

# Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy

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**STUDY QUESTION:** What is the prevalence of chronic endometritis (CE) in women with repeated unexplained implantation failure (RIF) at IVF, and how does antibiotic treatment affect the reproductive outcome?

**SUMMARY ANSWER:** Chronic endometritis, associated with infection with common bacteria or mycoplasma, is common in women complaining of RIF and antibiotic treatment significantly improves the reproductive outcome at a subsequent IVF cycle.

**WHAT IS KNOWN ALREADY:** We have reported that CE is a frequent finding in women with repeated pregnancy loss and a significantly higher rate of successful pregnancies was achieved after adequate antibiotic treatment. Moreover, CE was identified in 30.3% of patients with repeated implantation failure at IVF and women diagnosed with CE had lower implantation rates (11.5%) after IVF cycles. In contrast, other authors reported that the clinical implication of CE should be considered minimal and that the reproductive outcome at IVF/ICSI cycles was not negatively affected by CE.

**STUDY DESIGN, SIZE, DURATION:** A retrospective study was performed from January 2009 through June 2012 on 106 women with unexplained infertility and a history of RIF.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** All patients underwent hysteroscopy and endometrial sampling for histology and microbiological investigations. Women diagnosed with CE underwent antibiotic treatment and the effect of treatment was confirmed by hysteroscopy with biopsy. Within 6 months after treatment all women had a further IVF attempt. The IVF outcomes were compared in women without signs of CE (Group 1) and persistent CE (Group 2) after antibiotic treatment. Clinical pregnancy rate (PR), and live birth rate (LBR) were compared at post-treatment IVF attempt.

**MAIN RESULTS AND THE ROLE OF CHANCE:** Seventy (66.0%) women were diagnosed with CE at hysteroscopy. In 61 (57.5%) CE was confirmed by histology and 48 (45.0%) by cultures. Common bacteria and mycoplasma were the most prevalent agents. In 46 (75.4%) out of 61 women, with diagnosis of CE at hysteroscopy and histology, examinations were normal after appropriate antibiotic treatment control (Group 1) while in 15 (24.6%) cases signs of CE were still present (Group 2). At IVF attempt after treatment, a significantly higher PR and LBR was reported in women from Group 1 compared with women from Group 2 (65.2 versus 33.0%  $P = 0.039$ ; 60.8 versus 13.3%,  $P = 0.02$ , respectively).

**LIMITATIONS, REASONS FOR CAUTION:** Possible biases related to retrospective studies and to preferential referral of patients with CE, and limited number of cases.

**WIDER IMPLICATIONS OF THE FINDINGS:** A prospective randomized clinical trial is needed to confirm our findings but in women with RIF a hysteroscopic evaluation of the uterine cavity to exclude CE should be considered and appropriate antibiotic treatment should be given before submitting the patient to a further IVF attempt.

**STUDY FUNDING/COMPETING INTEREST(S):** This research was funded by the University personal research grants of Ettore Cicinelli. The authors have no competing interests to declare.

**Key words:** IVF/ICSI outcome / chronic endometritis / antibiotic treatment / repeated implantation failure / hysteroscopy

## Introduction

The pregnancy rate following one cycle of IVF and embryo transfer can be as high as 60% (Margalioth et al., 2006). However, even in the very successful units, some couples fail repeatedly. Failure could be caused by many different factors such as inappropriate ovarian stimulation, suboptimal laboratory culture conditions and faults in embryo transfer techniques. Repeated implantation failure (RIF) is defined as failure to conceive following two or three embryo transfer cycles, or cumulative transfer of > 10 good quality embryos (El-Toukhy and Taranissi, 2006). Implantation failure is related to either maternal factors or embryonic causes. Maternal factors include uterine anatomic abnormalities, thrombophilia, non-receptive endometrium and immunological factors (Salim et al., 2002). Although uterine abnormalities are considered to have a relevant impact on the chances to conceive through IVF, conventional infertility investigations, based on ultrasound and hysterosalpingography (HSG), may miss subtle intrauterine lesions (La Sala et al., 1998; Oliveira et al., 2003; Fatemi et al., 2010). At hysteroscopy prior to IVF the prevalence of unsuspected intrauterine abnormalities has been demonstrated to range between 11 and 45% (Fatemi et al., 2010); accordingly, recent reports suggest that hysteroscopy in the cycle preceding ovarian stimulation, could be useful for patients with RIF (Fatemi and Popovic-Todorovic, 2013). One of the abnormalities, which cannot be detected with ultrasound and HSG, is chronic endometritis (CE) that is a subtle pathology often asymptomatic or only accompanied by mild disturbances. Histological identification of plasma cells in the endometrial stroma is considered the gold standard for the diagnosis (Kasius et al., 2011) but due to the normal presence of leukocytes in the endometrium especially before menstruation, even histology may miss the diagnosis. In our previous papers, we demonstrated that fluid hysteroscopy reliably diagnoses CE based on the demonstration of specific signs such as micropolyps, stromal edema and focal or diffuse hyperemia (Cicinelli et al., 2005, 2008).

