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Full length article

Chronic endometritis identified by plasma cells can often be diagnosed in patients with recurrent implantation failure

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ABSTRACT

Purpose: Is plasma cell (PC)-diagnosed chronic endometritis a common phenomenon in patients with recurrent implantation failure, and what can be learned from it?

Methods: In this retrospective case–control study at Wiesbaden Fertility Centre, 147 patients with recurrent implantation failure (RIF) underwent endometrial biopsy (EB) between January 2017 and December 2021 to rule out chronic endometritis (CE). For diagnosis, samples were labelled immunohistochemically with anti-CD138 and anti-MUM1 antibodies for the detection of PCs. The cut-off for the diagnosis of CE was at least five PCs per 10 evaluated high-power fields (HPFs). Patients with four PCs or fewer in the endometrial stroma (ES) formed Group A. Patients with at least five PCs in the ES were subsequently treated with doxycycline for 14 days and formed Group B. Those patients in Group B with persistent findings in a control biopsy received a combination of ciprofloxacin and metronidazole for 7 days. The EB time was documented, and the outcomes of assisted reproductive technology (ART), pregnancy and birth within 9 months of diagnosis were evaluated, as well as patient characteristics for both groups.

Results: Following the calculation of a threshold value using receiver operating characteristic curve analysis to determine the optimal time for EB (between cycle days 8 and 9 in this study), 65 patients were excluded from further evaluation due to an inadequate EB time point. Of the remaining 82 patients, 49 (59.8%) were assigned to Group A and 33 (40.2%) were assigned to Group B. In four patients in Group B, the control biopsy revealed that they still had at least five PCs per 10 HPFs, and these patients were treated following the previously described protocol. A comparison of the two groups revealed no significant differences in terms of medical history, characteristics, ART used, and pregnancy and live birth rates. However, the timing of EB is crucial for a correct diagnosis of CE.

Conclusion: In this retrospective case–control study, patients with RIF frequently (40%) exhibited histological signs (at least five PCs per 10 HPFs in ES) of CE. These findings indicate that EB for the detection of CE should be performed in the late follicular phase or after day 8 of an ovulatory cycle if a cut-off value of at least five PCs is used. Antibiotic treatment with doxycycline led to a reduction in PCs in over 80% of cases. Persistent findings can be treated successfully with ciprofloxacin and metronidazole. After treatment of CE, the outcomes of patients were found to be comparable with those without CE. Therefore, an investigation to exclude CE using PCs in patients with RIF seems advisable before performing further ART.

Introduction

In-vitro fertilization (IVF) is the most effective treatment for infertility worldwide. Approximately 30 % of patients achieve pregnancy and 20 % achieve delivery with the first round of IVF treatment [1–4]. The limited success is probably due to uncontrollable molecular,

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biological and genetic characteristics within germ cells, embryos and the endometrium. Many aspects of this highly complex process are still poorly understood [5,6].

Despite normal findings and often after several attempts, some patients do not become pregnant. This is often referred to as 'recurrent implantation failure' (RIF). However, the number of embryos transferred in cases of RIF is not uniformly defined [7,8]. Various authors have defined RIF based on transfer of at least three embryos without pregnancy [7–11].

For some time, chronic endometritis (CE) has been suspected to be an important influencing factor impairing the success of IVF and the development of pregnancy by disruptive inflammatory reactions [12–17]. It is a phenomenon that is often overlooked due to the lack of symptoms, and is sometimes doubted as an independent clinical entity due to inconsistent definitions [18]. A plethora of micro-organisms, such as chlamydia, *Neisseria* spp., *Ureaplasma* spp., *Mycoplasma* spp., Enterobacteriaceae, *Corynebacterium* spp., enterococci, staphylococci and *Klebsiella pneumoniae* have been discussed [12,13].

In these cases, elevated plasma cells (PCs) in the endometrial stroma (ES) and decidua can be taken as a sign of increased and persistent inflammatory responses with the formation of antibodies and pro-inflammatory factors [19]. The values at which CE can be diagnosed are not uniformly defined in the literature. A threshold of at least five PCs per 10 evaluated high-power fields (HPFs) is currently considered applicable [13,14].

