber M, Koziróg E, Olszak O, Krygier-Kurz K, Kazmierczak J, Wilczynski J. Adenomyosis and infertility-review of medical and surgical approaches. *Int J Environ Res Public Health.* (2021) 18:1235. doi: 10.3390/ijerph18031235
- Elhelf IAS, Albahar H, Shah U, Oto A, Cressman E, Almekawy M. High intensity focused ultrasound: the fundamentals, clinical applications and research trends. *Diagn Interv Imaging.* (2018) 99:349–59. doi: 10.1016/j.diii.2018.03.001
- Njagi P, Groot W, Arsenijevic J, Dyer S, Mburu G, Kiarie J. Economic costs of infertility care for patients in low-income and middle-income countries: a systematic review protocol. *BMJ Open.* (2020) 10:e042951. doi: 10.1136/bmjopen-2020-042951
- Rooney KL, Domar AD. The relationship between stress and infertility. *Dialogues Clin Neurosci.* (2018) 20:41–7. doi: 10.31887/DCNS.2018.20.1/krooney
- Freire CVS, Campos LN, Rangel AG, Naus A, Wagemaker S, Brandão GR, et al. Uncovering gaps in women's authorship: a big data analysis in academic surgery. *World J Surg.* (2024) 48:2152–62. doi: 10.1002/wjs.12256
- Ninkov A, Frank JR, Maggio LA. Bibliometrics: methods for studying academic publishing. *Perspect Med Educ.* (2022) 11:173–6. doi: 10.1007/s40037-021-00695-4
- Ramakreshnan L, Aghamohammadi N, Fong CS, Sulaiman NM. A comprehensive bibliometrics of 'walkability' research landscape: visualization of the scientific progress and future prospects. *Environ Sci Pollut Res Int.* (2021) 28:1357–69. doi: 10.1007/s11356-020-11305-x
- van Oijen JCF, van Dongen-Leunis A, Postma J, van Leeuwen T, Bal R. Achieving research impact in medical research through collaboration across organizational

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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- boundaries: insights from a mixed methods study in the Netherlands. *Health Res Policy Syst.* (2024) 22:72. doi: 10.1186/s12961-024-01157-z
- Xu D, Wang YL, Wang KT, Wang Y, Dong XR, Tang J, et al. A scientometrics analysis and visualization of depressive disorder. *Curr Neuropsychopharmacol.* (2021) 19:766–86. doi: 10.2174/1570159X18666200905151333
- Zhu H, Shi L, Wang R, Cui L, Wang J, Tang M, et al. Global research trends on infertility and psychology from the past two decades: a bibliometric and visualized study. *Front Endocrinol.* (2022) 13:889845. doi: 10.3389/fendo.2022.889845
- Kemper JM, Rolnik DL, Mol BWJ, Ioannidis JPA. Reproducible research practices and transparency in reproductive endocrinology and infertility articles. *Fertil Steril.* (2020) 114:1322–9. doi: 10.1016/j.fertnstert.2020.05.020
- Jin X, Tian C, Kang M, Hu S, Zhao L, Zhang W. The 100 top-cited articles in menopausal syndrome: a bibliometric analysis. *Reprod Health.* (2024) 21:47. doi: 10.1186/s12978-024-01770-9
- Peng C, Kuang L, Zhao J, Ross AE, Wang Z, Ciolino JB. Bibliometric and visualized analysis of ocular drug delivery from 2001 to 2020. *J Control Release.* (2022) 345:625–45. doi: 10.1016/j.jconrel.2022.03.031
- Sun HL, Bai W, Li XH, Huang H, Cui XL, Cheung T, et al. Schizophrenia and inflammation research: a bibliometric analysis. *Front Immunol.* (2022) 13:907851. doi: 10.3389/fimmu.2022.907851
- Lin X, Wu G, Gao B, Wang S, Huang J. Bibliometric and visual analysis of coronary microvascular dysfunction. *Front Cardiovasc Med.* (2022) 9:1021346. doi: 10.3389/fcvm.2022.1021346
- You Y, Li W, Liu J, Li X, Fu Y, Ma X. Bibliometric review to explore emerging high-intensity interval training in health promotion: a new century picture. *Front Public Health.* (2021) 9:697633. doi: 10.3389/fpubh.2021.697633
- Zhou Q, Lu M, Li GS, Peng GL, Song YF. Knowledge mapping and visualization analysis of pelvic organ prolapse repair with mesh from 2001 to 2021. *Front Bioeng Biotechnol.* (2023) 11:1104724. doi: 10.3389/fbioe.2023.1104724
- Chen C. A glimpse of the first eight months of the COVID-19 literature on Microsoft academic graph: themes, citation contexts, and uncertainties. *Front Res Metr Anal.* (2020) 5:607286. doi: 10.3389/frma.2020.607286
- Liu X, Zhao S, Tan L, Tan Y, Wang Y, Ye Z, et al. Frontier and hot topics in electrochemiluminescence sensing technology based on CiteSpace bibliometric analysis. *Biosens Bioelectron.* (2022) 201:113932. doi: 10.1016/j.bios.2021.113932
- van Eck NJ, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics.* (2010) 84:523–38. doi: 10.1007/s11192-009-0146-3



