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Meta-Analysis

Gynaecology

Müllerian anomalies and endometriosis as potential explanatory models for the retrograde menstruation/implantation and the embryonic remnants/celomic metaplasia pathogenic theories: a systematic review and meta-analysis

Paolo Vercellini [1,2,*], Noemi Salmeri [3], Edgardo Somigliana [1,2], Martina Piccini [1], Francesca Caprara [1], Paola Viganò [1,2], and Sara De Matteis [3,4]

[1]Academic Center for Research on Adenomyosis and Endometriosis, Department of Clinical Sciences and Community Health, Università degli Studi, Milano, Italy
[2]Department of Maternal, Infant, and Child Health, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[3]Department of Obstetrics and Gynaecology, IRCCS San Raffaele Scientific Institute, Milan, Italy
[4]Department of Health Sciences, Università degli Studi, ASST Santi Paolo e Carlo, Milano, Italy

*Correspondence address. Department of Clinical Sciences and Community Health, Università degli Studi, Via Commenda 12, 20122 Milano, Italy.
E-mail: paolo.vercellini@unimi.it https://orcid.org/0000-0003-4195-0996

ABSTRACT

STUDY QUESTION: Does endometriosis prevalence differ in patients with obstructive Müllerian anomalies (OMA) versus those with nonobstructive Müllerian anomalies (NOMA), and in patients with NOMA versus those without Müllerian anomalies?

SUMMARY ANSWER: The quantitative synthesis of published data demonstrates a substantially increased prevalence of endometriosis in patients with OMA compared with those with NOMA, and a similar prevalence in patients with NOMA and those without Müllerian anomalies.

WHAT IS KNOWN ALREADY: The pathogenesis of endometriosis has not been definitively clarified yet. A higher prevalence of endometriosis in patients with OMA than in those with NOMA would support the retrograde menstruation (RM)/implantation theory, whereas a higher prevalence of endometriosis in the NOMA group than in the group without Müllerian anomalies would support the embryonic remnants/celomic metaplasia hypothesis.

STUDY DESIGN, SIZE, DURATION: This systematic review with meta-analysis was restricted to full-length, English-language articles published in peer-reviewed journals between 1980 and 2023. The PubMed and EMBASE databases were searched using the keyword 'endometriosis' in combination with 'Müllerian anomalies', 'obstructive Müllerian anomalies', 'female genital malformations', 'retrograde menstruation', 'infertility', 'pelvic pain', and 'classification'. References from relevant publications were screened, and PubMed's 'similar articles' and 'cited by' functions were used.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Studies were selected if they reported the prevalence of surgically confirmed endometriosis in either individuals with OMA compared to those with NOMA, or patients with NOMA compared to those without Müllerian anomalies. Cohort and case-control studies and case series were deemed eligible for inclusion. Noncomparative studies, studies not reporting both the number of individuals with endometriosis and the total number of those with Müllerian anomalies or with other gynecological conditions, those including exclusively data on patients with absent or uncertain menstrual function (e.g. complete Müllerian agenesis category), or with imperforate hymen were excluded. Two reviewers independently abstracted data. The risk of bias was assessed with the Risk of Bias In Non-randomized Studies of Exposures tool. The overall certainty of the evidence was graded according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines.

MAIN RESULTS AND THE ROLE OF CHANCE: Seven retrospective studies were included. The overall mean estimate of endometriosis prevalence was 47% (95% CI, 36–58%) in patients with OMA, and 19% (95% CI, 15–24%) in patients with NOMA, with a common odds ratio (OR) of 4.72 (95% CI, 2.54–8.77). The overall mean estimate of endometriosis prevalence in patients with NOMA was 23% (95% CI, 20–27%), and that in patients without Müllerian anomalies was 21% (95% CI, 20–22%), with a common OR of 0.95 (95% CI, 0.57–1.58). The overall certainty of the evidence according to GRADE guidelines was judged as low for both comparisons.

LIMITATIONS, REASON FOR CAUTION: Some NOMA subtypes may create a partial obstacle to menstrual efflux and/or generate dysfunctional myometrial contractions that favor transtubal reflux, thus increasing the risk of endometriosis and limiting the difference between OMA and NOMA. As infertility and pelvic pain are strongly associated with endometriosis, women with these symptoms are inappropriate controls. Confounding by indication could explain the lack of difference in endometriosis prevalence between patients with NOMA and those without Müllerian anomalies.

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WIDER IMPLICATIONS OF THE FINDINGS: The results of this meta-analysis support the validity of the RM theory but do not definitively rule out alternative hypotheses. Thus, RM may be considered the initiator for the development of endometriotic lesions, while not excluding the contribution of both inheritable and tissue-specific genetic and epigenetic modifications as disease-promoting factors.

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Introduction

The association between congenital Müllerian anomalies and endometriosis has been a topic of investigation and debate since the half of the past century, although scientific interest increased substantially during the eighties (Pitot *et al.*, 2020).

The reason for such interest lies substantially in the search for evidence supporting or refuting two of the principal pathogenic theories underpinning endometriosis onset: the retrograde menstruation (RM)/implantation theory, based on transtubal reflux and implantation of originally eutopic endometrium at ectopic sites; and the embryonic remnants/celomic metaplasia theory, based on the development of endometrial tissue primarily at ectopic sites directly from embryonic remnants and/or through celomic metaplasia (i.e. celomic epithelium-derived tissues differentiating into Müllerian-oriented epithelium such as tubal (serous) epithelium, endometrial epithelium, endosalpingiosis, endosalpingiomas, and endometriosis) (Lauchlan, 1972; Redwine, 1988; Fujii, 1991). This condition was originally ascribed by Sampson (1925) to 'developmentally misplaced endometrial tissue'.