The purpose of this retrospective case–control study was to determine the incidence of CE in a cohort of patients with RIF [9,11,18]. The diagnosis of CE was based on at least five PCs per 10 HPFs in ES. In addition, this study investigated whether the number of PCs could be reduced sufficiently with antibiotics, and how this affected subsequent clinical outcomes during a 9-month follow-up period.

Materials and methods

Patient recruitment

This study was approved by the Ethics Committee of Hesse State Medical Association (File No. 2019-1497-evBO). All patients with RIF who had undergone an endometrial biopsy (EB) between January 2017 and December 2021 were identified from the data archive of Wiesbaden Fertility Centre, and included in the study. RIF was defined as the occurrence of at least three embryo transfers (ETs) that did not result in pregnancy. In addition to RIF, the inclusion criteria encompassed immunohistochemical staining for PCs for diagnostic purposes, and a documented follow-up period of at least 9 months. The patients were contacted via telephone and e-mail and invited to participate in the study. Once written consent had been obtained, data analysis could commence.

The exclusion criteria were unclear pregnancy outcomes, hydrosalpinx, abnormalities of the uterine cavity detected on three-dimensional sonogram or hysteroscopy, acute urogenital infections in one or both partners, severe autoimmune diseases (lupus erythematosus, antiphospholipid antibody syndrome), and abnormal genetic findings in one or both partners.

Patients with four PCs or fewer in 10 HPFs in ES formed Group A, and patients with at least five PCs in 10 HPFs formed Group B. Patients with at least five PCs were considered to have CE and treated according to the protocol described below.

Patient characteristics

The following biometric data and findings at the time of diagnosis were recorded: age (years), body mass index (BMI; kg/m^2), years of infertility, number of previous pregnancies and births before starting IVF, concentration of anti-Müllerian hormone (AMH; ng/ml), endometriosis status, unilateral or bilateral tubal occlusion without hydrosalpinx, thrombophilic factors (heterozygous factor V Leiden and prothrombin mutation, reduction of antithrombin III, protein C and S, methylene tetrahydrofolate reductase polymorphism), autoantibodies (antiphospholipid antibodies, thyroid peroxidase antibodies and antinuclear antibodies), diabetes mellitus (types I and II), insulin resistance, polycystic ovary syndrome (PCOS), hypothyroidism, nicotine usage, thyroxine intake and metformin intake. Andrological subfertility was defined by two abnormal spermiograms, in accordance with the World Health Organization laboratory manual [20].

Similarly, in conventional IVF, intracytoplasmic sperm injection (ICSI) and frozen embryo transfer (FET) performed in the past, the number of embryos transferred previously and embryo morphology were documented. The European Society of Human Reproduction and Embryology classification was used to assess embryological morphology [21].

Nine months post EB, the following treatments and outcomes were recorded: IVF, ICSI, FET, spontaneous conception, number of embryos transferred, number of ETs performed, and embryo morphology. In addition, the thickness of the endometrium, measured by sonography during ovulation induction, was recorded. The pregnancy rate was calculated as the quotient of proven clinical intrauterine pregnancies (chorionic cavities with vital embryoblasts) per group. The birth rate was based on the proportion of documented live births in patients with intrauterine pregnancies. Multiple pregnancies or births were evaluated as one event. Miscarriages were recorded as a documented loss of pregnancy before reaching extrauterine viability. Ectopic pregnancies were recorded based on documented surgical and/or chemotherapeutic interventions, and reported proportionately in the subgroups.

Diagnosis and treatment of CE

PCs were used as marker cells to detect CE. Detection was based on antibodies against the surface receptor for CD138, or syndecan-1, which is expressed on the surface of PCs, among others. Furthermore, antibodies against melanoma associated antigen (mutated) 1 (MUM1) were used, which is also expressed in PCs. A minimum of five PCs per 10 HPFs was used to diagnose CE [13,22–24].