25. van Eck NJ, Waltman L. Citation-based clustering of publications using CitNetExplorer and VOSviewer. *Scientometrics*. (2017) 111:1053–70. doi: 10.1007/s11192-017-2300-7
26. Liu B, Zhou CJ, Ma HW, Gong B. Mapping the youth soccer: a bibliometric analysis using R-tool. *Digit Health*. (2023) 9:20552076231183550. doi: 10.1177/20552076231183550
27. Javed Ali M, Djalilian A. Readership awareness series—paper 7: an overview of impact factor and other scientometrics. *Semin Ophthalmol*. (2024) 39:1–5. doi: 10.1080/08820538.2023.2259180
28. Shanmugasundaram S, Huy B, Shihora D, Lamparello N, Kumar A, Shukla P. Evaluation of *H*-index in academic interventional radiology. *Acad Radiol*. (2023) 30:1426–32. doi: 10.1016/j.acra.2022.09.020
29. Pados G, Gordts S, Sorrentino F, Nisolle M, Nappi L, Daniilidis A. Adenomyosis and infertility: a literature review. *Medicina*. (2023) 59:1551. doi: 10.3390/medicina59091551
30. Calero MJ, Villanueva MRB, Joshaghani N, Villa N, Badla O, Goit R, et al. Fertility and pregnancy outcomes in patients with adenomyosis: is adenomyosis synonymous with infertility? *Cureus*. (2022) 14:e30310. doi: 10.7759/cureus.30310
31. Harada T, Khine YM, Kaponis A, Nikellis T, Decavalas G, Taniguchi F. The impact of adenomyosis on women's fertility. *Obstet Gynecol Surv*. (2016) 71:557–68. doi: 10.1097/OGX.00000000000000346
32. Abu Hashim H, Elaraby S, Fouda AA, Rakhawy ME. The prevalence of adenomyosis in an infertile population: a cross-sectional study. *Reprod Biomed Online*. (2020) 40:842–50. doi: 10.1016/j.rbmo.2020.02.011
33. Moawad G, Kheil MH, Ayoubi JM, Klebanoff JS, Rahman S, Sharara FI. Adenomyosis and infertility. *J Assist Reprod Genet*. (2022) 39:1027–31. doi: 10.1007/s10815-022-02476-2
34. Vercellini P, Viganò P, Bandini V, Buggio L, Berlanda N, Somigliana E. Association of endometriosis and adenomyosis with pregnancy and infertility. *Fertil Steril*. (2023) 119:727–40. doi: 10.1016/j.fertnstert.2023.03.018
35. Maheshwari A, Gurunath S, Fatima F, Bhattacharya S. Adenomyosis and subfertility: a systematic review of prevalence, diagnosis, treatment and fertility outcomes. *Hum Reprod Update*. (2012) 18:374–92. doi: 10.1093/humupd/dms006
36. Cozzolino M, Tartaglia S, Pellegrini L, Troiano G, Rizzo G, Petraglia F. The effect of uterine adenomyosis on IVF outcomes: a systematic review and meta-analysis. *Reprod Sci*. (2022) 29:3177–93. doi: 10.1007/s43032-021-00818-6
37. Younes G, Tulandi T. Effects of adenomyosis on *in vitro* fertilization treatment outcomes: a meta-analysis. *Fertil Steril*. (2017) 108:483–490.e3. doi: 10.1016/j.fertnstert.2017.06.025
38. Vercellini P, Consonni D, Drudi D, Bracco B, Frattaruolo MP, Somigliana E. Uterine adenomyosis and *in vitro* fertilization outcome: a systematic review and meta-analysis. *Hum Reprod*. (2014) 29:964–77. doi: 10.1093/humrep/deu041
39. Mavrelis D, Holland TK, O'Donovan O, Khalil M, Ploumpidis G, Jurkovic D, et al. The impact of adenomyosis on the outcome of IVF-embryo transfer. *Reprod Biomed Online*. (2017) 35:549–54. doi: 10.1016/j.rbmo.2017.06.026
40. Ye L, Liang R, Liu X, Li J, Yue J, Zhang X. Frailty and sarcopenia: a bibliometric analysis of their association and potential targets for intervention. *Ageing Res Rev*. (2023) 92:102111. doi: 10.1016/j.arr.2023.102111
41. Ofer D, Kaufman H, Linial M. What's next? Forecasting scientific research trends. *Heliyon*. (2024) 10:e23781. doi: 10.1016/j.heliyon.2023.e23781
42. Dueholm M, Lundorf E. Transvaginal ultrasound or MRI for diagnosis of adenomyosis. *Curr Opin Obstet Gynecol*. (2007) 19:505–12. doi: 10.1097/GCO.0b013e3282f1bf00
43. Benagiano G, Brosens I. The endometrium in adenomyosis. *Womens Health*. (2012) 8:301–12. doi: 10.2217/WHE.12.8
44. Buggio L, Monti E, Gattei U, Drudi D, Vercellini P. Adenomyosis: fertility and obstetric outcome. A comprehensive literature review. *Minerva Ginecol*. (2018) 70:295–302. doi: 10.23736/S0026-4784.17.04163-6
45. Benagiano G, Brosens I, Habiba M. Adenomyosis: a life-cycle approach. *Reprod Biomed Online*. (2015) 30:220–32. doi: 10.1016/j.rbmo.2014.11.005
46. Wang S, Duan H. The role of the junctional zone in the management of adenomyosis with infertility. *Front Endocrinol*. (2023) 14:1246819. doi: 10.3389/fendo.2023.1246819
47. Allegra A, Marino A. Adenomyosis and infertility: a possible glimmer of light in the dark. *Fertil Steril*. (2024) 121:795–6. doi: 10.1016/j.fertnstert.2024.02.016
48. Salmeri N, di Stefano G, Viganò P, Stratton P, Somigliana E, Vercellini P. Functional determinants of uterine contractility in endometriosis and adenomyosis: a systematic review and meta-analysis. *Fertil Steril*. (2024) 122:1063–78. doi: 10.1016/j.fertnstert.2024.07.026
49. Vannuccini S, Tosti C, Carmona F, Huang SJ, Chapron C, Guo SW, et al. Pathogenesis of adenomyosis: an update on molecular mechanisms. *Reprod Biomed Online*. (2017) 35:592–601. doi: 10.1016/j.rbmo.2017.06.016
50. Fischer CP, Kayisili U, Taylor HS. HOXA10 expression is decreased in endometrium of women with adenomyosis. *Fertil Steril*. (2011) 95:1133–6. doi: 10.1016/j.fertnstert.2010.09.060
51. Jiang Y, Jiang R, Cheng X, Zhang Q, Hu Y, Zhang H, et al. Decreased expression of NR4A nuclear receptors in adenomyosis impairs endometrial decidualization. *Mol Hum Reprod*. (2016) 22:655–68. doi: 10.1093/molehr/gaw042
52. Xiao Y, Li T, Xia E, Yang X, Sun X, Zhou Y. Expression of integrin beta3 and osteopontin in the eutopic endometrium of adenomyosis during the implantation window. *Eur J Obstet Gynecol Reprod Biol*. (2013) 170:419–22. doi: 10.1016/j.ejogrb.2013.05.007
53. Ota H, Igarashi S, Sato N, Tanaka H, Tanaka T. Involvement of catalase in the endometrium of patients with endometriosis and adenomyosis. *Fertil Steril*. (2002) 78:804–9. doi: 10.1016/S0015-0282(02)03344-7
54. Orazov MR, Radzinsky VE, Nosenko EN, Khamoshina MB, Dukhin AO, Lebedeva MG. Immune-inflammatory predictors of the pelvic pain syndrome associated with adenomyosis. *Gynecol Endocrinol*. (2017) 33:44–6. doi: 10.1080/09513590.2017.1399696
55. Ishikawa M, Nakata T, Yaginuma Y, Nishiwaki K, Goishi K, Saitoh S. Expression of superoxide dismutase (SOD) in adenomyosis. *Am J Obstet Gynecol*. (1993) 169:730–4. doi: 10.1016/0002-9378(93)90653-Z
56. Lazzeri L, Andersson KL, Angioni S, Arena A, Arena S, Bartiromo L, et al. How to manage endometriosis in adolescence: the endometriosis treatment Italian Club approach. *J Minim Invasive Gynecol*. (2023) 30:616–26. doi: 10.1016/j.jmig.2023.03.017
57. Horton J, Sterrenburg M, Lane S, Maheshwari A, Li TC, Cheong Y. Reproductive, obstetric, and perinatal outcomes of women with adenomyosis and endometriosis: a systematic review and meta-analysis. *Hum Reprod Update*. (2019) 25:592–632. doi: 10.1093/humupd/dmz012
58. Günther V, Allahqoli L, Gitas G, Maass N, Tesch K, Ackermann J, et al. Impact of Adenomyosis on infertile patients-therapy options and reproductive outcomes. *Biomedicine*. (2022) 10:3245. doi: 10.3390/biomedicine10123245
59. Bourdon M, Santulli P, Oliveira J, Marcellin L, Maignien C, Melka L, et al. Focal adenomyosis is associated with primary infertility. *Fertil Steril*. (2020) 114:1271–7. doi: 10.1016/j.fertnstert.2020.06.018
60. Vannuccini S, Luisi S, Tosti C, Sorbi F, Petraglia F. Role of medical therapy in the management of uterine adenomyosis. *Fertil Steril*. (2018) 109:398–405. doi: 10.1016/j.fertnstert.2018.01.013
61. Vannuccini S, Petraglia F. Recent advances in understanding and managing adenomyosis. *F1000Res*. (2019) 8:283. doi: 10.12688/f1000research.17242.1
62. Grimbizis GF, Mikos T, Tarlatzis B. Uterus-sparing operative treatment for adenomyosis. *Fertil Steril*. (2014) 101:472–87. doi: 10.1016/j.fertnstert.2013.10.025
63. Tan J, Moriarty S, Taskin O, Allaire C, Williams C, Yong P, et al. Reproductive outcomes after fertility-sparing surgery for focal and diffuse adenomyosis: a systematic review. *J Minim Invasive Gynecol*. (2018) 25:608–21. doi: 10.1016/j.jmig.2017.12.020
64. Mijatovic V, Florijn E, Halim N, Schats R, Hompes P. Adenomyosis has no adverse effects on IVF/ICSI outcomes in women with endometriosis treated with long-term pituitary down-regulation before IVF/ICSI. *Eur J Obstet Gynecol Reprod Biol*. (2010) 151:62–5. doi: 10.1016/j.ejogrb.2010.02.047
65. Pecorella G, Nigdelis MP, Sparic R, Morciano A, Tinelli A. Adenomyosis and fertility-sparing surgery: a literature appraisal. *Int J Gynaecol Obstet*. (2024) 166:512–26. doi: 10.1002/ijgo.15389
66. Dueholm M. Uterine adenomyosis and infertility, review of reproductive outcome after *in vitro* fertilization and surgery. *Acta Obstet Gynecol Scand*. (2017) 96:715–26. doi: 10.1111/aogs.13158