The comparison of endometriosis prevalence in patients with obstructive Müllerian anomalies (OMA) and those with nonobstructive Müllerian anomalies (NOMA) could constitute an explanatory model based on the existence of a biological gradient between the amount of refluxed blood and endometrial fragments and the risk of developing endometriosis. In other words, women with OMA would constitute a natural 'quasi-experimental' group with pathologically increased menstrual reflux, whereas patients with NOMA would constitute a 'control' group characterized by a physiologic amount of menstrual reflux. The choice of women with NOMA as controls should limit confounding, as cases and controls are selected from basically the same population, i.e. women with a defect in the development, or in the process of descent, fusion, and medial resorption, of Müllerian ducts during embryogenesis.

A comparison between patients with NOMA and those without Müllerian anomalies is also aimed at verifying the relation between RM and endometriosis. A strong positive association between NOMA and endometriosis would partially argue against the RM theory and instead support the embryonic remnants/celomic metaplasia hypothesis. If genetic derangements or abnormal intrauterine environmental exposures cause both uterine malformations and endometriosis, a higher prevalence of endometriosis would be observed in the NOMA group than in the group without Müllerian anomalies. In this case, endometriosis could originate from vestigial Müllerian cells embedded in the celomic mesothelium during the organogenetic derailment (Fedele *et al.*, 1992). This condition has also been termed Mülleriosis (Redwine, 1988). Conversely, if the same prevalence were observed in individuals with NOMA and those without genital anomalies, the hypothesis of an embryologic etiology of endometriosis would lose credibility.

Finally, if the natural OMA experimental model is biologically valid, correction of outflow obstruction without destruction of endometriotic lesions should be followed by the usual course of endometriosis observed in patients without Müllerian anomalies, e.g. high pain and lesion persistence/recurrence rates in the absence of postoperative hormonal suppression. Instead, prompt spontaneous endometriosis resolution without the need for surgical or medical interventions after obstruction removal would support the hypothesis that the pathogenesis of such 'secondary' endometriosis may differ from that of the classic disease, and would scale back the importance of the amount of RM in spontaneous 'primary' forms, thus emphasizing the role of additional promoting factors.

To try to partly disentangle the above uncertainties, we reviewed the literature data on the prevalence of endometriosis in women with Müllerian anomalies published since 1980. In particular, we aimed at pooling the available evidence on the prevalence of endometriosis in patients with OMA versus NOMA, as well as in individuals with NOMA versus those without Müllerian anomalies.

Materials and methods

Search strategy and eligibility criteria

This review was restricted to full-length, English-language articles published in peer-reviewed journals between 1 January 1980 and 1 December 2023. Information was identified by systematically searching the electronic PubMed and EMBASE databases in December 2023 using the keyword 'endometriosis' in combination with 'Müllerian anomalies', 'obstructive Müllerian anomalies', 'female genital malformations', 'retrograde menstruation', 'infertility', 'pelvic pain', and 'classification'. References from relevant publications were systematically screened and further articles were searched using PubMed's 'similar articles' and 'cited by' functions.

Studies were included only if they reported the prevalence of surgically confirmed endometriosis in either individuals with obstructive (OMA) compared to those with NOMA, or patients with NOMA compared to those without Müllerian anomalies. Only observational studies involving humans, including cohort, case–control studies, and case series, were deemed eligible for inclusion in the review.

Individual case reports were excluded, as well as editorials, opinions, reviews, and abstracts presented at meetings. Noncomparative studies and studies not investigating the presence or absence of endometriosis, not reporting both the

numerator (subjects with endometriosis) and the denominator (total number of individuals with Müllerian anomalies or with other gynecological conditions), those reporting exclusively data on patients with absent or uncertain menstrual function (e.g. complete Müllerian agenesis category, Mayer–Rokitanksy–Küster–Hauser (MRKH) syndrome), or those with imperforate hymen who generally do not undergo abdominal exploration were excluded. When the series included patients with and without abdominal exploration, only the former subgroup was considered.

The study was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations.

Data extraction

Two observers (M.P. and F.C.) independently evaluated all retrieved articles; discrepancies between the two were addressed through discussion with a third reviewer (P.Ve.).

The two reviewers independently abstracted the following data, using a standardized form: year of publication, country, study design, Müllerian anomalies classification adopted, endometriosis classification used, malformation category, number of cases with each OMA and/or NOMA malformation, number of patients with endometriosis in the two groups of each comparison, and, when available, endometriosis disease stage.

Assessment of risk of bias and certainty of the evidence

The risk of bias within and across included studies was assessed by adopting the Risk of Bias In Non-randomized Studies of Exposures (ROBINS-E) tool from the Cochrane collaboration guidelines (Higgins, 2023). The seven bias domains of the tool were evaluated independently by two reviewers (N.S. and P.Vi.), and any disagreement was resolved by discussion with a third reviewer (P.Ve.). Each of the included studies was classified as at low, moderate, or serious risk of bias.

The overall certainty of the evidence was graded into four levels (high, moderate, low, or very low) according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines (Guyatt et al., 2008).

Data synthesis

For each study, a 2×2 table was generated that included the number of patients with and without endometriosis in the two considered study groups (OMA versus NOMA; NOMA versus patients without Müllerian anomalies). The pooled proportion of endometriosis in each study group was calculated and reported as overall (mean) estimate, in percentage (%), with corresponding 95% CIs.