Chronic endometritis may hamper endometrial receptivity and may cause infertility because the endometrium is characterized by an abnormal pattern of lymphocyte subsets and, consequently, an aberrant endometrial microenvironment (Matteo et al., 2009). In a recent paper, we demonstrated that in women with repeated abortions, CE is a frequent finding and that women who received adequate antibiotic treatment had a significantly higher rate of successful pregnancies compared with women who were not treated or with persistent disease (Cicinelli et al., 2014). Moreover, CE was identified in 30.3% of patients with repeated implantation failure at IVF and women diagnosed with CE had lower implantation rates (11.5%) after an IVF cycle (Quaas and Dokras, 2008). In contrast, Kasius and coworkers reported that the clinical implication of CE seems minimal since they diagnosed this condition in ~2% of asymptomatic infertile patients with a normal transvaginal

ultrasound examination (TVS) (Kasius et al., 2011). The same author reported that the reproductive outcome at IVF/ICSI cycles was not negatively affected by CE and that the low prevalence and unknown clinical significance of endometritis warrants further study (Kasius et al., 2011, 2012).

Due to the controversial role of CE in assisted reproduction and the vast experience of our group in hysteroscopic and histological fields, in the present study we wanted to evaluate the effects of CE on RIF at IVF. For this purpose, we evaluated retrospectively the prevalence of CE at hysteroscopy, histology and endometrial cultures, in a population of women who experienced RIF at IVF due to unexplained infertility and the IVF outcome after specific antibiotic treatment. In detail, we compared the live birth rates at the first IVF attempt within 6 months after treatment between women in whom CE was successfully treated and those in whom it was still present after treatment.

## Materials and Methods

### Subjects

Two hundred and fifty-six women were referred to our department for hysteroscopy due to previous IVF failure in the period January 2009–June 2012. We analyzed retrospectively the charts of 106 of these who were affected by unexplained infertility and RIF as defined above and who were known to have planned a further attempt of IVF within 6 months after our assessment. Inclusion criteria were: absence of any abnormality at transvaginal ultrasound and at HSG, age < 40 years, at least six good quality embryos transferred in three or more previous IVF/ICSI cycles without signs of implantation, normal response with at least six oocytes retrieved with standard induction protocol, normal parental peripheral karyotype. Exclusion criteria were as follows: FSH on day 3 > 10 mUI/ml, BMI > 30 mUI/ml, history of clinical repeated pregnancy loss, previous surgery for myoma and/or endometriosis, ultrasound diagnosis of ovarian endometriomas, corticosteroid treatment or other medical treatments known to interfere with immune system, known clinical autoimmune disease, antiphospholipid syndrome, thrombophilic condition requiring anticoagulant therapy, presence of antisperm antibodies, unwillingness to give informed consent. All infertile women had undergone the following examinations recommended for the basic infertility evaluation (Quaas and Dokras, 2008): semen analysis of the partner, testing for detection of ovulation (mid luteal progesterone, LH kit), assessment of ovarian reserve, transvaginal ultrasound, and hysterosalpingography.

### Diagnosis and treatment of CE

In our department, women underwent diagnostic mini-hysteroscopy in the follicular phase of menstrual cycle. Mini-hysteroscopy was performed using a lens-based 3 mm OD mini-telescope, 105° angle of visual field equipped with a 3.5 mm OD single-flow diagnostic sheath (Karl Storz, Tuttlingen, Germany) as previously described (Cicinelli et al., 2008). Saline was employed to distend the uterine cavity. A 300 W light source with a xenon