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# Clinical efficacy of etonogestrel implants on relieving dysmenorrhea in endometriosis and adenomyosis women for up to 3 years

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**Background:** Dysmenorrhea and menstrual disorders caused by endometriosis (EM) and adenomyosis (AM) have significantly affected the quality of life of a large number of women. As a highly effective clinical contraceptive measure, etonogestrel implants have been previously reported to relieve dysmenorrhea. However, the dysmenorrhea treatment and menstrual regulation effects of etonogestrel implants in AM and EM patients have not been systematically studied.

**Methods:** This retrospective study followed up 100 patients with etonogestrel implants from May 2015 to October 2016, including 44 patients with EM and 56 patients with AM. The VAS scores of dysmenorrhea, menstrual volume, and related adverse events were measured at 12, 24, and 36 months after etonogestrel implantation in these patients.

**Results:** In 100 EM and AM patients, dysmenorrhea significantly improved, with moderate and severe cases decreasing from 50 to 16 and 0% at 36 months. Amenorrhea increased over time, and frequent bleeding declined. Adverse reactions included weight gain (21%), acne (13%), and decreased sexual desire (10%). Serum CA125 levels dropped, confirming therapeutic efficacy.

**Conclusion:** Etonogestrel implantation significantly alleviated dysmenorrhea symptoms in AM and EM patients.

## KEYWORDS

etonogestrel implants, endometriosis, adenomyosis, dysmenorrhea, women

## Introduction

The presence of endometriosis (EM) and adenomyosis (AM) affects 10 to 15% of the female population (1). Although AM and EM are not fatal, they are still the leading cause of pelvic pain and subfertility, and the leading cause of gynecological hospitalizations (2). AM and EM negatively affected women's quality of life, work productivity, sexual relationships, and self-esteem (3). AM and EM not only cause pelvic pain, dyspareunia, amenorrhea, dysmenorrhea, and infertility, but also increase the risk of gynecologic malignancies (4, 5). Women with AM and EM can be asymptomatic, and some EM and AM lesions may heal on their own without diagnosis (6). Some patients with AM and EM can ultimately only be accurately diagnosed by laparoscopy, laparotomy, or hysterectomy, resulting in an immeasurable public health burden (7).



Painful symptoms play a critical role in AM and EM, serving not only as major clinical manifestations but also as key indicators for diagnosis and treatment evaluation (PMID: 34205040). Symptoms such as dysmenorrhea, chronic pelvic pain, dyspareunia, and cyclical or persistent abdominal pain significantly impact patients' quality of life, work productivity, and psychological well-being (8). Dysmenorrhea, in particular, is one of the most common symptoms and often the primary reason for patients seeking medical attention (9). The occurrence of pain is primarily associated with abnormal uterine smooth muscle contractions, proliferation of nerve fibers, and localized inflammatory responses (10). Moreover, the severity of pain is often unrelated to the extent or depth of the lesions, making its management particularly challenging (11). Given the profound impact of pain on patients' lives, investigating effective strategies to alleviate pain symptoms in AM and EM is of significant clinical importance, which is a central focus of this study on the efficacy of etonogestrel implants.

As an estrogen-dependent disease, AM (with or without EM) is sensitive to hormone-related drugs (12). Drug treatments for AM and EM include a range of options, such as oral contraceptive pills, oral progestin-only therapy, the levonorgestrel intrauterine system (Mirena), gestrinone, danazol, and gonadotropin-releasing hormone agonists (GnRH- $\alpha$ ) (13). These therapies, however, are often associated with limitations, including long treatment durations, high recurrence rates after discontinuation, and various adverse effects, which can reduce patient tolerance and compliance (14). Etonogestrel implants have been widely used in clinical contraception with a 1-year unintended pregnancy rate of 0–0.5%. In clinical application, many patients with primary and secondary dysmenorrhea have been found to have significant relief of dysmenorrhea after placement of etonogestrel implants (15). Etonogestrel implants are very useful for patients with AM and EM who resist surgery or who still have unbearable menstrual cramps after surgery (16). Most patients with EM and AM still need contraception (17). Choosing a drug that is both contraceptive and relieving dysmenorrhea would be of greater benefit to the vast majority of women with EM and AM. In this study, we hypothesized that etonogestrel implants could effectively relieve dysmenorrhea in AM and EM patients. CA125 is a high-molecular-weight glycoprotein and a membrane antigen found on the surface of endometriotic lesion cells. Studies have shown that ectopic endometrial tissue has a robust ability to synthesize and secrete CA125, up to four times higher than normal endometrial tissue (18). Adenomyosis can lead to elevated serum CA125 levels due to secretion by ectopic endometrial tissue (19). Therefore, in this study, CA125 was used as an indicator for evaluating EM and AM, indirectly reflecting the activity and functional changes in EM and AM before and after different treatments. We hope our research will lead to further applications of etonogestrel implants in AM and EM therapy.