Data on binary outcome measures (presence or absence of endometriosis in each group of comparison) were used to calculate odds ratios (ORs) and their 95% CIs. For each meta-analysis, the summary risk estimate was calculated using the DerSimonian and Laird method (DerSimonian and Laird 1986; DerSimonian and Kacker 2007) for random-effects models. The fixed-effect model with the Mantel–Haenszel method (1959) was further implemented in case of low heterogeneity, to help the reader address the impact of pooling models on summary effect estimates.

The I^2 statistics, which describe the proportion of the total variation of estimates across studies caused by heterogeneity rather than chance (Higgins et al., 2003), were then calculated. Negative values of I^2 are set equal to zero so that I^2 lies between 0% and 100%. A value of 0% indicates no observed heterogeneity, whereas I^2 values of 25%, 50%, and 75% indicate low, moderate, and high heterogeneity, respectively (Higgins et al., 2003). When statistically and biologically relevant, sensitivity analyses were conducted to evaluate the influence of individual studies on the overall pooled estimate. Small-study effect bias was tested using the Egger's test (Egger et al., 1997). All analyses were performed using Stata software, version 17.0 (StataCorp, 2022).

The present review was exempt from Institutional Review Board approval as exclusively published, de-identified data were used.

Results

The flow diagram of the literature search results is shown in Fig. 1. Out of a total of 104 records assessed for eligibility, 96 non-comparative studies not meeting the inclusion criteria were excluded. The main reason for exclusion was that these studies did not specifically address the presence or absence of endometriosis (e.g. Rock and Jones, 1980; Rock et al., 1984, 2010; Song et al., 2016). Additionally, one study (Fedele et al. 1989) describing patients included in a subsequent larger series was also excluded. A total of seven comparative studies published between 1986 and 2014 were finally included in the assessment of endometriosis prevalence in patients with OMA versus those with NOMA (Acién, 1986; Olive and Henderson, 1987; Uğur et al., 1995; Tong et al., 2014), and in those with NOMA versus those without Müllerian anomalies (Fedele et al., 1992; Uğur et al., 1995; Nawroth et al., 2006; Demir et al., 2011). One study recruited three types of patients (OMA, NOMA, and no Müllerian anomalies) at the same time (Uğur et al., 1995). Consequently, this study has been included in both the preplanned comparisons. All studies were retrospective reviews of case series. A total of 76 patients had OMA, 668 had NOMA, and 4462 had various non-Müllerian gynecologic conditions. The characteristics of studies included in the two comparisons are shown in Tables 1 and 2.

OMA versus NOMA

Acién (1986) described the surgical findings in 46 patients with various genital anomalies. Excluding four cases of gonadal dysgenesis and three cases of MRKH syndrome, endometriosis was detected in 5/39 (13%) cases, 1/10 (10%) in the obstructive anomalies group, and 4/29 (14%) in the nonobstructive anomalies group ($P = 1.00$). Endometriosis was not found in two patients with transverse vaginal septum.

Olive and Henderson (1987) observed endometriosis in 10/13 (77%) women with OMA versus 16/43 (37%) of those with NOMA ($P < 0.01$). Eight of nine patients with hematocolpos or hematometra had endometriosis.

In the series of Uğur et al. (1995), endometriosis was detected in 15/26 (58%) patients with outflow obstruction versus 21/119 (18%) of those with NOMA ($P < 0.001$). Endometriosis was in minimal or mild forms, except for four cases of moderate disease, all in the obstructive anomalies group.

Tong et al. (2014) diagnosed endometriosis in 18/94 (19%) young women who underwent surgery for obstructed hemivagina and ipsilateral renal anomaly (OHVIRA) syndrome (Smith and Laufer, 2007). The prevalence of endometriosis was significantly higher in patients with complete hemivaginal outflow obstruction (10/27, 37%) than in those with incomplete obstruction (8/67, 12%; $P = 0.012$). In 14 individuals, an endometrioma was present in the ovary ipsilateral to the hemivaginal obstruction.

Among the above four comparative studies, the overall mean estimate of the prevalence of endometriosis in patients with OMA was 47% (95% CI, 36–58%), and that in patients with NOMA was 19% (95% CI, 15–24%).

