Chronic endometritis: correlation among hysteroscopic, histologic, and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies

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Objective: To evaluate the type and etiopathogenic role of infectious agents detected in endometrial cultures obtained from women with chronic endometritis (CE).

Design: Prospective controlled study.

Setting: University hospital.

Patient(s): 2190 women undergoing hysteroscopy for different indications.

Intervention(s): Vaginal and endometrial samples were collected from 438 women with a CE diagnosis at hysteroscopy and 100 women with no signs of CE (controls).

Main Outcome Measure(s): Histology and cultures for common bacteria, Neisseria gonorrhoeae and Mycoplasma, and molecular biology testing for Chlamydia were performed.

Result(s): We compared results of vaginal and intrauterine cultures obtained from women with and without CE. Histologic results were positive in 388 of these cases (88.6%), and at least one microorganism was found in 320 endometrial samples (73.1%). In the control group, histologic results and endometrial culture were positive in only 6% and 5% of cases, respectively. The most frequent infectious agents detected at the endometrial level were common bacteria (58% of cases). Ureaplasma urealyticum was detected in 10% and Chlamydia in only 2.7% of positive endometrial cultures. In only 143 (32.6%) cases were the same infectious agent isolated in endometrial and vaginal cultures.

Conclusion(s): More than 70% of CE cases resulted from nongonococcal, nonchlamydial infections. Common bacteria and Mycoplasma were the most frequent etiologic agents. Vaginal cultures have low concordance with endometrial cultures. (Fertil Steril 2008;89:677–84. ©2008 by American Society for Reproductive Medicine.)

Key Words: Infectious agents, chronic endometritis, infertility, bacteria, Ureaplasma, Chlamydia

Chronic endometritis (CE) is a persistent inflammation of the endometrial lining. Histologically, the diagnosis of CE is generally based on finding an excessive number of neutrophils and plasma cell infiltrates in endometrial biopsies (1–3). In most of cases, the diagnosis of CE is an incidental finding based on histologic specimens obtained for various gynecologic indications (such as abnormal uterine bleeding or infertility). Chronic endometritis is often asymptomatic or accompanied by mild symptoms, which include pelvic pain, dysfunctional uterine bleeding, dyspareunia, and leukorrhea (1–3).

Although it often is clinically silent, CE may hamper the reproductive capacity of spontaneous and in vitro fertilization (IVF) cycles (4, 5). Moreover, there is compelling evidence that CE may also cause spontaneous preterm labor and premature birth (6–8). Because of its subtle nature, the actual prevalence of this pathology in the general population is unknown, with estimates ranging from 0.8% to 19.0% (9, 10). It was found in up to 72% of histologic specimens collected from women seeking care in a sexually transmitted disease clinic (11). Thus, the prevalence of CE may be much higher in specific populations such as in infertile women.

In a recent study, we demonstrated that hysteroscopy using fluid for distending the uterine cavity is a useful and reliable technique for detecting CE. Taking the presence of hyperemia, mucosal edema, and micropolyps as diagnostic parameters, hysteroscopy showed a diagnostic accuracy of 93.4% (12, 13). Using these criteria, we found signs of CE in approximately 17.4% of the women referred for diagnostic hysteroscopy for various indications (12).

Not only making the diagnosis but also determining the etiology of CE is a challenging issue. Publications in literature...
on the etiology of CE provide conflicting data. Results of traditional cultures are laboratory dependent and even when specific devices are used, contamination from vaginal and endocervical content cannot be excluded. Finally, demonstration of infectious agents in the endometrial cavity does not necessarily imply a pathogenic significance (8). As a further contribution to the existing confusion, the slew of publications suggesting an infectious etiology (14–16) have been recently challenged by a study by Andrews et al. (17) that failed to demonstrate a preventive effect of preconception antibiotic therapy in women with recent pregnancy loss or preterm labor.

Our study investigated the potential etiologic role of infectious agents detected by endometrial and vaginal cultures by prospectively comparing findings made in women diagnosed with CE with those made in women without signs of CE.