Prerequisites for all EBs were the exclusion of pregnancy and at least a 9-mm proliferative endometrium in an ovulatory cycle on sonography. No specific instructions were set for a particular cycle day. In cases of anovulation and missing endometrial proliferation, patients were treated with daily stimulation of 50 IU of recombinant follicle-stimulating hormone (rec-FSH) or, in the case of luteinizing hormone (LH) deficiency, 50 IU of human menopausal gonadotropin (hMG).

All endometrial samples were collected during diagnostic hysteroscopy with a 4-mm biopsy curette. Patients with abnormal hysteroscopic findings were excluded from further analysis. Patients with four PCs or fewer in 10 HPFs formed Group A. Patients with at least five PCs in 10 HPFs formed Group B; these patients were diagnosed with CE, and received doxycycline 200 mg/day for 14 days, followed by vaginal administration of lactobacilli. After treatment, a control biopsy was performed using a pipelle under sonographic control. Fewer than five PCs was interpreted as cure. In the case of persistent findings, a combination of ciprofloxacin 500 mg/day and metronidazole 400 mg/day was used for 7 days, followed by subsequent administration of lactobacilli. A second control biopsy, using a pipelle under sonographic control, was performed 3 weeks after the completion of antibiotic therapy, and samples were examined to confirm that the CE had been cured.

At the Centre for Histology, Cytology and Molecular Pathology, Wiesbaden, formalin-fixed samples were dehydrated, embedded in paraffin, sliced, and stained with haematoxylin and eosin. In addition, immunohistochemical labelling was performed on hydrated and dewaxed sections with antibodies against CD138 and MUM1 for the determination of PCs. Light microscopy was evaluated at 400 × magnification by a pathologist who is a recognized researcher and clinician in the field

of gynaecological pathology.

Assisted reproductive technology

IVF and ICSI cycles were performed in accordance with the antagonist protocol. rec-FSH was used for stimulation, starting on cycle day 2 with a dose of 150–225 IU based on age, body weight, AMH concentration and antral follicle count. Patients with LH deficiency received an additional 75 IU of LH. Gonadotrophin-releasing hormone antagonist treatment was started on cycle day 6. If more than three follicles reached a diameter of $\geq$ 17 mm, final oocyte maturation was induced with recombinant human chorionic gonadotrophin 250 µg or, in the case of impending ovarian hyperstimulation syndrome (OHSS), with 3 x 0.1 mg of triptorelin. Oocytes were collected after 36 h. Conventional IVF or ICSI was used depending on the sperm findings and the results of previous IVF cycles. Patients received progesterone 600 mg intravaginally from the evening of oocyte retrieval until pregnancy. In the case of impending OHSS, fertilized oocytes were frozen in liquid nitrogen, and ET was postponed for 4 weeks. With a soft preheated catheter and under abdominal ultrasound guidance, ET occurred 3–5 days after fertilization. The pregnancy test was performed 14 days after ET, and sonography was performed 30 days after ET.

Frozen fertilized oocytes and/or embryos were derived from cycles with supernumerary embryos or imminent OHSS. FET was performed in modified natural cycles. In cases of anovulation, the endometrium was prepared with daily stimulation of rec-FSH 50 IU or oestradiol 6 mg orally per day. Ovulation induction, progesterone administration, ET and pregnancy detection tests were carried out in the same way as the IVF and ICSI cycles.

In the case of proven thrombophilic factors (heterozygous factor V Leiden and prothrombin mutation, reduction of antithrombin III, protein C and S, methylene tetrahydrofolate reductase polymorphism, antiphospholipid antibodies), low molecular weight heparin (LMWH) was added daily, starting with stimulation. Acetylsalicylic acid 100 mg daily was combined with LMWH in the case of repeated positive antiphospholipid antibody titres, and was administered until 32 weeks of pregnancy.

Statistical analysis

Quantitative variables are presented as mean and standard deviation (SD), and qualitative variables are presented as relative and absolute frequencies. Normal distribution of the quantitative variables was checked both graphically with histograms and with a statistical test procedure (Shapiro–Wilk test). The mean values of Groups A and B were compared with the independent samples *t*-test in the case of a normal distribution; otherwise, the Mann–Whitney *U* test was used for two-group comparison. If significant differences were found between the calculated mean values in the group comparisons, binomial logistic regression was used to examine influencing factors, and to indicate the relative risk (RR) and 95 % confidence interval (CI).