## Methods

### Study design

This study is a follow-up observational study conducted from May 2015 to October 2016 on AM and EM patients who had etonogestrel

implants placed. From May 2015 to October 2016, 400 contraceptive patients who requested contraception and were placed etonogestrel implants in Hangzhou Obstetrics and Gynecology Hospital and Hangzhou Lin'an District Maternal and Child Health Hospital outpatient clinic were analyzed. One hundred patients diagnosed with EM and AM by clinical symptoms, signs, transvaginal color Doppler ultrasonography, and serum CA125 levels were selected as the research subjects. There were 44 EM patients and 56 AM patients. 11 cases of etonogestrel implants were taken out after 12 months, and the continuation rate in 12 months was 89.0%. Between 13 and 24 months, an additional 10 patients had the implants removed. Therefore, a total of 21 removals occurred over the 0–24-month period, leading to a continuation rate of 79.0% at 24 months. One case was taken out in the third year. The main reasons for removal were bleeding or amenorrhea, weight gain, planning to become pregnant, etc. The relevant details and research process were shown in Figure 1.

### Diagnosis criteria

Patients in this study were diagnosed with AM or EM prior to the placement of etonogestrel implants, based on clinical symptoms, signs, transvaginal color Doppler ultrasonography, and serum CA125 levels. The diagnostic criteria followed the 2015 Guidelines for the Diagnosis and Treatment of Endometriosis issued by the Endometriosis Collaboration Group of the Obstetrics and Gynecology Branch of the Chinese Medical Association.

### Diagnostic criteria for EM

**Clinical Symptoms and Signs:** Pelvic pain, infertility, and menstrual irregularities. **Imaging:** Transvaginal ultrasound is valuable for diagnosing ovarian endometriotic cysts, typically presenting as anechoic regions with dense internal echoes. Transvaginal or rectal ultrasound, CT, and MRI are useful for identifying deep infiltrating lesions in the rectum or rectovaginal septum. **Laparoscopy:** The gold standard for diagnosis, allowing direct observation of lesion morphology. Examination should include detailed assessment of the pelvic cavity, particularly the uterosacral ligament and ovarian fossa. **Histopathology** confirming endometrial glands and stroma, along with inflammatory responses and fibrosis, is required for definitive diagnosis. **Serum CA125:** Elevated CA125 levels are more commonly associated with severe EM, significant pelvic inflammation, ruptured ovarian endometriotic cysts, or coexisting adenomyosis. It is not useful for early-stage EM diagnosis. **Cystoscopy or Colonoscopy:** Indicated for suspected bladder or intestinal EM to exclude malignancy, with biopsy confirmation rates of 10–15%.

### Diagnostic criteria for AM

**Symptoms and Pelvic Examination:** Suggestive findings include an enlarged uterus with a firm, irregular shape. **Imaging:** Ultrasound: Shows uterine enlargement and thickened myometrium, often more pronounced posteriorly. Echogenic spots or streaks within the lesion may be seen, with indistinct boundaries. MRI: Identifies low-signal-intensity lesions on T1-weighted images and high-signal-intensity

Abbreviations: EM, endometriosis; AM, adenomyosis; VAS, Visual analogue scale; GnRH- $\alpha$ , gonadotropin-releasing hormone agonist.

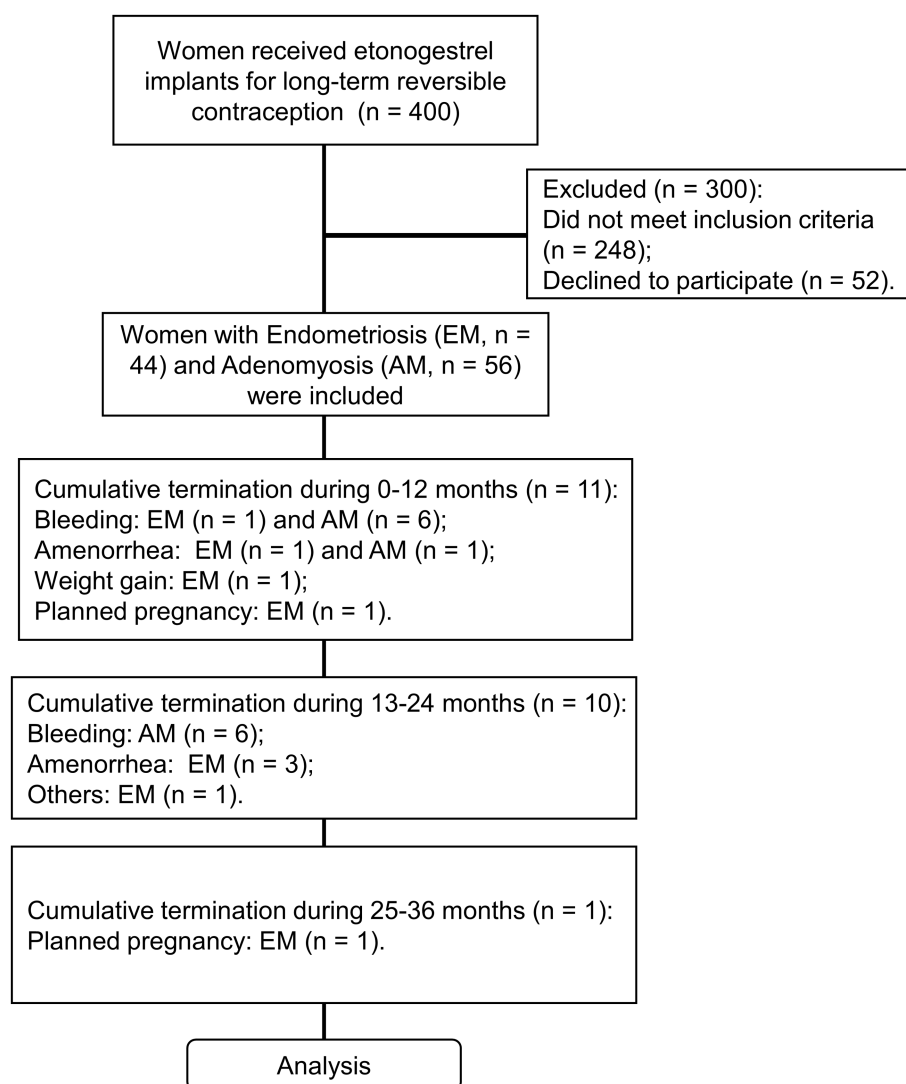


FIGURE 1  
The flow chart diagram of this study.

lesions on T2-weighted images. The junctional zone thickness exceeding 12 mm is a key indicator. Serum CA125: Elevated in most cases. Pathology: Histopathological confirmation is definitive.

## Participants

**Inclusion criteria:** Patients who visited Hangzhou Obstetrics and Gynecology Hospital and Hangzhou Lin'an District Maternal and Child Health Hospital outpatient clinic; patients who were placed etonogestrel implants for contraception; patients were diagnosed as AM or EM before surgery based on clinical symptoms, signs, transvaginal color Doppler ultrasound, or serum CA125 level.