bulb, a digital camera (Karl Storz, Tuttlingen, Germany) and a 21 inch video color screen 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. All hysteroscopies were performed by two of the authors (E.C., M.M.) and pictures were recorded in digital format. In all women enrolled in the study both authors agreed about the diagnosis of CE based on the demonstration of micro-polyps, polypoid endometrium, stromal edema and focal or diffuse hyperemia, as previously published (Cicinelli et al., 2005, 2008). In the follicular phase of the subsequent cycle, an endometrial biopsy using a 3 mm Novak's curette connected to a 20 ml syringe was performed for cultural and histological purposes to search for infectious agents such as common bacteria, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma* species, *Ureaplasma urealyticum* and yeast. In order to minimize the risk that endometrial cultures could be contaminated in the vagina, after placing a vaginal speculum and cleaning external uterine ostium by using a gauze soaked in iodine solution, 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 into 2 ml of saline and divided into two aliquots, one for infectious agent investigations and the other placed in formalin for histological examination. Based on the infectious agent detected and on the antibiogram result, an appropriate antibiotic treatment was prescribed. In the follicular phase of the cycle following the therapy all women were asked to attend the clinic again to re-evaluate uterine cavity by hysteroscopy for signs of CE and to collect endometrial samples for histology and culture. Treatment was repeated for a maximum of three cycles but stopped earlier if the cultures were negative and both hysteroscopic and histologic findings had normalized. At the end of treatment, women had their planned IVF treatment cycle at the same center as their previous attempts.

### Microbiological analysis

Endometrial specimens for *N. gonorrhoeae* were immediately placed in Stuart's transport medium and transported to the laboratory. In the laboratory, endometrial specimens were Gram-negative stained; then the endometrial specimens were plated into appropriate agar medium, 5% sheep blood Columbia Agar Base, Chocolate Agar, Mannitol Salt Agar and MacConkey Agar (Bio Merieux, Rome, Italy) and the presence of microorganisms was evaluated. The plates were incubated for 48 h in air or 5% carbon dioxide. Bacteria were identified using published criteria (Dade International, Inc., Milan, Italy). Genital mycoplasmas were quantitatively detected by immunoassay (Mycoplasma-IST, Biomerieux, and Rome, Italy). Chlamydia was tested by RT-PCR.

For yeast's isolation, specimens were plated into Sabouraud Chloramphenicol Agar and identification was made by using commercial kits (API-C System, Biomerieux, Rome, Italy).

### Histological analysis

Endometrial samples were fixed in neutral formalin and later embedded in paraffin for histological analysis. Five-micrometer sections were stained with hematoxylin-eosin. Histological diagnosis of CE was based on criteria previously described (Cicinelli et al., 2009). Attention was paid to the following features: superficial stromal edema, increased stromal density, pleomorphic stromal inflammatory infiltrate dominated by lymphocytes and plasma cells.

For this study in order to eliminate the inter-observer variability in all patients considered the histological examinations were re-evaluated by a single author (L.R.) who was unaware of previous histological and hysteroscopic findings. In all cases considered, the re-evaluation confirmed the previous results.

### IVF protocol

All patients underwent IVF in one of two high-volume (>500 procedures/year) centers in Italy. Ovulation induction was performed by recombinant FSH (175–225 IU/day) starting on Day 2 of the menstrual cycle. GnRH antagonist (0.125 mg/day, Cetorelix, Serono) was given beginning on Day 5 of rFSH injection, or when the leading follicle reached 10 mm, to the day of hCG administration. Once at least two leading follicles reached a size of  $\geq 17$  mm, 10 000 IU of urinary hCG was administered to trigger ovulation. Ovum retrieval was performed 34 h after the hCG injection. No more than three embryos with at least one of good quality per procedure were transferred transvaginally on Day 3 of culture. The luteal phase was supported with vaginal progesterone.

### Reproductive parameters calculation

All women diagnosed with CE at hysteroscopy and histology were contacted by telephone and asked about the reproductive outcome at the first IVF cycle performed within 6 months after therapy. The following outcomes were retrospectively evaluated: implantation rate, clinical pregnancy rate (PR), first trimester miscarriage and live birth rate (LBR). Implantation rate was defined as the percentage of embryos implanting successfully relative to the total number of embryos transferred. Clinical pregnancy rate per transfers was defined as the proportion of transfers that resulted in at least one intrauterine gestational sac documented by ultrasound. Live birth rate was defined as the number of transfers that resulted in live birth. All women confirmed their consent to use their data, anonymously, for research purposes.