**MATERIALS AND METHODS**

In the period from January 2005 to April 2006, we enrolled 2190 women referred to our department for diagnostic hysteroscopy. Clinical characteristics of women enrolled in the study are displayed in Table 1. The study was approved by our institutional review board, and all women gave their informed consent.

All women underwent diagnostic office hysteroscopy in the follicular phase of the menstrual cycle. Office hysteroscopy was performed using a lens-based 2.7-mm outer diameter (OD) minitelescope, 105° angle of visual field equipped with a 3.5-mm OD single-flow diagnostic sheath (Slim-line Hysteroscope; ACMI, Southborough, MA). To minimize the risk that the hysteroscopy itself could contaminate the endometrial cavity, all examinations were performed after placing a vaginal speculum and performing external uterine ostium cleaning with a gauze soaked in iodine solution. Saline was employed to distend the uterine cavity at a pressure generated by simple drop from a bag suspended 1 m above the patient. A 300 W light source with a xenon bulb (ACMI), a 3 CCD digital camera (Micro-Digital IIIe; ACMI), and a 21-inch video color screen (Sony Trinitron, PVM-20M2MDE, Sinigawa-Ku, Tokyo, Japan) were used.

The exploration of the uterine cavity consisted of a panoramic view of the cavity followed by a thorough evaluation of the endometrial mucosa as previously described. Diagnosis of CE was based on criteria previously published elsewhere (12, 13) (Fig. 1). All hysteroscopies were performed by two of the authors (E.C., R.N.).

In the follicular phase of the subsequent cycle we looked for the presence of common bacteria, *Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma* species, *Ureaplasma urealyticum*, and yeast at the vaginal and endometrial levels. For this, women had a vaginal swab taken and an endometrial sampling using a 3-mm Novak’s curette connected to a 20-mL syringe. To minimize the risk that endometrial cultures could be contaminated in the vagina after placing a vaginal speculum, the Novak’s cannula was inserted under visual control into the uterine cavity taking care to avoid any contact with vaginal walls. Endometrial samples were diluted in 10 mL of saline and divided into two aliquots, one for cultures and the other for histologic examination.

Specimens for *N. gonorrhoeae* were immediately placed in Stuart’s transport medium and transported to the laboratory. Specimens for *Chlamydia* were placed into transport medium for specific enzyme immunoassay (Idea Tm, Dako, Milan, Italy).

In the laboratory, vaginal and endometrial specimens were gram-stained; then the swabs and the endometrial specimens were plated into appropriate agar medium, 5% sheep blood.
Columbia Agar Base, Chocolate Agar, Mannitol Salt Agar, and Mac Conkey Agar (Biomerieux, Rome, Italy), and the presence of microorganisms was evaluated. The plates were incubated for 48 hours in air or 5% carbon dioxide. The identification of bacteria was made by using published criteria (Dade International Inc., Milan, Italy).

Genital mycoplasmas were quantitatively detected by immunoassay (Mycoplasma-IST; Biomerieux). For yeast isolation, specimens were plated into Saboraud Chloramphenicol Agar and identification was made by using commercial kits (API-C System; Biomerieux).

Finely, endometrial samples were fixed in neutral formalin and later embedded in paraffin for histologic analysis. Five microsections were stained with hematoxylin and eosin. The histologic examinations were performed by a single operator (L.R.) who was unaware of the hysteroscopic findings. Histologic diagnosis of CE was based on criteria previously described in the literature (1, 3, 9). Attention was paid to the following features: superficial stromal edema, increased stromal density, and pleomorphic stromal inflammatory infiltrate dominated by lymphocytes and plasma cells.

As controls we enrolled 100 consecutive women for whom an endometrial biopsy had been indicated due to endometrial abnormality with no sign of CE at hysteroscopy.

Group characteristics were compared using Student’s t-test and the chi-square test. Concordance between hysteroscopic diagnosis of CE and positive histology and/or endometrial cultures was evaluated by chi-square test. Results of vaginal and endometrial cultures in women with hysteroscopic diagnosis of CE were compared by chi-square test. Statistical analysis was performed by using Epi Info 6.04 (Centers for Disease Control and Prevention, Atlanta, GA). P < .05 was considered statistically significant.