**Exclusion criteria:** patients with abnormal vaginal bleeding; patients diagnosed with malignant tumors of uterine origin or precancerous lesions through diagnostic curettage or cervical biopsy.

All patients were followed up 12 months, 24 months, and 36 months after placement of etonogestrel implants. Telephone, outpatient follow-up and other methods were used to follow up patients. Designated staff responsible for conducting follow-up reviews and

recording data were responsible for reviewing and recording. These tasks include assessing the severity of dysmenorrhea, evaluating menstrual conditions (through menstrual card analysis), documenting other adverse reactions and reasons for implant removal, as well as measuring serum CA125 concentrations. The mentioned activities are part of the routine evaluations performed during patients' regular follow-up visits.

## Etonogestrel implants

The etonogestrel implant used in this study (produced by Organon, the Netherlands, trade name Ebanon, contains 68 mg of etonogestrel, production batch number: 211587/294559) has an effective duration of 3 years. The patients underwent gynecological examination and breast examination before etonogestrel placement, and their blood routine, blood biochemistry, and coagulation function were normal. Cervical cytology examination was used to rule out contraindications to the placement of etonogestrel. The operating doctor explained to the patient in detail the possible adverse reactions and precautions after etonogestrel implantation. Patients signed informed consent. On the

1st to 5th day of menstruation, the etonogestrel implant was placed by a trained and qualified surgeon and the etonogestrel implant special placer was used during the placement operation.

## Dysmenorrhea score

Pain scores were recorded by the VAS pain scale during each patient's menstrual period. 0 points for no pain, 1–3 points for mild pain, 4–6 points for moderate pain, and 7–10 points for severe pain.

## Menstrual bleeding

Patients were asked to assess changes in menstrual bleeding patterns between the two surveys at each survey. Amenorrhea: No bleeding. Infrequent bleeding: 1–2 episodes of bleeding and/or spotting, frequent bleeding: >5 bleeds, regular bleeding: 3–5 bleeds and/or spotting, prolonged bleeding: >14 days continuous bleeding and spotting, and spotting: spotting alone.

## Statistical analysis

Data analysis was performed with SPSS 20.0 statistical software. Normal distribution is expressed as mean  $\pm$  standard deviation. Prior to conducting the comparative analysis, a Kolmogorov–Smirnov test was performed to evaluate the normality of the data distribution. The results demonstrated that the data did not conform to a normal distribution. Consequently, the Friedman test, followed by Dunn's multiple comparisons test, was employed for the comparative analysis.

## Results

### Characteristics of the women with EM and AM

This study included 100 patients with preoperative diagnosis of EM and AM by clinical symptoms, signs, transvaginal color Doppler ultrasonography, and serum CA125 level detection. There were 44 EM patients and 56 AM patients. As shown in Table 1, the age of these patients was 20–45 years ( $33.81 \pm 5.24$ ), the pregnancies were 0–9 with an average of 2.8, and the parity was 0–3 with an average of 1.2. The patients were all married and had no reproductive requirements at present, and required contraception. Among the 100 study subjects, 34 patients had severe dysmenorrhea before placement, and needed to take painkillers or intramuscular analgesics for pain relief. There were 12 patients with AM who had been treated with Mirena before placement, and etonogestrel implants were placed after the device moved down or fell off.

### Alleviation of dysmenorrhea among women with EM and AM post etonogestrel implants

An analysis of the dysmenorrhea of the patients was presented in Table 2. Among the 100 patients, 81 had dysmenorrhea and 19 had no

**TABLE 1** Characteristics of the women with endometriosis (EM,  $n = 44$ ) and adenomyosis (AM,  $n = 56$ ) who received etonogestrel implants for long-term reversible contraception.

| Number of patients                      | 100              |
|-----------------------------------------|------------------|
| Age at consent for implantation (years) | $33.81 \pm 5.24$ |
| BMI (kg/cm <sup>2</sup> )               | $24.73 \pm 4.92$ |
| Gravidity ( $n$ , %)                    |                  |
| 0                                       | 2 (2%)           |
| 1                                       | 9 (9%)           |
| 2                                       | 36 (36%)         |
| $\geq 3$                                | 53 (53%)         |
| Parity ( $n$ , %)                       |                  |
| 0                                       | 7 (7%)           |
| 1                                       | 46 (46%)         |
| 2                                       | 34 (34%)         |
| 3                                       | 13 (13%)         |
| Marriage ( $n$ , %)                     |                  |
| Yes                                     | 100 (100%)       |
| No                                      | 0 (0%)           |
| Previous contraception methods          |                  |
| No contraception control                | 14 (14%)         |
| Intrauterine device                     | 22 (22%)         |
| Condom                                  | 43 (43%)         |
| Oral contraceptive                      | 9 (9%)           |
| Mirena                                  | 12 (12%)         |

BMI: body mass index. Values were expressed as  $n$  (percentage, %) or mean  $\pm$  SD.

dysmenorrhea. The dysmenorrhea was significantly relieved 12 months, 24 months, and 36 months after etonogestrel placement. Sixteen (16%) patients had moderate dysmenorrhea and 34 (34%) patients had severe dysmenorrhea before implantation of the etonogestrel. The proportion of patients without dysmenorrhea at 12 months, 24 months, and 36 months after operation continued to increase, and the proportion of moderate to severe dysmenorrhea continued to decrease, and the difference was statistically significant compared with preoperative ones. These data suggested that etonogestrel implants significantly relieved or eliminated dysmenorrhea symptoms quickly and lastingly.

### Bleeding patterns among women with EM and AM post etonogestrel implants

The menstrual bleeding patterns of EM and AM patients at 12, 24, and 36 months after etonogestrel implants were analyzed and summarized in Table 3. Menstrual bleeding was significantly lower in patients 12 months after etonogestrel implants compared to baseline levels. Consistently, the number of amenorrhea patients with etonogestrel implants increased significantly after 24 and 36 months, and the number of patients with frequent or prolonged bleeding decreased significantly. These data demonstrated that etonogestrel implants could significantly reduce menstrual flow in AM and EM patients.

TABLE 2 Improvement of dysmenorrhea among women with endometriosis and adenomyosis who received etonogestrel implants for long-term reversible contraception.

| Time point         | No pain    | Mild       | Moderate   | Severe     | p value            |
|--------------------|------------|------------|------------|------------|--------------------|
| Baseline (n = 100) | 19 (19%)   | 16 (16%)   | 31 (31%)   | 34 (34%)   | /                  |
| 12 months (n = 89) | 31 (34.8%) | 20 (22.5%) | 22 (24.7%) | 16 (18.0%) | 0.013*             |
| 24 months (n = 79) | 37 (46.8%) | 22 (27.8%) | 17 (21.5%) | 3 (3.8%)   | 0.022 <sup>†</sup> |
| 36 months (n = 78) | 53 (67.9%) | 16 (20.5%) | 9 (11.5%)  | 0 (0%)     | 0.026 <sup>‡</sup> |

Values were expressed as n (percentage, %). p value was derived from Chi-square test. \*Compared to baseline, <sup>†</sup>compared to 12 months, <sup>‡</sup>compared to 24 months.