### Statistical analyses

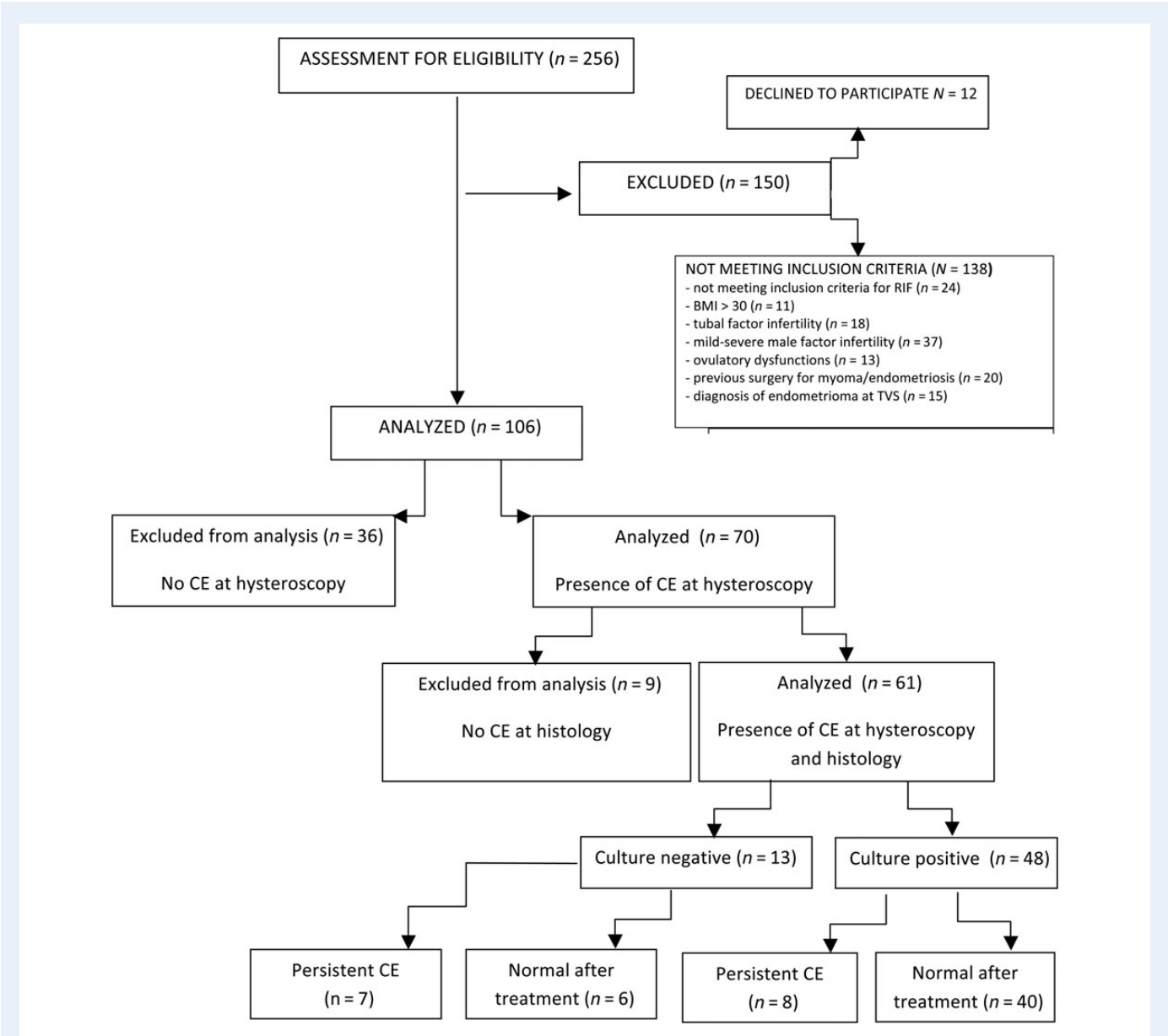
Clinical features were compared by using Chi-square test. Chi-square test and Fisher exact test, where appropriate, were used to compare the implantation rate, the clinical pregnancy rate, the live birth rate (LBR) and the number of miscarriages. Statistical analysis was performed by using Epi Info 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA); a  $P < 0.05$  was considered as the limit for significance.

### Ethical approval

This retrospective study was approved by the local ethical committee. All women gave their informed consent to use, anonymously, their data for research purposes.