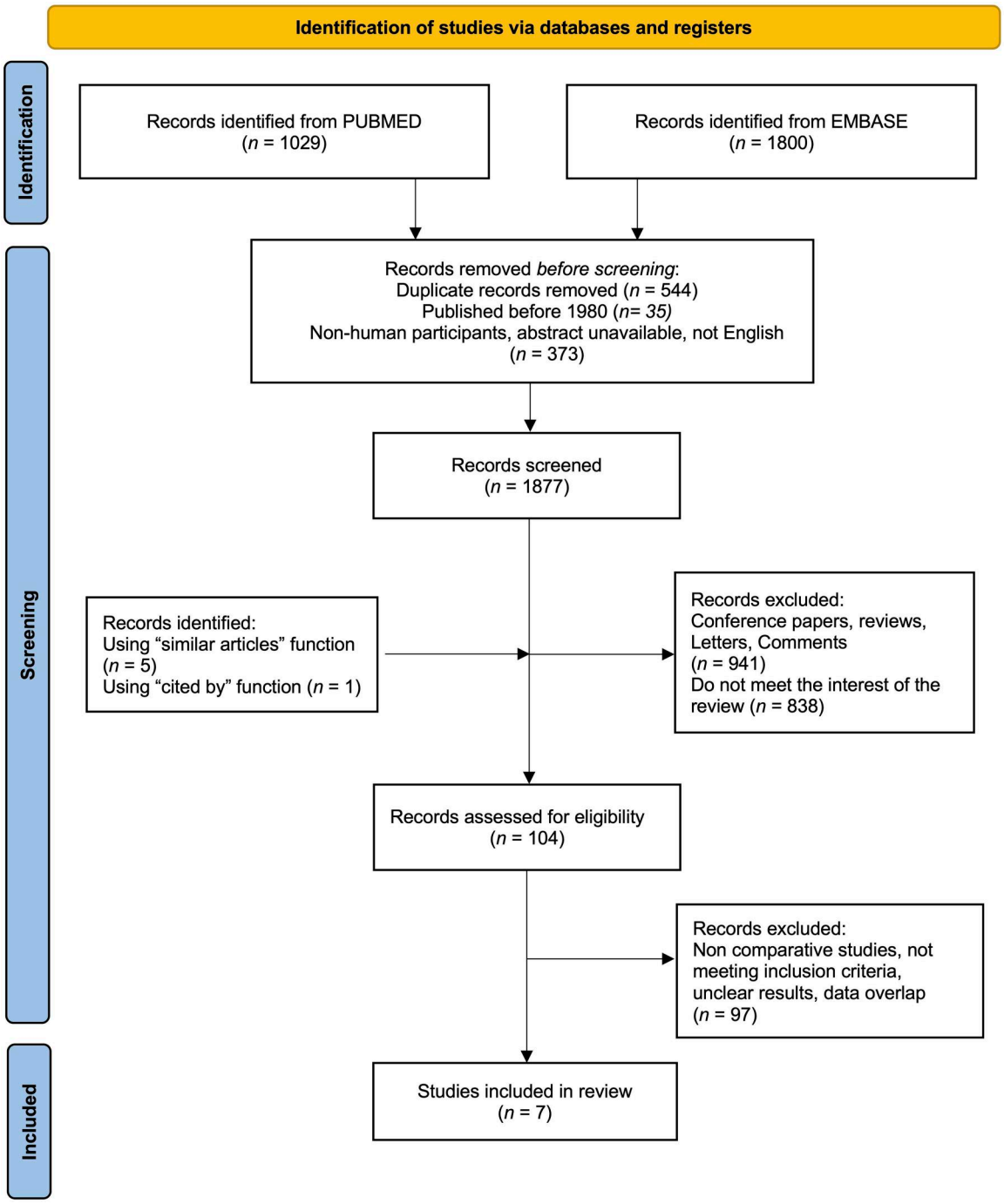

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of the literature search and study selection process for a systematic review and meta-analysis of Müllerian anomalies and endometriosis.

The OR of endometriosis ranged from 0.69 (Acién, 1986) to 6.36 (Uğur *et al.*, 1995). Pooling of the results derived from the included reports yielded a common OR of 4.72 (95% CI, 2.54–8.77) at the random-effects meta-analysis, with very low heterogeneity ($I^2 = 3.75\%$) and a non-significant small-study effect (Egger's test, $P = 0.125$) (Fig. 2). Similar results were obtained by applying the fixed-effects model (OR, 4.54; 95% CI, 2.53–8.15; $I^2 = 4.42\%$; Supplementary Fig. S1), consistently demonstrating a substantial increase in the risk of endometriosis in patients with OMA compared with those with NOMA. The risk of bias assessments for the studies included in this comparison is shown in Supplementary Fig. S2. The overall certainty of the evidence according to GRADE guidelines was judged as low.

NOMA versus no Müllerian anomalies

Fedele *et al.* (1992) detected endometriosis in 61/198 (31%) patients with NOMA and 210/545 (39%) of those without Müllerian anomalies ($P = 0.209$), who all underwent laparoscopy for infertility. The prevalence of endometriosis was significantly higher in individuals with unicornuate uterus (55%) compared with those with other malformation classes (28%; $P < 0.05$), but not compared with the control group without anomalies.

Table 1. Characteristics and results of studies comparing the prevalence of endometriosis in patients with obstructive Müllerian anomalies and in patients with nonobstructive Müllerian anomalies (literature data, 1986–2014).

Source, year	Country	Study design	Müllerian anomalies classification	Endometriosis classification	OMA, n (class)	NOMA, n (class)	Endometriosis in OMA, n (stage)	Endometriosis in NOMA, n (stage)	Comments
Acién (1986)	Spain	Retrospective	None	Acosta *et al.* (1973)	10 (OHVIRA, n = 8; transverse vaginal septum, n = 2)	29 (unicornis, n = 6; bicornis bicollis, n = 6; bicornis unicollis, n = 9; subseptus, n = 8)	1 (severe, n = 1)	4 (mild, n = 2; moderate, n = 1; severe, n = 1)	Cases with gonadal dysgenesis (n = 4) and Rokitansky syndrome (n = 1) are excluded
Olive and Henderson (1987)	U.S.A.	Retrospective	Buttram and Gibbons (1979)	None	13 (partial agenesis, n = 12; unicornuate with cavitated, noncommunicating rudimentary horn, n = 1)	43 (unicornuate, n = 9; didelphys, n = 10; bicornuate, n = 13; septate, n = 11)	10	16	Degree of severity of endometriosis not reported
Uğur *et al.* (1995)	Turkey	Retrospective	The American Fertility Society (1988)	Unclear	26 (cervico-vaginal agenesis, n = 6; transverse vaginal septum, n = 13; unicornuate with cavitated, non-communicating rudimentary horn, n = 3; unclear, n = 4)	119 (unicornuate, n=unclear; didelphys, n = 12; bicornuate, n = 29; septate, n = 62; arcuate, n = 9)	15 (mild, n = 5; moderate, n = 6; severe, n = 3; unclear, n = 1)	21 (mild, n = 12; moderate, n = 8; severe, n = 1)	Number of OMA and NOMA not clearly identifiable in class I (hypoplasis/agenesis) and class II (unicornuate). Endometriosis stage indicated as 1, 2, and 3 without reference to a published classification
Tong *et al.* (2014)	China	Retrospective	None	None	27 (OHVIRA with complete hemivaginal obstruction)	67 (OHVIRA with incomplete hemivaginal obstruction)	10	8	A total of 14 patients had ovarian endometriomas ipsilateral to the obstructed hemivagina, and 4 had bilateral endometriomas

NOMA, nonobstructive Müllerian anomalies; OHVIRA, obstructed hemivagina with uterus didelphys and ipsilateral renal anomaly; OMA, obstructive Müllerian anomalies.