RESULTS

Hysteroscopy diagnosed CE in 438 of 2190 (20.0%) women. Study group and controls were homogeneous with regard to age and parity, but the indications for hysteroscopy were different.

The results of the histology and bacteriology findings in endometrial specimens of patients with signs of CE at hysteroscopy and in 100 women (control group) without hysteroscopic signs of inflammation are displayed in Table 2. The histology confirmation was positive for CE in 388 (88.6%) of 438 positive hysteroscopy cases, and the endometrial samples were positive for at least one microorganism in 320 cases (73.1%). In the control group, the histology and endometrial cultures were positive in only 6% (P < .000001, odds ratio [OR] = 121.6; 95% confidence interval [CI], 48.2–213.6) and 5% (P < .000001, OR = 51.5; 95% CI, 19.6–95.9) of cases, respectively.

In women with CE, the number of positive vaginal cultures was statistically significantly lower (254 cases, 58%) than at the endometrial level. Yet there were more positive vaginal cultures in women with CE than in the control group (P < .005, OR = 2.0; 95% CI, 1.2–3.2) (see Table 2). After excluding from calculation the patients who had negative biopsy results for endometritis but positive visual diagnosis, the results did not change substantially. Following this adjustment, the frequency of positive vaginal cultures was 60.1% versus 41% in the CE and control groups, respectively.

The number of cases positive for specific etiologic agents at vaginal and endometrial investigations in women with and without evidence of CE at hysteroscopy is shown in Table 3. Even after excluding cases that had a similar pathogen on vaginal and endometrial cultures, implying possible contamination from the vagina, the difference in positive endometrial cultures between women diagnosed with CE and controls was still highly statistically significant (295 vs. 2 cases; P < .000001, OR = 101.1; 95% CI, 24.1–218.4). Table 3 shows the cases in which infertility was the indication for hysteroscopy for each infectious agent detected at endometrial level in women diagnosed with CE.

The prevalence of each microorganism in endometrial and vaginal samples in women with diagnosis of CE is depicted in Figure 2. The most frequent infectious agents detected at the endometrial level were common bacteria, which accounted for 58% of all cases. In particular, streptococci were found in 27.9% of cases, and bacteria from intestinal flora (Enterococcus faecalis and Escherichia coli) was recovered in 25.5% of cases. Ureaplasma urealyticum was detected in 10.0%,
and Chlamydia in only 2.7% of cases. No cases of N gonorrhoeae were found.

When comparing results of endometrial and vaginal cultures in women diagnosed with CE, the percentage of concordance differed depending on the etiologic agent (see Fig. 2). In fact, the endometrium to vagina positivity ratio for E coli, streptococci, staphylococci, E faecalis, and Chlamydia was greater than 1, but for Ureaplasma and yeast it was lower than 1. Specifically, the ratio of positive endometrial and vaginal cultures was 1.3 for E coli, 1.5 for streptococci, 2.8 for E faecalis, and 5.4 for Chlamydia; on the contrary, for U urealyticum and yeast the same ratio was 0.5 and 0.4, respectively. Notably, the vaginal culture was positive for staphylococci in no cases out of 20 of endometrial positivity.

It is worth underlining that in 143 (32.6%) cases the infectious agent isolated in the endometrial culture was the same as that found in the vaginal culture, but in 295 cases (67.4%) results were discordant. In accordance with the different distribution ratios of each agent, the rate of concordance between vaginal and endometrial culture varied with each etiologic agent. The odds of finding the same