TABLE 3 Changes in bleeding patterns among women with endometriosis and adenomyosis who received etonogestrel implants for long-term reversible contraception.

| Time point         | Amenorrhea | Infrequent bleeding | Regular bleeding | Frequent bleeding | Prolonged bleeding | p value            |
|--------------------|------------|---------------------|------------------|-------------------|--------------------|--------------------|
| Baseline (n = 100) | 7 (7%)     | 12 (12%)            | 19 (19%)         | 29 (29%)          | 33 (33%)           |                    |
| 12 months (n = 89) | 18 (20.2%) | 21 (23.6%)          | 21 (23.6%)       | 20 (22.5%)        | 9 (10.1%)          | 0.000*             |
| 24 months (n = 79) | 23 (29.1%) | 29 (36.7%)          | 17 (21.5%)       | 8 (10.1%)         | 2 (2.5%)           | 0.023 <sup>†</sup> |
| 36 months (n = 78) | 27 (34.6%) | 32 (41.1%)          | 16 (20.5%)       | 3 (3.8%)          | 0 (0%)             | 0.312 <sup>‡</sup> |

Values were expressed as n (percentage, %). p value was derived from Chi-square test. \*Compared to baseline, <sup>†</sup>compared to 12 months, <sup>‡</sup>compared to 24 months.

Adverse reactions in patients post etonogestrel implants

During the three-year follow-up period, the adverse reaction statistics of the patients were shown in Table 4. The main adverse reactions were weight gain in 21 cases (21%), acne in 13 cases (13.0%), breast tenderness in 9 cases (9%), abdominal pain in 6 cases (6%), mood changes in 4 cases (4%), sexual desire Decreased in 10 cases (10%), sleep disorder in 4 cases (4%), pigmentation in 2 cases (2.0%), etc. After placing etonogestrel implants in 100 patients with EM and AM, the dysmenorrhea of the patients was significantly relieved or even disappeared, and the menstrual flow was significantly reduced, suggesting that etonogestrel implants had a significant effect on the treatment of EM and AM.

Changes in VAS scores and serum CA125 in AM and EM patients

VAS scores and changes in serum CA125 for all patients (n = 78) at the end of the three-year follow-up endpoint are shown in Figure 2. As shown in Figure 2A, the VAS of patients with dysmenorrhea decreases year by year, which further confirms that the patient’s dysmenorrhea was significantly relieved or even disappeared after placing etonogestrel implants in the previously reported data. In addition, the serum CA125 level of etonogestrel implants in AM and EM patients also gradually decreased, suggesting the therapeutic effect of etonogestrel implants in AM and EM (Figure 2B).

Discussion

The purpose of this study was to investigate the clinical efficacy and adverse effects of etonogestrel implants for the relief of dysmenorrhea in patients with EM and AM (20). EM and AM are common diseases in obstetrics and gynecology, with an incidence of

2–48% (21). In recent years, the incidence of EM and AM has increased significantly. EM and AM are generally seen in women of reproductive age, and are more common in women aged 25–45. About 15–40% of AM patients have EM (22). Women with EM or AM are often accompanied by dysmenorrhea and increased menstrual bleeding, which seriously affects their quality of life and future fertility (23). The main causes of dysmenorrhea and miscarriage caused by AM and EM are endocrine dysfunction, decreased endometrial receptivity and immune factors (24). In addition, AM leads to impaired uterine spiral arterial remodeling and structural dysfunction of the uterine junction zone, which increases the reproductive risk in women (25). At present, hysterectomy is the accepted cure for AM. Although surgical treatment is straightforward, the subsequent loss of fertility, early perimenopausal symptoms, and pelvic floor dysfunction have a serious impact on the psychological and physical health of patients (26). Drug therapy for AM and EM mainly includes levonorgestrel intrauterine sustained-release system (Mirena), gestrinone, danazol, and gonadotropin-releasing hormone agonist (GnRH-α), etc. (27). However, EM and AM have a high recurrence rate after drug treatment.

Etonogestrel implants have been widely used in clinical contraception since it was approved by the U.S. Food and Drug Administration (FDA) in 2006 (28). The 1-year unintended pregnancy rate of etonogestrel implants is less than 0.5%, which is close to sterilization (29). In addition to the exact contraceptive effect, etonogestrel implants also have the advantages of high efficiency, good tolerance, fewer symptoms of estrogen deficiency, and quick recovery of fertility after removal (30). In clinical applications, etonogestrel implants have been found to be significantly relieved by etonogestrel implants in many patients with primary and secondary dysmenorrhea after placement, which is very valuable for patients with AM and EM who resist hysterectomy (31). Etonogestrel implants are thus a valuable supplemental treatment modality in some AM patients with EM who may still have unbearable dysmenorrhea after hysterectomy. The mechanism of action of etonogestrel implants for clinical contraception is the inhibition of ovulation. It can also induce endometrial atrophy, reduce menstrual flow, or even cause



amenorrhea, and thus relieve dysmenorrhea (31). EM and AM are common and intractable diseases in women of reproductive age. At the same time, most patients with EM and AM still need contraception due to their poor intrauterine structure (32). Choosing a clinical strategy for both contraception and the treatment of dysmenorrhea will bring greater benefits to the majority of women with EM and AM.

This study followed up 100 patients with etonogestrel implants between May 2015 and October 2016, including 44 patients with EM and 56 patients with AM. We investigated the VAS score of dysmenorrhea, menstrual volume and related adverse reactions in patients with EM and AM after etonogestrel implantation at 12, 24, and 36 months. We found that in 19 patients with dysmenorrhea, dysmenorrhea was significantly relieved at 12, 24, and 36 months after the placement of etonogestrel implants. In addition, the statistics show that etonogestrel implants can significantly reduce menstrual bleeding. In this study, 22 patients had etonogestrel implants removed, of which 13 were removed due to vaginal bleeding, accounting for 59.09%. The main presentation in these patients was irregular bleeding and spotting after placement of etonogestrel implants. Vaginal spot bleeding is a very tricky