## Results

The patients were aged 23–40 years; mean  $\pm$  SD  $31.9 \pm 4.1$  years. Seventy (66.0%) out of 106 women enrolled were diagnosed with CE at hysteroscopy, 61 (57.5%) cases were also positive at histology, while 48 (45%) were positive also at cultures (Fig. 1). In 36 women at hysteroscopy no evidence of CE was found; accordingly histology was also negative for CE (Fig. 1). In 34 (70.7%) out of 48 cases positive at cultures, common bacteria (*Escherichia Coli*, *Enterococcus faecalis*, *Streptococcus agalactiae*, etc.) were found. *Mycoplasma* and *Ureaplasma urealyticum* were detected in 14 (29.6%) cases (in 3 cases *Ureaplasma* and common bacteria coexisted) and *Chlamydia* in 4 cases (8.4%) (Table I). In 40 (83.3%) out of 48 patients with positive cultures, after antibiogram guided antibiotic treatment the control hysteroscopy as well as histology and cultures became negative while in the other 8 (17%) cases, signs of CE were still present at the last hysteroscopic and histologic evaluation. In 13 cases which, at first evaluation, were positive at hysteroscopy and histology but not at cultures, a broad spectrum antibiotic therapy based on Centers for Disease Control guidelines was proposed; after therapy in 6 out 13 (46%)



**Figure 1** Diagram of the study and distribution of the population investigated. CE means chronic endometritis.

women both hysteroscopy and the histology were normal while in the remaining 7 (54%) women signs of CE were still present (Fig. 1).

**Antibiotic treatment**

In most of the cases positive for Gram negative bacteria Ciprofloxacin 500 mg twice a day for 10 days was employed as first line therapy. In case of Gram positive bacteria, Amoxicillin + Clavulanate 1 g twice a day for 8 days was prescribed. Mycoplasma and *U. urealyticum* were treated with Josamycin 1 g twice a day for 12 days while, in case of persistence, Minocycline 100 mg twice a day for 12 days was employed. In women with negative cultures a treatment based on Ceftriaxone 250 mg IM in a single dose plus Doxycycline 100 mg orally twice a day for 14 days with Metronidazole 500 mg orally twice a day for 14 days, according to Centers for Disease Control guidelines, was administered. In case of persistence of signs of CE at subsequent hysteroscopy, the

protocol was repeated up to three times. In detail, in 17 cases (37%) signs of CE were negative after a single treatment, in 14 cases (30.4%) two treatments and in 15 cases (32.6%) three treatments.

The 61 women who were initially positive for CE by both hysteroscopy and histology were divided into two groups based on findings of the final hysteroscopic and histologic examination: 46 patients with a normal hysteroscopic and histologic pattern (Group 1) and 15 with persistence of CE at hysteroscopy and histology (Group 2). Clinical features of both groups are reported in Table II. We contacted the women by telephone and asked the outcome of their first IVF cycle within 6 months after treatment.

**Reproductive outcome**

All 61 patients analyzed at the first IVF cycle after treatment had a successful ovarian stimulation and underwent to the embryo transfer

**Table I** Infectious agents found in endometrial cultures from 48 women with RIF.

	No-(%)
* <i>Enterococcus faecalis</i>	16-(33)
<i>Mycoplasma/Ureaplasma</i>	14-(30)
* <i>Escherichia coli</i>	11-(23)
* <i>Streptococcus agalactiae</i>	5-(10)
<i>Chlamydia</i>	4-(8)
<i>Streptococcus bovis</i>	2-(4)
<i>Candida</i>	1-(2)
<i>Klebsiella pneumoniae</i>	1-(2)
<i>Staphylococcus epidermidis</i>	1-(2)
<i>Staphylococcus aureus</i>	1-(2)
<i>Streptococcus milleri</i>	1-(2)

Forty patients tested positive for a single agent and had multiple positivity. In three cases ureaplasma and common bacteria coexisted.

\*Infectious agents with persistent positivity.

**Table II** Clinical characteristics of women, number of previous IVF attempts and results in terms of implantation rate, clinical pregnancy rate, live birth rate (LBR) and number of miscarriages at the first IVF attempt within 6 months after treatment.

	Group 1 (n = 46)	Group 2 (n = 15)	P-value
Age (years)	31.7 ± 4.2	32.0 ± 4.6	NS
Partner age (years)	33.9 ± 5.4	34.6 ± 4.7	NS
BMI (Kg/m <sup>2</sup> )	23.0 ± 1.9	22.9 ± 2.1	NS
Smokers (%)	26	27	NS
FSH day 3 mUI/ml	7.1 ± 1.6	7.1 ± 1.7	NS
No. of previous IVF attempts	4.1 ± 1.0	4.1 ± 0.9	NS
No. of embryos transferred	1.95 ± 0.5	1.93 ± 0.4	NS
No. of good quality embryos transferred	1.3 ± 0.4	1.4 ± 0.5	NS
Implantation rate (%)	37	17	NS
Clinical pregnancy rate at first IVF after treatment (%)	65	33	0.039*
LBR at first IVF after treatment (%)	61	13	0.02*
First trimester miscarriage (%)	4	20	NS

Data are expressed as means ± SD unless stated otherwise. The limit of significance is a P value ≤ 0.05.