<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>Vagina</th>
<th>Endometrium</th>
<th>Vagina</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>38</td>
<td>50 (20)</td>
<td>15</td>
<td>1</td>
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<tr>
<td>Streptococci</td>
<td>80</td>
<td>122 (60)</td>
<td>16</td>
<td>2</td>
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<tr>
<td>Staphylococci</td>
<td>0</td>
<td>20 (17)</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Enterococcus faecalis</td>
<td>22</td>
<td>62 (26)</td>
<td>7</td>
<td>1</td>
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<tr>
<td>Chlamydia</td>
<td>2</td>
<td>12 (11)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Ureaplasma</td>
<td>86</td>
<td>44 (26)</td>
<td>5</td>
<td>1</td>
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<tr>
<td>Yeast</td>
<td>26</td>
<td>10 (3)</td>
<td>10</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>254</td>
<td>320</td>
<td>54</td>
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*The number in parenthesis indicates the cases in which infertility was the indication for the hysteroscopy for each infectious agent detected at the endometrial level in women with chronic endometritis.*
microorganisms in the whole internal genital tract or only at endometrial level expressed by the ratio of positive cases at both sites over those at endometrial level only ranged from 0 to 100% for each infectious agent; notably, the odds were only 19.3% for *E faecalis*, 16.7% for Chlamydia, and, more remarkably, 0 for *Staphylococcus* (Fig. 3).

**DISCUSSION**

Chronic endometritis is a subtle pathology that is difficult to both diagnose and treat. In previous publications, we described the diagnostic criteria for CE. On hysteroscopy, these are constituted by the presence of micropolyps, focal or diffuse hyperemia, and stromal edema (12, 13). We also have demonstrated the high diagnostic reliability of this approach for diagnosing CE based on inflammation as the combination of the hyperemia, edema, and micropolyps; the diagnostic accuracy is 93.4% (12).

The data of our present study provide an insight into the etiopathogenesis of CE, suggesting a relevant role of common bacteria in this pathology. Many reports have investigated the microbiological environment of the uterine cavity and its clinical implications. However, results of most of these studies have had limitations related to the sampling technique, the value of traditional cultures employed, the uncertain significance of the demonstration of microorganisms in the uterine cavity, the histologic criteria employed, or the difficulty of dealing with reproductive outcome of patients (6, 8). Our study compared the results of endometrial and vaginal cultures with both histologic and hysteroscopic findings to affirm the reliability with which CE was diagnosed.

In the women in whom CE was positively diagnosed, we found at least one microorganism in about 73% of cases. On the contrary, the rate of positive endometrial findings was statistically significantly lower (5%) in women without evidence of CE. Hence, these results strongly speak in favor of an infectious etiology of CE as diagnosed by hysteroscopic and histologic evidence of chronic inflammation.

Regarding the type of infectious agent, it is worth underlining that at the endometrial level the most frequent agents found were common bacteria, accounting for about 60% of cases; *U urealyticum* was detected in 10% of cases. Unexpectedly, Chlamydia was demonstrated in only 2.7% of positive endometrial cultures. No cases of *N gonorrhoeae* were found; this probably was related to the specific characteristics of the population studied (women undergoing hysteroscopy for various indications) and probably also to ethnic factors.

The results of this study are in accordance with those of the PEACH study, which showed that approximately 60% of women with pelvic inflammatory disease have nongonococcal, nonchlamydial infection (18). This is not surprising considering the high prevalence in the population of bacterial vaginosis and the knowledge that ascending bacteria can colonize the uterine cavity (8, 16–21). Our data agree with previous reports that suggested a potential pathogenic role...
of common bacteria (streptococci, staphylococci, E coli, E faecalis, etc.). Anaerobic gram-negative rods and bacterial vaginosis have been associated with pelvic inflammatory disease (1, 19).

Bacterial vaginosis may decrease conception rates and increase early pregnancy losses in IVF (14, 20); the presence of bacterial vaginosis may also increase the risk of preterm birth (6). Salim et al. (15) reported that failure to conceive in assisted reproduction is significantly associated with bacterial colonization of the uterine cervix; any gram-negative colonization was associated with no conception. Notably, Kamiyama et al. (16) demonstrated an association of high endotoxin levels in the menstrual blood and low pregnancy rates after IVF, suggesting that bacterial endotoxin may be responsible for failure of implantation of an otherwise healthy embryo. Our data are in partial agreement with those of Andrews et al. (22) on nonpregnant women with asymptomatic bacterial vaginosis, where endometrial cultures were positive for at least one microorganism in 83% (637 out of 769) of the women, and plasma cell endometritis was present in 39% (190 out of 482). They found a consistent and significant association between bacterial vaginosis and an increased frequency of endometrial colonization with bacterial vaginosis-associated microorganisms (relative risk [RR] ranged from 1.96–4.22).