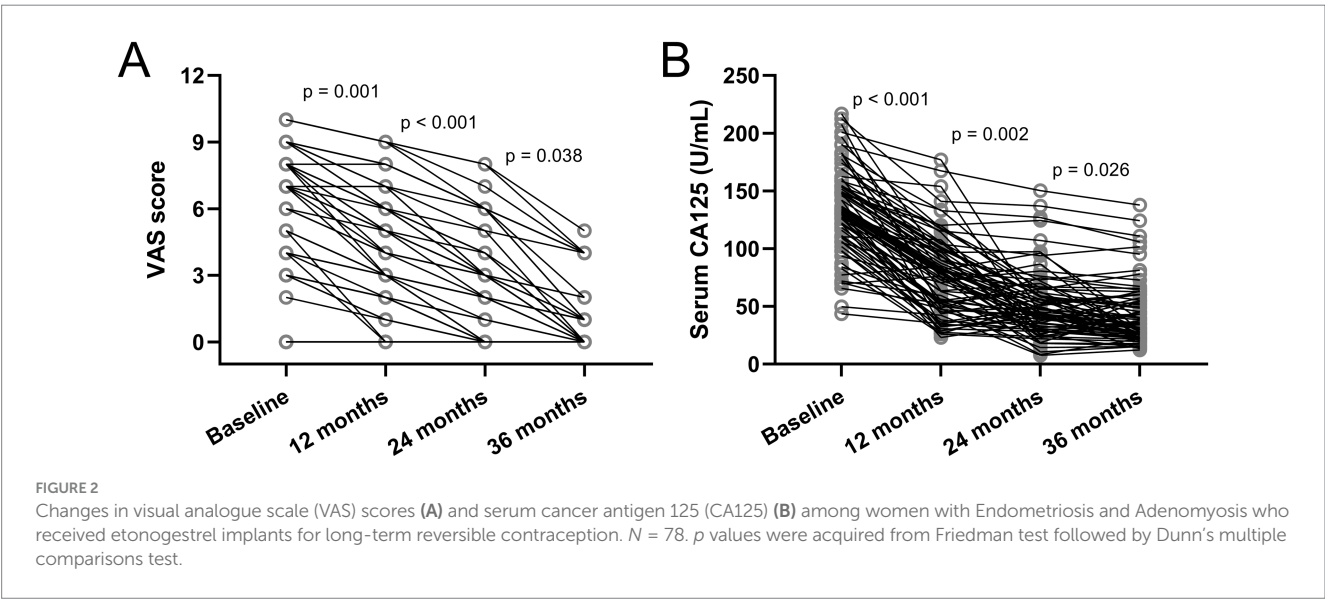
problem in the use of etonogestrel implants (33). There is no proper solution yet, but the amount of bleeding is very small. Generally, no special treatment is required, and it does not affect daily life of the patients. Vaginal bleeding from etonogestrel implants is more likely to be accepted by patients after adequate counseling and explanation. Other adverse reactions of etonogestrel implants are mainly weight gain, acne, breast tenderness, mood changes, loss of libido, etc. However, these adverse reactions did not affect the continuation rate of etonogestrel implants. By analyzing the data in this study, we believe that etonogestrel implants are characterized by easy placement and long duration of treatment. The use of etonogestrel implants overcomes the characteristics of long-term oral tolerance or poor compliance of traditional dysmenorrhea drugs and frequent recurrence after drug withdrawal. Etonogestrel implants have few systemic adverse reactions and a good contraceptive effect. In addition, we also analyzed the CA125 levels in different time points of the two groups of patients. Since CA125 is abundantly present on the cell membrane surface of metaplastic epithelial tissues and can indirectly reflect the activity and function of ectopic endometrial tissue, it serves as a valuable indicator for observing EM and AM. It can indirectly reflect the effects of different treatments and pre- and post-treatment changes on the activity and function of EM and AM. Therefore, we believe that etonogestrel implants are a feasible way to treat dysmenorrhea in patients with EM and AM.

This study has several limitations. Its retrospective design introduces potential recall and selection biases, especially as 22 patients with incomplete data were excluded. The small sample size and lack of a control group limit the generalizability and comparability of the findings, while reliance on subjective outcome measures, such as VAS scores and self-reported bleeding patterns, may introduce reporting bias. Additionally, the study was conducted in a single center, which may reduce its broader applicability. Adverse reactions were assessed only over the three-year follow-up, leaving long-term safety and efficacy unaddressed. Serum CA125, used as a marker of disease activity, may have limited specificity, and the study did not evaluate the impact of etonogestrel implants on fertility, a key concern

TABLE 4 Adverse reactions among women with endometriosis and adenomyosis who received etonogestrel implants for long-term reversible contraception.

| Number of patients | 100      |
|--------------------|----------|
| Weight gain        | 21 (21%) |
| Acen               | 13 (13%) |
| Breast pain        | 9 (9%)   |
| Abdominal pain     | 6 (%)    |
| Emotional lability | 4 (4%)   |
| Hypaphrodisia      | 10 (10%) |
| Sleep disorders    | 4 (4%)   |
| Skin pigmentation  | 2 (2%)   |

Values were expressed as n (percentage, %).



for many patients with EM and AM. Lastly, while the follow-up period was sufficient to observe medium-term effects, it does not provide insight into the long-term sustainability of the treatment benefits.

## Conclusion

In conclusion, we analyzed the therapeutic effect of etonogestrel implants on dysmenorrhea and irregular menstruation in AM and EM patients and its adverse effects in this study. We show that etonogestrel implants can significantly relieve dysmenorrhea and reduce menstrual bleeding in AM and EM patients. The main adverse effects of etonogestrel implants are irregular bleeding and spotting after placement, which are acceptable in most patients. We believe our study provides possible therapeutic options for the management of dysmenorrhea and menstrual irregularities in AM and EM patients with contraceptive needs.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## Ethics statement

The studies involving humans were approved by Hangzhou Obstetrics and Gynecology Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## References