Table 2. Characteristics and results of studies comparing the prevalence of endometriosis in patients with nonobstructive Müllerian anomalies and in patients without Müllerian anomalies (literature data, 1992–2011).

Source, year	Country	Study design	Müllerian anomalies classification	Endometriosis classification	NOMA, n (class)	No Müllerian anomalies, n (surgical indication)	Endometriosis in NOMA, n (stage)	Endometriosis in no Müllerian anomalies, n (stage)	Comments
Fedele *et al.* (1992)	Italy	Retrospective	Buttram and Gibbons (1979)	Revised American Fertility Society (1985)	198 (unicornuate, n = 20; didelphys, n = 17; bicornuate, n = 46; septate, n = 115)	545 (primary infertility, n = 376; secondary infertility, n = 147; repeated abortion, n = 22;)	61 (I, n = 39; II, n = 10; III, n = 11; IV, n = 1)	210 (stage not reported)	Prevalence of endometriosis higher in unicornuate class (55%) compared with other classes (28%)
Uğur *et al.* (1995)	Turkey	Retrospective	The American Fertility Society (1988)	Unclear	119 (unicornuate, n=unclear; didelphys, n = 12; bicornuate, n = 29; septate, n = 62; arcuate, n = 9)	3240 (infertility, n = 2535; recurrent pregnancy loss, n = 99; pelvic pain, n = 292; amenorrhea, n = 34; adnexal mass, n = 221; not classified, n = 79)	21 (mild, n = 12; moderate, n = 8; severe, n = 1)	619 (stage not reported)	Number of NOMA not clearly identifiable in class I (hypoplasis/agenesis) and class II (unicornuate). The sum of surgical indications in the no anomalies group does not add up to the total. Endometriosis stage indicated as 1, 2, and 3 without reference to a published classification
Nawroth *et al.* (2006)	Germany	Retrospective	The American Fertility Society (1988)	American Society for Reproductive Medicine (1997)	120 (complete septate uterus, n = 24; partial septate uterus, n = 96)	486 (primary infertility, n = 252; secondary infertility, n = 234)	31 (minimal-mild, n = 31)	74 (minimal-mild, n = 71; moderate, n = 3)	The NOMA group is composed exclusively by patients with class V (septate uterus) Müllerian anomalies
Demir *et al.* (2011)	Turkey	Retrospective	The American Fertility Society (1988)	American Society for Reproductive Medicine (1997)	92 (complete septate uterus, n = 39; partial septate uterus, n = 53)	191 (primary infertility, n = 124; secondary infertility, n = 67)	9 (minimal-mild, n = 8; severe, n = 1)	30 (minimal-mild, n = 27; severe, n = 3)	The NOMA group is composed exclusively by patients with class V (septate uterus) Müllerian anomalies.

NOMA, nonobstructive Müllerian anomalies.

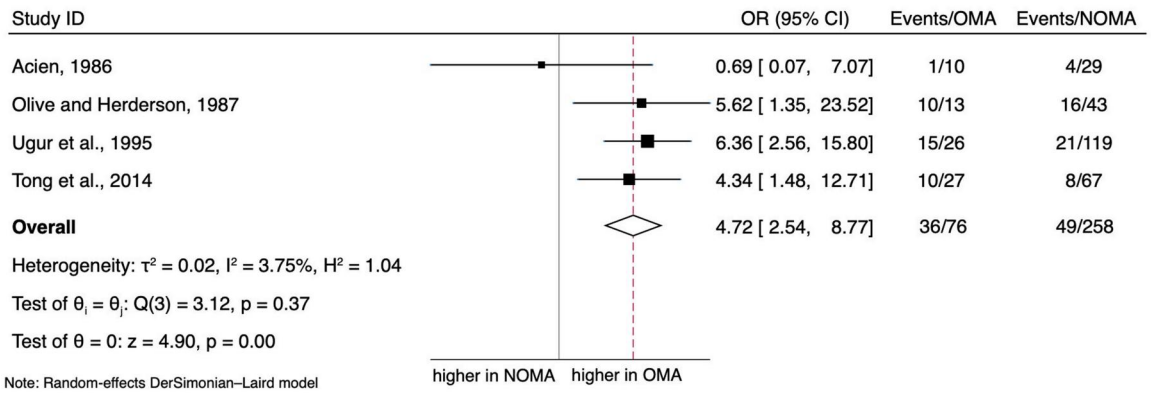

Figure 2. Results of studies comparing the prevalence of endometriosis in patients with obstructive Müllerian anomalies and those with nonobstructive Müllerian anomalies. Pooled effect estimate calculated according to random-effects DerSimonian–Laird model. Horizontal lines indicate 95% CI; boxes show the study-specific weight; the rhombus represents combined effect size; and the dashed line indicates the overall estimate. NOMA, nonobstructive Müllerian anomalies; OMA, obstructive Müllerian anomalies; OR, odds ratio.

In the large cohort of Uğur *et al.* (1995), endometriosis was detected in 21/119 (18%) of patients with NOMA and 619/3240 (19%) of those without Müllerian anomalies ($P > 0.05$).