The results of our study suggest that in approximately 10% of cases U urealyticum may be responsible for CE. It was detectable in the vagina of 86 (19.6%) women with CE. In about 10% of cases, Ureaplasma coexisted with other pathogens. The pathogenic role of Mycoplasma is in agreement with the study from Haggerty et al. (23), which found Mycoplasma genitalium in 12% of cervical specimens and in 8% of endometrial specimens from women affected by nongonococcal, nonchlamydial endometritis.

At the endometrial level, we found Chlamydia in only 2.7% of cases, with a prevalence lower than the approximate 14% reported in the PEACH study of women with chronic pelvic inflammatory disease (18). In only one case was Chlamydia detected in the vagina. Ethnic differences and the characteristics of the population enrolled in our study may account for this discrepancy. Our data fully agree with that of Stern et al. (24), who detected the presence of Chlamydia by polymerase chain reaction in only one out 43 specimens of histologically diagnosed CE. They concluded that C trachomatis had a limited role, if any, in the origin of mild or moderate CE.

Our study had the intrinsic limitations of all studies that rely on bacteriological cultures of the endometrial cavity and use traditional culture techniques and transcervical sampling. Only microorganisms able to grow under conventional conditions used in microbiology laboratories can be recovered, which may yield biased microbial findings. The present study was undertaken to investigate the infectious agents causing CE, not to study the microbiology of the uterine cavity. Hence, the results of our study focused on the presence of
microorganisms that could be significantly correlated with CE, but this does not exclude the possibility that other microorganisms (such as anaerobic bacteria or viruses) might also coexist and play a role. It is of note that the endometrial cultures were negative in approximately 12% of cases in our series.

Our study and control groups were similar in regards to age and parity, but the distribution of the various indications for hysteroscopy varied between the two groups; however, we think that it is unlikely that this factor affected the study’s results and meaningfulness. Our study’s primary objective was to evaluate the role of infectious agents in the pathogenesis of CE, not to correlate the presence of infectious agents with specific clinical findings or indications for hysteroscopy. Moreover, compared with the study group, the incidence of positive endometrial cultures was negligible in the control group.

The risk of contamination during endometrial sampling needs to be considered because the surface of the cervix is normally colonized by microorganisms, which could be carried into the uterus by an instrument passing through the endocervical canal. To exclude this possibility, many investigators have used custom-designed double-lumen (25) and triple-lumen (26) catheters, which minimize but do not totally exclude the risk of contamination. In our study, the possibility that reliability of endometrial results could be significantly affected by contamination seemed low for several reasons. First, when performing endometrial sampling, extreme care was employed to avoid any contact of the curette and vaginal walls. Second, in only approximately 32% of cases was the microorganism found at vaginal level the same as that found in the endometrium. Third, in approximately 20% of cases the endometrial culture was the only positive results. Fourth, the endometrium to vagina positive rate varied widely, depending on the etiologic agent; positive findings for E. coli, streptococci, staphylococci, *E. faecalis*, and *Chlamydia* were greater at the endometrial level than vaginal level, but for *Ureaplasma* and yeast it was the opposite. Fifth, the rate of concordance between vaginal and endometrial culture for each etiologic agent was not the same but rather showed great variability.

The great variability in concordance of endometrial and vaginal cultures for each microorganism suggests that these may have different tropism and capacity to segregate into biological microenvironments. Alternatively, different germs may have different resistance to treatment such that some may survive in the endometrium and not in the vagina or vice versa. It is interesting that none of the cases with positive *Staphylococcus* endometrial cultures also had positive vaginal findings, and <20% of cases positive for *E. faecalis* and *Chlamydia* at endometrial level were also positive at vaginal level. In contrast, in most cases positive for *Ureaplasma* and yeast, the microorganisms were detected also at vaginal level.