on Day 3. The mean number of embryos transferred at the first IVF cycle after treatment was  $1.95 \pm 0.5$  in group 1 and  $1.93 \pm 0.4$  in group 2. No difference was observed between the two groups in the total number of embryos transferred nor in the mean number of good quality embryos transferred ( $1.3 \pm 0.4$  in group 1 versus  $1.4 \pm 0.5$  in group 2). The clinical pregnancy rate per transfer was significantly higher in Group 1 compared with Group 2 (65 versus 33%; Fisher exact test:  $P = 0.039$ ). (Table II)

Accordingly, in women from Group 1 the LBR was significantly higher compared with Group 2 (61 versus 13%, respectively,  $P = 0.02$ ) (Table II). The implantation rate was higher in Group 1 compared with Group 2 (37 versus 17%) although difference did not reach statistical significance (Chi-square test  $P = 0.069$ ). No significant difference was observed in the number of first trimester miscarriages, two cases were recorded in Group 1 and 3 cases in Group 2 (Table II). The percentage of successful IVF at 6 months after treatment in women previously diagnosed with CE and treated based on antibiogram results or Centers for Disease Control guidelines is displayed in Fig. 2. Results were divided based on resolution or persistence of signs of CE at the end of treatment.

No significant difference in LBR was observed between women whose CE resolved after a single treatment and those whose CE required two or three treatments to resolve.

## Discussion

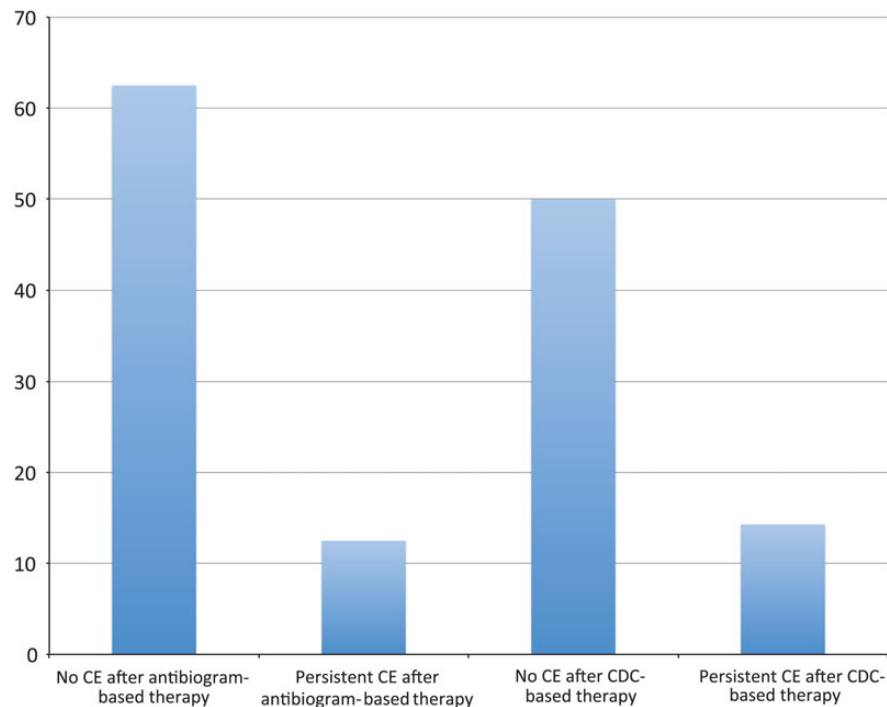
The results of this retrospective study add further evidence to the relationship between CE and impaired endometrial receptivity. The study demonstrates that CE is a common finding in women complaining of RIF and that common bacteria and mycoplasmas are the most frequently involved infectious agents. Moreover, this study suggests that hysteroscopy is a useful diagnostic technique in women with RIF as it reliably diagnoses the presence of CE, and makes it possible to evaluate the effectiveness of the antibiotic treatment in restoring a normal hysteroscopic and histologic appearance of the endometrial cavity. Finally, the reproductive outcome at IVF was significantly improved in those patients in whom antibiotic treatment was able to normalize both hysteroscopic and histologic endometrial pattern.

Regarding the first point, the finding that CE may have a role in the RIF etiology is in accordance with many experimental and clinical papers that underline the role of endometrial inflammation in the etiopathogenesis of RIF (Strandell et al., 1994; Feghali et al., 2003; Espinoza et al., 2006; Johnston-MacAnanny et al., 2010). Indeed, untreated CE has been suggested to diminish the success rates of both spontaneous conception and IVF cycles, as well as to contribute to adverse obstetrical outcomes (Feghali et al., 2003; Espinoza et al., 2006).