In the study by Nawroth *et al.* (2006), the prevalence of endometriosis was significantly higher in patients with subseptate uterus (31/120; 26%) than in those undergoing laparoscopy for infertility (74/486; 15%; $P = 0.006$).

Demir *et al.* (2011) observed endometriosis in 9/92 (10%) patients with complete or partial septate uterus compared with 30/191 (16%) patients with a normal uterus and who underwent laparoscopy for primary or secondary infertility ($P = 0.39$).

Among the above four comparative studies, the overall mean estimate of the prevalence of endometriosis in patients with NOMA was 23% (95% CI, 20–27%), and that in patients without Müllerian anomalies was 21% (95% CI, 20–22%).

The OR of endometriosis ranged from 0.58 (Demir *et al.*, 2011) to 1.94 (Nawroth *et al.*, 2006), with significant heterogeneity among studies ($I^2 = 76.5$). Pooling of the results derived from the included reports yielded a common OR of 0.95 (95% CI, 0.57–1.58) and non-significant small-study effects (Egger's test: $P = 0.671$), demonstrating no significant difference in risk of endometriosis in patients with NOMA compared with those without Müllerian anomalies (Fig. 3). Sensitivity analysis conducted after omitting Nawroth *et al.* (2006) showed a slightly higher risk of endometriosis in controls compared to NOMA (OR, 0.75; 95% CI, 0.57–0.98; $I^2 = 0\%$), with no significant publication bias detected (Egger's test, $P = 0.792$). The risk of bias assessments for the studies included in this comparison is shown in Supplementary Fig. S3. The overall certainty of the evidence according to GRADE guidelines was judged as low.

Discussion

The findings of the present systematic review confirm that the prevalence of endometriosis is substantially and significantly higher in patients with OMA compared with that in patients with NOMA. The magnitude of the association is high (common OR > 4). This suggests a robust relation rather than being explained by confounders (Grimes and Schulz, 2012), and appears to support the RM hypothesis of a positive association between the amount of transtubal reflux and the risk of endometriosis.

However, the interpretation of the evidence is not straightforward because, on one side, a biological gradient based on the amount of RM as a determinant of endometriosis onset seems established and, on the other side, such an amount can be considered quantitatively massive and non-physiologic. In other words, endometriosis could affect individuals with OMA regardless of the presence of other biological mechanisms that generally favor the development of the disease in women without uterine malformations. These patients may not necessarily be also biologically predisposed to the development of spontaneous, nonobstructive endometriosis. Thus, it can be questioned whether data observed in these 'quasi-experimental', though natural, conditions are generalizable to the entire endometriosis population, presumably composed mainly of biologically susceptible subjects that develop the disease in the absence of chronic cryptomenorrhea. In addition, the characteristics of the patients with OMA or NOMA are not comparable, as the former are generally much younger and with a different surgical indication (postmenarcheal acute colicky pain) compared with the latter individuals (Smith and Laufer, 2007).

Overall, less than half of the patients with OMA had coexistent endometriosis. This percentage might appear too low to support RM as the exclusive pathogenic factor, otherwise almost all patients with OMA should have developed endometriosis. However, in most patients the outflow obstruction was surgically corrected soon after the onset of symptoms (Tong *et al.*, 2014) and, theoretically, there might have not been enough menstrual episodes to systematically induce endometriosis development (Sanfilippo *et al.*, 1986). As an example, in the study by Tong *et al.* (2014), the mean age at diagnosis of endometriosis and of OHVIRA syndrome was, respectively, 13.7 and 14.8 years in those with complete obstruction and 19.7 and 21.5 years in those with incomplete obstruction. Moreover, the mean time between menarche and the diagnosis of endometriosis was 2.5 years in the former group and 8 years in the latter ($P < 0.05$ for all comparisons). Of relevance here, Song *et al.* (2016) reported that, in a large series of adolescents with cervical atresia and functioning endometrium, the only variable significantly associated with the presence of pelvic endometriosis was the extent of the delay from symptom onset to surgery. The prevalence of endometriosis was 30% (7/23) in patients with a delay of 1 year or less, and 63% (45/71) in those with a delay of >1 year ($P = 0.006$).

Furthermore, according to Rock *et al.* (1982), the lower the obstruction, such as in cases of transverse septum of the middle third of the vaginal canal or low-lying obstructed hemivaginas, the better the possibility to accommodate large amounts of trapped menstrual debris in the developing hematocolpos before hematometra and hematosalpinx ensue with the resulting aberrant pelvic reflux. Unfortunately, data are too small and/or