When comparing the two groups, a significant difference was found regarding the mean cycle day on which the biopsy was taken (day 11 for Group A vs day 14 for Group B; $p = 0.006$) (RR 1.09, 95 % CI 1.02–1.20).

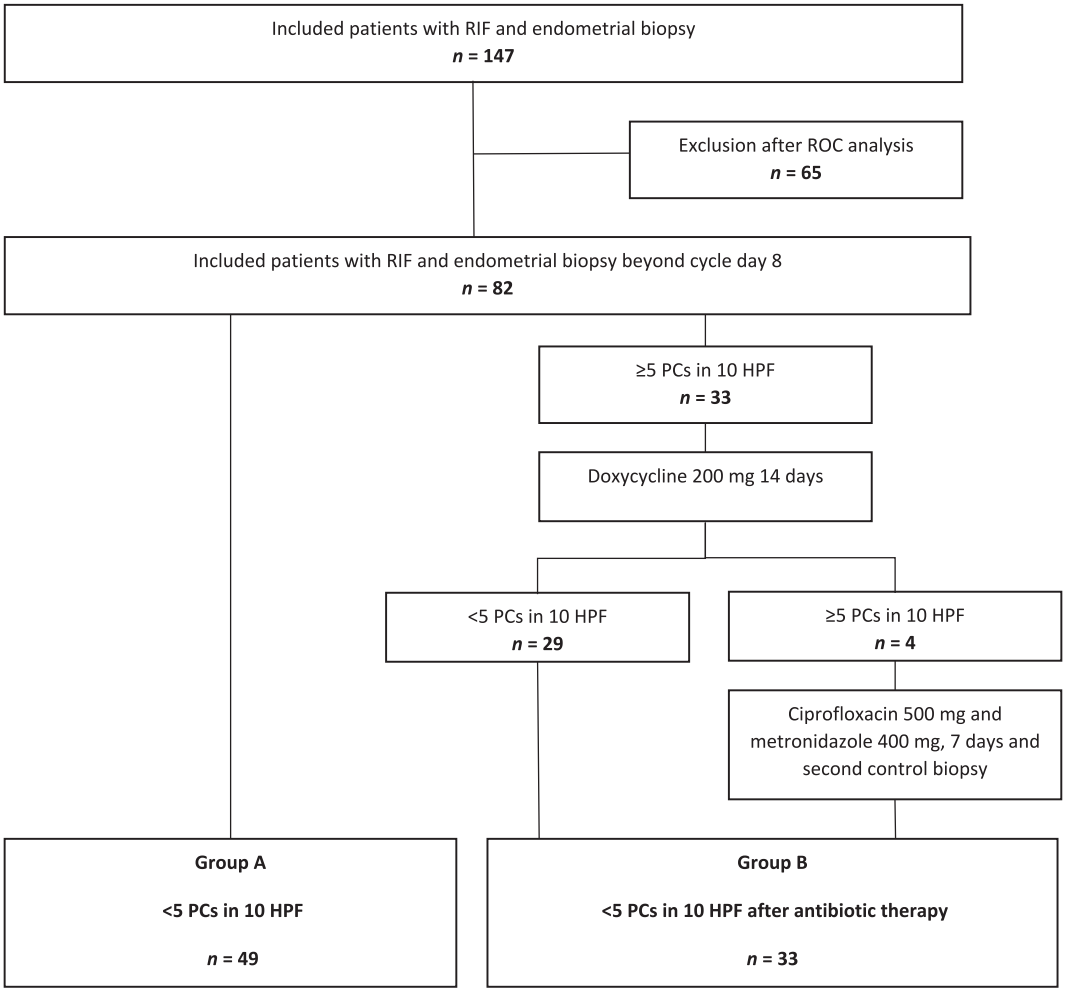

Fig. 1. Study flow chart. RIF, recurrent implantation failure; PC, plasma cells; HPF, high-power fields; ROC, receiver operating characteristic curve.