- Gruber TM, Ortlieb L, Henrich W, Mechsner S. Deep infiltrating endometriosis and Adenomyosis: implications on pregnancy and outcome. *J Clin Med.* (2021) 11:157. doi: 10.3390/jcm11010157
- Shi J, Dai Y, Zhang J, Li X, Jia S, Leng J. Pregnancy outcomes in women with infertility and coexisting endometriosis and adenomyosis after laparoscopic surgery: a long-term retrospective follow-up study. *BMC Pregnancy Childbirth.* (2021) 21:383. doi: 10.1186/s12884-021-03851-0
- Sun TT, Li XY, Shi JH, Wu YS, Gu ZY, Leng JH. Clinical features and long-term outcomes after laparoscopic surgery in patients co-existing with endometriosis and Adenomyosis. *Front Med (Lausanne).* (2021) 8:696374. doi: 10.3389/fmed.2021.696374
- Deng S, Lang JH, Leng JH, Liu ZF, Sun DW, Zhu L. Effects of levonorgestrel-releasing intrauterine system on pain and recurrence associated with endometriosis and adenomyosis. *Zhonghua Fu Chan Ke Za Zhi.* (2006) 41:664–8.
- Xia W, Zhang D, Ouyang J, Liang Y, Zhang H, Huang Z, et al. Effects of pelvic endometriosis and adenomyosis on ciliary beat frequency and muscular contractions in the human fallopian tube. *Reprod Biol Endocrinol.* (2018) 16:48. doi: 10.1186/s12958-018-0361-y
- Chao X, Liu Y, Fan Q, Shi H, Wang S, Lang J. The role of the vaginal microbiome in distinguishing female chronic pelvic pain caused by endometriosis/adenomyosis. *Ann Transl Med.* (2021) 9:771. doi: 10.21037/atm-20-4586
- Qi H, Zhang H, Zhang D, Li J, Huang Z, Zhao X, et al. Reassessment of prevalence of tubal endometriosis, and its associated clinicopathologic features and risk factors in premenopausal women received salpingectomy. *Eur J Obstet Gynecol Reprod Biol X.* (2019) 4:100074. doi: 10.1016/j.eurox.2019.100074
- Schrager S, Yogendran L, Marquez CM, Sadowski EA. Adenomyosis: diagnosis and management. *Am Fam Physician.* (2022) 105:33–8.
- Bernardi M, Lazzeri L, Perelli F, Reis FM, Petraglia F. Dysmenorrhea and related disorders. *F1000Res.* (2017) 6:1645. doi: 10.12688/f1000research.11682.1
- Chapron C, Vannuccini S, Santulli P, Abrao MS, Carmona F, Fraser IS, et al. Diagnosing adenomyosis: an integrated clinical and imaging approach. *Hum Reprod Update.* (2020) 26:392–411. doi: 10.1093/humupd/dmz049
- Martire FG, Piccione E, Exacoustos C, Zupi E. Endometriosis and adolescence: the impact of dysmenorrhea. *J Clin Med.* (2023) 12:624. doi: 10.3390/jcm12175624
- Scheerer C, Bauer P, Chiantera V, Sehoul J, Kaufmann A, Mechsner S. Characterization of endometriosis-associated immune cell infiltrates (EMaICI). *Arch Gynecol Obstet.* (2016) 294:657–64. doi: 10.1007/s00404-016-4142-6
- Kittai AS, Blank J, Graff JN. Gonadotropin-releasing hormone antagonists in prostate Cancer. *Oncology (Williston Park).* (2018) 32:604–596.
- Pienkowski C, Tauber M. Gonadotropin-releasing hormone agonist treatment in sexual precocity. *Endocr Dev.* (2016) 29:214–29. doi: 10.1159/000438893
- Rocca ML, Palumbo AR, Visconti F, Di Carlo C. Safety and benefits of contraceptives implants: a systematic review. *Pharmaceuticals (Basel).* (2021) 14:548. doi: 10.3390/ph14060548
- El-Hadad S, Villars P, Kahr MK, Leeners B. Removal of nonpalpable etonogestrel implants after fixation with a curved needle—a case series. *Contraception.* (2021) 104:577–80. doi: 10.1016/j.contraception.2021.07.001
- Moray KV, Chaurasia H, Sachin O, Joshi B. A systematic review on clinical effectiveness, side-effect profile and meta-analysis on continuation rate of etonogestrel contraceptive implant. *Reprod Health.* (2021) 18:4. doi: 10.1186/s12978-020-01054-y
- Wang Y, Yi YC, Guu HF, Chen YF, Kung HF, Chang JC, et al. Impact of adenomyosis and endometriosis on IVF/ICSI pregnancy outcome in patients undergoing gonadotropin-releasing hormone agonist treatment and frozen embryo transfer. *Sci Rep.* (2023) 13:6741. doi: 10.1038/s41598-023-34045-7

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YL: Data curation, Funding acquisition, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. DL: Data curation, Validation, Writing – original draft, Writing – review & editing. TF: Data curation, Validation, Writing – original draft, Writing – review & editing. CW: Data curation, Validation, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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19. Kil K, Chung JE, Pak HJ, Jeung IC, Kim JH, Jo HH, et al. Usefulness of CA125 in the differential diagnosis of uterine adenomyosis and myoma. *Eur J Obstet Gynecol Reprod Biol.* (2015) 185:131–5. doi: 10.1016/j.ejogrb.2014.12.008
20. Just PA, Moret S, Borghese B, Chapron C. Endometriosis and adenomyosis. *Ann Pathol.* (2021) 41:521–34. doi: 10.1016/j.annpat.2021.03.012
21. Van Den Bosch T, Van Schoubroeck D. Ultrasound diagnosis of endometriosis and adenomyosis: state of the art. *Best Pract Res Clin Obstet Gynaecol.* (2018) 51:16–24. doi: 10.1016/j.bpobgyn.2018.01.013
22. Bordonne C, Puntot J, Maitrot-Mantelet L, Bourdon M, Marcellin L, Dion E, et al. Imaging for evaluation of endometriosis and adenomyosis. *Minerva Obstet Gynecol.* (2021) 73:290–303. doi: 10.23736/S2724-606X.21.04710-9
23. Maruyama S, Imanaka S, Nagayasu M, Kimura M, Kobayashi H. Relationship between adenomyosis and endometriosis; different phenotypes of a single disease? *Eur J Obstet Gynecol Reprod Biol.* (2020) 253:191–7. doi: 10.1016/j.ejogrb.2020.08.019
24. Gruber TM, Mechsner S. Pathogenesis of endometriosis: the origin of pain and subfertility. *Cells.* (2021) 10:1381. doi: 10.3390/cells10061381
25. Vannuccini S, Petraglia F. Recent advances in understanding and managing adenomyosis. *F1000Res.* (2019) 8:283. doi: 10.12688/f1000research.17242.1
26. Bulun SE, Yildiz S, Adli M, Wei JJ. Adenomyosis pathogenesis: insights from next-generation sequencing. *Hum Reprod Update.* (2021) 27:1086–97. doi: 10.1093/humupd/dmab017
27. Horton J, Sterrenburg M, Lane S, Maheshwari A, Li TC, Cheong Y. Reproductive, obstetric, and perinatal outcomes of women with adenomyosis and endometriosis: a systematic review and meta-analysis. *Hum Reprod Update.* (2019) 25:592–632. doi: 10.1093/humupd/dmz012
28. Wong S, Naresh A. Etonogestrel subdermal implant-associated regression of endometrial intraepithelial neoplasia. *Obstet Gynecol.* (2019) 133:780–2. doi: 10.1097/AOG.0000000000003152
29. Dugre N, Choksi N, Kirkwood J. Etonogestrel implant effectiveness. *Can Fam Physician.* (2022) 68:594. doi: 10.46747/cfp.6808594
30. Berlan ED, Richards MJ, Vieira CS, Creinin MD, Kaunitz AM, Fraser IS, et al. Best practices for counseling adolescents about the Etonogestrel implant. *J Pediatr Adolesc Gynecol.* (2020) 33:448–54. doi: 10.1016/j.jpag.2020.06.022
31. Carvalho N, Margatho D, Cursino K, Benetti-Pinto CL, Bahamondes L. Control of endometriosis-associated pain with etonogestrel-releasing contraceptive implant and 52-mg levonorgestrel-releasing intrauterine system: randomized clinical trial. *Fertil Steril.* (2018) 110:1129–36. doi: 10.1016/j.fertnstert.2018.07.003
32. Bishop IJ, Gertz AM, Simon B, Tawe L, Lechiile K, Liu S, et al. Etonogestrel concentrations among contraceptive implant users in Botswana using and not using dolutegravir-based antiretroviral therapy. *Contraception.* (2020) 102:174–9. doi: 10.1016/j.contraception.2020.04.019
33. Zigler RE, McNicholas C. Unscheduled vaginal bleeding with progestin-only contraceptive use. *Am J Obstet Gynecol.* (2017) 216:443–50. doi: 10.1016/j.ajog.2016.12.008

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