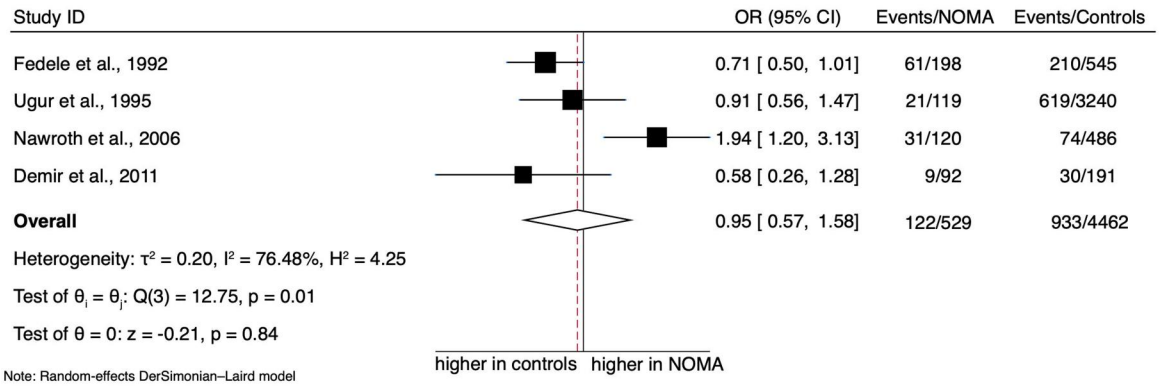

Figure 3. Results of studies comparing the prevalence of endometriosis in patients with nonobstructive Müllerian anomalies and those without Müllerian anomalies (controls). Pooled effect estimate calculated according to random-effects DerSimonian–Laird model. Horizontal lines indicate 95% CI; boxes show the study-specific weight; the rhombus represents combined effect size; and the dashed line indicates the overall estimate. NOMA, nonobstructive Müllerian anomalies; OR, odds ratio.

unclear to permit reliable subgroup analyses based on the level of the outlet obstruction. Finally, histological demonstration of endometriosis was not always reported and supposedly biopsies were not systematically performed at laparoscopy. Therefore, it may not be excluded that some cases of presumed peritoneal endometriosis in patients with OMA were mere hemosiderin deposits (Fedele *et al.*, 1992).

Regardless of statistical significance, one out of five patients with NOMA had endometriosis, and such a high prevalence is probably even more unexpected than the relatively limited estimate found in patients with OMA. Selection biases in the recruitment of subjects in the included studies may explain this finding. Moreover, it may not be excluded that some Müllerian anomalies, although classified as nonobstructive, may create a partial and subclinical obstacle to menstrual efflux. In this case, stronger myometrial contractions would be needed to generate a pressure gradient between the uterine cavity and the vaginal canal sufficient to prompt a transcervical flow (e.g. a single substenotic cervical canal in patients with didelphys uteri, bicornuate uteri with double cervix, or completely septate uteri with a septate cervix). The retrograde flow would thus also augment, increasing the likelihood of endometriosis onset that would explain the unusually high prevalence estimates reported. In addition, the same embryologic disorder that results in aberrant development of Müllerian ducts hypothetically may also determine a myometrial dysfunction leading to loss or partial reversal of the physiologic fundo-cervical uterine contraction polarity during menses (uterine dysperistalsis) that would favor RM and increase the risk of endometriosis.

Concerning the second preplanned comparison, no significant difference was observed in the prevalence of endometriosis among patients with NOMA and those without Müllerian anomalies. Thus, at *prima facie*, it is tempting to reject the hypothesis of a common embryologic pathogenic pathway leading to both Müllerian anomalies and endometriosis (Redwine, 1988), because, if this was the case, a significantly higher prevalence of endometriosis would have been expected in the former group (Fedele *et al.*, 1992).

However, the comparison between patients with NOMA and those without Müllerian anomalies is also not straightforward. Patients with anovulation and tubal factors were not always excluded from the NOMA group, thus potentially reducing the real prevalence of endometriosis in this specific subpopulation. In addition, the considered study period was not always the same for cases and controls (e.g. Nawroth *et al.*, 2006), and surgeon awareness, diagnostic abilities, and accuracy in reporting early endometriotic lesions may have changed over time.

Moreover, the 21% mean prevalence of endometriosis in the no Müllerian anomalies group obtained by pooling the individual estimates of the selected studies was substantially higher than the 3%-5% observed in the general female population of reproductive age (Ghiasi *et al.*, 2020; Parazzini *et al.*, 2020; Sarria-Santamera *et al.*, 2020). Removal from the analysis of a single very large series (Uğur *et al.*, 1995) did not substantially change the result.

Indeed, the so-called control group may not be representative of the general female population. The indications for surgery in patients with NOMA and those without Müllerian anomalies differed and some conditions strictly associated with endometriosis (e.g. infertility) sometimes were unevenly distributed (Demir *et al.*, 2011). As an example, in the series published by Fedele *et al.* (1992), primary infertility was the surgical indication for laparoscopy in 23% of patients with NOMA and 69% of those without Müllerian anomalies ($P < 0.0001$). Conversely, the proportion of women with a history of repeated abortion was 52% and 4%, respectively ($P < 0.0001$). The primary infertility rates reported in the control groups in the studies by Demir *et al.* (2011; 64.9%), Fedele *et al.* (1992; 69%), and Uğur *et al.* (1995; 78.2%) are higher than that in the control group in the study by Nawroth *et al.* (2006; 51.9%). This could justify the slightly higher prevalence of endometriosis in controls compared with patients with NOMA observed when excluding the study by Nawroth *et al.* (2006) at sensitivity analysis. Indeed, the frequency of endometriosis in individuals with primary infertility and few previous conceptions is expected *a priori* to be high, thus spuriously nullifying a potentially significant difference with patients with NOMA. More, in general, NOMA may interfere with the pregnancy course, but rarely impede conception, and do not cause pelvic pain. Thus, comparing two groups with different surgical indications introduces confounding, as most individuals in the control group underwent laparoscopy precisely because of the two conditions, i.e. infertility and pelvic pain, most strongly associated with endometriosis. In addition, we could not exclude the presence of undiagnosed retroperitoneal endometriosis in controls considered to be without endometriosis, representing an additional potential confounding factor in our quantification of the pooled estimate.

The only data that would reliably answer the question of whether an excess in the prevalence of endometriosis exists in patients with NOMA would be a comparison with the prevalence

observed in the general population in individuals without surgical indications. This is impossible, especially if one aims at including also superficial peritoneal implants because these lesions cannot be detected without direct visualization.