Accordingly, it is known that the implantation rates at IVF-ET are lower in patients with hydrosalpinx compared with those of couples with unexplained or male sterility. This is thought to be related to the leakage of hydrosalpinx fluid, containing leukocytes and inflammatory cytokines, which deteriorate the intrauterine environment and consequently leads to implantation failure (Strandell et al., 1994). The prevalence of CE in women with RIF in our study (66.0%) was about double compared with 30.3% reported by Johnston-MacAnanny et al. (2010). This discrepancy could be explained by the very strict selection criteria employed in this study and by the expertise of our group in hysteroscopic and histological diagnosis of CE. Anyway, we cannot exclude a selection bias due to the retrospective design of the study and the possibility of preferential referral to our center of women in whom a CE was suspected.

We have also to remark that, in order to rule out any confounding factor and inflammatory conditions, we considered clinical or ultrasonographic evidence of ovarian endometrioma as an exclusion criterion; however, the possibility of peritoneal endometriosis cannot be eliminated completely. This may have relevance as recently a close relationship between CE and endometriosis has been demonstrated (Takebayashi et al., 2014).





**Figure 2** The live birth rate achieved in the first IVF cycle within 6 months after treatment in women previously diagnosed with chronic endometritis (CE) and treated based on antibiogram results or Centers for Disease Control guidelines. Results were divided based on resolution or persistence of signs of CE at the end of treatment.

We found that common bacteria and mycoplasma are the most frequent etiological agents for CE in women with RIF. The high prevalence of common bacteria is not surprising considering the high prevalence of bacterial vaginosis and the knowledge that ascending bacteria can colonize the uterine cavity (Kamiyama et al., 2004; Cicinelli et al., 2012). Indeed, in IVF cycles the presence of bacterial vaginosis may decrease conception rates, increase early pregnancy losses and may also increase the risk of preterm birth (Salim et al., 2002; Eckert et al., 2003).

The results also showed that in ~29% of cases, *Mycoplasma* and *Ureaplasma urealyticum* may be responsible for CE. The pathogenic role of *Mycoplasma* is in accordance with a previous paper reporting the prevalence of *U. urealyticum* and *Mycoplasma hominis* in the endocervix at the time of oocyte collection in women undergoing *in vitro* fertilization (IVF) (Witkin et al., 1995) and with paper from Haggerty and coworkers who found *Mycoplasma genitalium* in 12% of cervical specimen and in 8% of endometrial specimen from women affected by non-gonococcal, non-*Chlamydia* endometritis (Haggerty et al., 2006).

*Chlamydia* was demonstrated in only about 8% of positive endometrial cultures. Our data are in full accordance with Stern and coworkers who detected the presence of *Chlamydia* by PCR in only one out of 43 specimens of histologically diagnosed CE, concluding for a limited role, if any, of *Chlamydia* in the origin of chronic endometritis (Stern et al., 1996). No case of *N. gonorrhoeae* was found and this is probably due to the specific characteristics of the population studied.

Our study has the intrinsic limitations of all studies that rely on bacteriological cultures of the endometrial cavity using traditional culture techniques and transcervical sampling. Only micro-organisms able to grow under conventional microbiology laboratory conditions can be

recovered and the procedure may therefore yield biased microbial findings. Thus, it cannot be excluded that other micro-organisms (anaerobic bacteria, viruses, etc.) might also coexist and play a role. In our previous paper we observed a significant discordance between the infectious agents found at vaginal and endometrial levels, concluding that the possibility that endometrial results could be biased by vaginal contamination was low (Cicinelli et al., 2009). Moreover, extreme care was employed during endometrial sampling in order to avoid any contact between the curette and vaginal walls. Finally, the fact that antibiotic treatment against infectious agent detected at endometrial culture resulted in a normalization of hysteroscopic finding and in a significant improvement in live births rate, speaks in favor of the actual endometrial origin of infectious agents found in endometrial specimens. The data of the present paper are in accord with the recent view that uterine cavity is normally not sterile and that presence of micro-organisms does not mean inflammation (Cowling et al., 1992). Hence, it is not just the presence of infectious agents within the internal genital tract but rather the interactions between infectious agents and endometrial environment that is seen today as the most critical issue that determines the presence of pathology (Eckert et al., 2003). Another important issue emerging from this study is the importance in women undergoing IVF of performing a careful hysteroscopic evaluation of the uterine cavity in order to detect subtle lesions like CE; this is of particular importance in women with RIF (La Sala et al., 1998; Oliveira et al., 2003; Bohlmann et al., 2010; Bosteels et al., 2010). This statement is supported by the improved reproductive outcome that we found in women with RIF, when treatment was able to normalize the hysteroscopic endometrial pattern after an antibiogram guided antibiotic therapy as well as after treatment based on Centers for Disease Control