The data from our study do not disagree with recent views that uterine cavity is normally not sterile and that the presence of microorganisms does not mean inflammation (8). Microorganisms have been recovered from the endometrial cavity of nonpregnant women at hysterectomy under experimental conditions that minimize the risk of specimen contamination (20). Moreover, as reported by Espinoza et al. (8), it is difficult to envisage that mucosa could be free of bacteria when it is continuously exposed to the microorganisms present in the internal genital tract and is invaded by sperm, which can carry microorganisms into the upper genital tract. Hence, it is not just the presence of infectious agents within the internal genital tract but rather the interactions between infectious agents and the endometrial environment that now is seen as the most critical issue that determines the presence of pathology.

One could argue that hysteroscopic investigation itself could determine transport of microorganisms up into the uterine cavity. For that reason, we tried to minimize risk of simple contamination not only by avoiding any contact with the vagina and cleansing the cervix before introducing the hysteroscope but also by postponing investigations after the subsequent menstruation. The highly statistically significant difference in endometrial positive rates between women with CE and controls made the hypothesis of contamination very unlikely. Because cultures were conducted in the cycle that followed the one in which the hysteroscopies were performed, infection occurring after the procedure cannot be formally excluded, but we believe posthysteroscopy infections were unlikely. Our assertion is based on the large difference in positive endometrial cultures between women with and without signs of CE at hysteroscopy and the short time interval (3 to 4 weeks, on average) between hysteroscopies and cultures.

Concerning the clinical aspects of CE, as shown in Table 1, results of our study confirmed previous data that had indicated that CE has a high prevalence in populations who complain of abnormal uterine bleeding and infertility (3, 5, 12). In this population, fluid hysteroscopy with miniendoscopes allows a reliable diagnosis of CE. Bearing in mind the ease, the low risk, and the great acceptability of fluid hysteroscopy (27), we are convinced that fluid minihysteroscopy should be performed routinely in women who complain of abnormal uterine bleeding or infertility, especially if the condition is unexplained.

Our study demonstrates that three quarters of women diagnosed with CE at hysteroscopy have a positive endometrial culture and that vaginal culture has a low concordance with endometrial culture (in only 32% of cases was the microorganism found in the vagina the same as that found in the endometrium). For simplifying treatment and avoiding endometrial biopsy, one thus could consider administering a broad-spectrum antibiotic treatment randomly, without performing cultures. Although a definitive answer could be obtained only by a specific trial, in our opinion this choice is likely to be unsuccessful. Indeed, the antibiotic regimens proposed for pelvic inflammatory disease are mainly directed against *N. gonorrhoeae* and *Chlamydia*, and our results...
demonstrate that these infectious agents play a marginal role in the genesis of CE. More than 25% of the positive endometrial cultures showed the presence of Enterobacteriaceae, which are frequently resistant to most commonly employed antibiotics for pelvic inflammatory disease; moreover, it is known that *Mycoplasma*, which accounted for 10% of the positive endometrial cultures and approximately 20% of the vaginal cultures, may be resistant to doxycycline (23). Bearing in mind that the demonstration of an infectious agent in the uterine cavity does not mean infection and that we found a 5% of positive rate in women without evidence of CE, we believe that we can prescribe the proper therapy only by combining fluid minihysteroscopy, which provides the evidence of inflammation, and endometrial culture, which indicates the infectious agent at play.

Our study demonstrated that inflammatory changes of the endometrial mucosa identified by hysteroscopy, which constitute the basis for diagnosing CE, are strongly associated with the presence of microorganisms in the uterine cavity. This, therefore, suggests an instrumental role for the infectious agents in the genesis of the pathology. Approximately three quarters of the cases of CE were positive for nongonococcal, nonchlamydial infectious agents, with common bacteria and *Mycoplasma* as the most frequently detected etiologic agents. The agents detected may be resistant to the usual antibiotic treatments for pelvic inflammatory disease. Although ad hoc trials are needed to draw definitive conclusions, the great discordance observed between the results of vaginal and endometrial cultures in our trial data strongly suggest that endometrial cultures are warranted to guide the treatment of women with CE.

REFERENCES