Another approach to studying the association between NOMA and endometriosis has been adopted by some investigators who, instead of evaluating the prevalence of endometriosis in individuals with and without NOMA, assessed the prevalence of NOMA in patients with and without endometriosis undergoing hysteroscopy and laparoscopy/laparotomy for infertility (Matalliotakis *et al.*, 2010; LaMonica *et al.*, 2016). A higher prevalence of NOMA (mostly septate uteri) in the endometriosis compared to the non-endometriosis group was observed in both studies, although the reported estimates were inconsistent, being 3% in Matalliotakis *et al.*'s study (2010) and 37% in LaMonica *et al.*'s study (2016), even though the sample size was fairly large in the two series (n = 625 and n = 343, respectively), and the proportion of women with endometriosis similar (68% and 66%, respectively). Additionally, the magnitude of the association was modest and the statistical significance was marginal, thus making confounding the most plausible explanation.

Though any inference seems unwarranted, these data appear to confirm an absence of a strong association between endometriosis and septate uteri. Indeed, endometriosis seems associated with infertility rather than with NOMA. Boujenah *et al.* (2017) observed a higher prevalence of endometriosis in infertile patients with Müllerian anomalies (18/41, 44%) compared with fertile women with similar Müllerian anomalies (3/11, 27%). However, the difference was not statistically significant.

A third question prompted our review, that is does secondary, obstructive endometriosis rapidly resolve once the outflow obstruction is removed or, instead, does it share the same disease course, in terms of pain, infertility, lesion recurrences, need for medical treatments, and reoperations, of primary, spontaneous endometriosis caused by classic RM (Mulchahey, 2002)? Unfortunately, we searched but could not identify studies comparing the outcomes of endometriosis in patients with OMA after surgical correction of outflow obstruction with or without concomitant eradication of endometriotic lesions. Therefore, no conclusions can be drawn regarding the effect of outlet obstruction correction, as the popular wisdom that restoring a normal menstrual efflux is followed by endometriosis resolution (Sanfilippo *et al.*, 1986) is based on very few anecdotal cases. Actually, in several, although much less emphasized, instances this has not occurred, as second-line laparoscopy for recurrent pain revealed persistence or recurrence of endometriosis 6 months to 2 years after the successful removal of outflow obstruction (Pinsonneault and Goldstein, 1985; Taylor and McComb, 2007; Silveira and Laufer, 2013).

Conclusion

In conclusion, the difference in the prevalence of endometriosis in patients with OMA compared with those with NOMA supports the validity of the RM theory but, in our opinion, cannot definitively rule out alternative hypotheses, as less than half of women with obstructed outlet had the disease and the magnitude of the difference was less than expected if RM was the only determinant of endometriosis. However, the presence of endometriosis was assessed at a much younger age in patients with OMA compared with those with NOMA, and the obstruction was generally corrected after a limited number of ovulatory menses. Moreover, it cannot be excluded that some NOMA subtypes also create a partial obstacle to menstrual efflux and/or generate dysfunctional myometrial contractions that favor transtubal reflux, thus increasing the risk of endometriosis and limiting the difference between OMA and NOMA.

The Müllerian embryonic remnants/celomic metaplasia hypothesis also cannot be reasonably rejected merely based on a lack of a significant difference in endometriosis prevalence between patients with NOMA and those with infertility or pelvic pain, as these latter conditions are strongly associated with the outcome of interest, and women with these symptoms cannot be considered proper controls. Thus, confounding by indication could explain the findings. In addition, endometriosis has been reported in premenarcheal girls without OMA (Marsh and Laufer, 2005).

When assessing the clinical relevance of our findings, it is crucial to recognize that endometriosis is a multistep phenomenon influenced by a broad spectrum of factors, including genetic and epigenetic modifications, and immunological and hormonal influences, as well as lifestyle and environmental exposures, all of which can contribute to the complex, multisystemic phenotype of endometriosis (Koninckx *et al.*, 2019; Zondervan *et al.*, 2020). The findings of this meta-analysis support the role of RM as the initiator for the development of endometriotic lesions, while not excluding the contribution of both inheritable (germline) and tissue-specific (cellular acquired) genetic and epigenetic modifications as disease-promoting factors.

The results of our appraisal of the available evidence on the association between different types of Müllerian anomalies and endometriosis remain open to alternative interpretations and the long-lasting debate (Sampson, 1925; Redwine, 1988) that prompted this systematic review seems unsettled. Whereas the robust relation between OMA and endometriosis is indisputable, several collateral but important questions remain unanswered. Unfortunately, the methodological limitations that here prevent definitive conclusions appear difficult to circumvent even in future studies.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data included in this article were extracted as published in the available original articles. No new data were generated to support this paper.

Authors' roles

P.Ve. conceived the study and drafted the original version of the article. M.P., F.C., and P.Ve. contributed to acquisition of the data and drafting of the manuscript. E.S. and P.Vi. participated in conceiving and drafting part of the article and critically revising the paper. S.D.M. processed and analyzed the data. N.S. participated in methodological assessments and data analysis and critically revised the manuscript. All authors revised critically the drafts of the manuscript and approved its final version. All authors agree to be accountable for all aspects of the work.

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Conflict of interest

P.Ve. is a member of the Editorial Board of *Human Reproduction Open*, the *Journal of Obstetrics and Gynaecology Canada*, and the International Editorial Board of *Acta Obstetricia et Gynecologica Scandinavica*; has received royalties from Wolters Kluwer for chapters on endometriosis management in the clinical decision support resource UpToDate; and maintains both a public and private gynecological practice. E.S. discloses payments from Ferring for research grants and honoraria from Merck-Serono for lectures. All other authors declare they have no conflict of interest.

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