guidelines. In fact the clinical PR and the LBR were significantly higher in women of Group I. The implantation rate was different between the two groups with a positive trend for Group I, even if the difference did not reach statistical significance. This data could be explained with the limited number of patients analyzed, but we cannot exclude that treatment of CE could improve the reproductive outcome by preventing adverse obstetrical events without affecting implantation (Feghali *et al.*, 2003; Espinoza *et al.*, 2006). A 61% (28/46) LBR in group I after treatment may seem too high. However, the strict selection criteria employed to select a specific subset of women affected by CE and the absence of additional factors interfering with IVF results could concur to explain our results. A high LBR in a specific subset of patients has been observed elsewhere, in women with RIF with expanded circulating NK and/or NKT-like cells a LBR improvement from 17.9 to 80.0% after intravenous immunoglobulin therapy has been recently demonstrated (Ramos Medina *et al.*, 2014). Regarding the absence of additional interfering factor with IVF results, old female age, duration of infertility, poor ovarian response, male factor and anovulation as indication for IVF have been reported to represent factors associated with poor outcome at IVF and low LBR compared with women undergoing IVF for unexplained infertility (Battacharya *et al.*, 2013). Accordingly, the mean age of women in group I was 31.7 years, all had a good ovarian reserve and in all cases the indication for IVF was unexplained infertility. Finally, we have to consider that due to the small number of patients in our study, also the chance factor may play a role in explaining so high results the 95% confidence limits from the binomial distribution for the LBR in group I are 45.4–74.9. The discrepancy with results of the Johnston-MacAnanny study (Johnston-MacAnanny *et al.*, 2010) in which patients with CE continued to have persistently lower implantation rates despite successful treatment may be partially explained by procedural differences. In their study the diagnosis of CE was performed only based on histologic findings without performing either hysteroscopy or endometrial cultures. However, it is known that due to normal presence of leukocytes in the endometrium especially before menstruation, histology may miss the diagnosis of CE (Resta *et al.*, 2012). Notably, in our series of women with RIF and diagnosed with CE at hysteroscopy, 13 out of 61 (21, 3%) cases positive at both hysteroscopy and histology were negative at cultures. This finding may have different explanations and may be related to technical procedures or to the presence of micro-aerophils or anaerobic bacteria which cannot be cultivated with conventional techniques (Haggerty *et al.*, 2006). This allows us to speculate that the hysteroscopic evaluation of the endometrial inflammatory disease could have a higher sensitivity than the endometrial cultures in detecting CE, and that a normal endometrial pattern at hysteroscopy could be more accurate in predicting the possibility of a successful pregnancy after IVF. When considering the problem of the cost effectiveness and the risk benefit of our approach to diagnosis and treatment of CE, we underline that fluid mini-hysteroscopy is a minimally invasive technique that can be performed in an office setting without anesthesia (Cicinelli *et al.*, 2008), so that the advantages in terms of diagnosis and treatment amply overcome the costs of the procedure. Moreover, in case of persistent CE no more than three cycles of treatment were performed so, although we cannot exclude the possibility to generate antibiotic resistance, the potential benefit for patients outweighs the risk.

In conclusion, even with potential limitations related to retrospective studies, results of this pilot research demonstrate that CE is a condition frequently associated with RIF. In our population the most prevalent infectious agents are common bacteria and mycoplasma. In women with

RIF hysteroscopy reliably detected the existence of CE. The normalization of the hysteroscopic endometrial pattern was associated with a significant improvement of the reproductive outcome of the IVF cycle performed after treatment.

However, prospective controlled clinical trials are needed to confirm the role of CE in RIF and to evaluate if the antibiotic treatment could actually influence IVF outcomes and improve LBR by restoring the endometrial competence

## Authors' roles

E.C. and M.M. were responsible for the study concept and participated in designing the study, interpreting data, in writing and reviewing the manuscript and approved the final version. P.G. and L.R. took part in interpreting data, in writing and reviewing the manuscript. U.I. performed statistical analysis. R.T. took part in interpreting data and in reviewing the manuscript. R.A. and A.L. took part in collecting and interpreting data.

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## Conflict of interest

None declared.

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