Therefore, the possibility of missing endometritis may increase if samples are taken too early. A receiver operating characteristic curve analysis was used to calculate a cut-off value for the cycle day of the EB from which CE could be diagnosed with the greatest possible reliability, and the Youden Index and the closest top left criterion were determined. This resulted in a cut-off value for cycle day 8.5. Hereinafter, all samples taken before cycle day 8.5 were excluded from further analysis (50 in Group A and 15 in Group B; Fig. 1). Both groups were compared again regarding all patient criteria and showed no significant differences; as such, it was possible to assume comparable populations when detecting CE. Data were analysed using SPSS Version 29.0.2.0 (IBM Corp., Armonk, NY, USA), and $p < 0.05$ was considered to indicate significance.

Results

In total, there were 49 patients in Group A and 33 patients in Group B, which corresponded to an incidence of CE of 40.2 %. All patients with at least five PCs were treated with doxycycline and biopsied again to check the findings after treatment. Four patients were still found to have at least five PCs, and these patients were categorized as not cured. This corresponded to an incidence of 12 %. After combination treatment with ciprofloxacin and metronidazole, the patients were examined again; none of the samples showed five PCs or more (Fig. 1). No complications due to antibiotic therapy were reported.

At the time of the study, the mean age of the patients in Groups A and B was 35.04 (SD 3.24) years and 35.48 (SD 3.53) years, respectively. Mean BMI was 23.79 (SD 4.91) kg/m^2 and 24.03 (SD 4.62) kg/m^2, respectively, and mean AMH concentration was 3.99 (SD 3.98) ng/ml and 2.88 (SD 2.37) ng/ml, respectively. The values did not differ significantly between the subgroups. Before their infertility, 53 % and 54 % of the women in Groups A and B, respectively, had been pregnant, and 20 % and 27 %, respectively, had given birth. The mean duration of infertility was 7.31 (SD 2.63) years and 6.55 (SD 2.21) years in Groups A and B, respectively (Table 1).

Five (10 %) patients in Group A and seven (21 %) patients in Group B had a history of endometriosis ($p = 0.17$), and 22 % and 21 %, respectively, had unilateral or bilateral tubal occlusion without hydrosalpinx ($p = 0.89$). Thrombophilic factors were found in 55 % and 51 % patients in Groups A and B, respectively. A total of 10 % and 21 % of patients in Groups A and B had autoantibodies, respectively ($p = 0.16$). Two diabetic patients were included in Group A, and there were no diabetic patients in Group B. PCOS was comparatively common in 10 % and 9 % of patients in Groups A and B, respectively ($p = 0.17$). In total, 26 (53 %) and 17 (51 %) patients in Groups A and B, respectively, presented with latent hypothyroidism ($p = 0.89$), 48 % and 54 % of whom received thyroxine, respectively ($p = 0.181$). Metformin was used in 10 % and 6 % of patients in Groups A and B, respectively (Table 1). The incidence of andrological subfertility was comparable in Groups A and B at 69 % and 81 %, respectively ($p = 0.21$) (Table 1).

The patients in Group A had undergone seven IVF (5.1 %), 118 ICSI (85.5 %) and 13 FET (9.4 %) cycles. The patients in Group B had undergone 14 IVF (13%), 82 ICSI (79%) and seven FET cycles (7%). The mean number of embryos transferred was 5.78 (SD 2.51) and 6.03 (SD 2.39) in Groups A and B, respectively. In terms of embryo morphology, 2.71 (SD 1.80) and 2.91 (SD 1.10) transferred embryos were classified as grade A, 2.55 (SD 1.72) and 2.64 (SD 2.52) were classified as grade B, and 0.51 (SD 0.65) and 0.48 (SD 0.75) were classified as grade C in Groups A and B, respectively. The groups did not differ significantly in these aspects (Table 2).

Table 3 shows the type of cycle and which day the endometrial sample was taken [mean 15.08 (SD 4.97) vs 13.65 (SD 5.26) for Groups A and B, respectively; $p = 0.11$]. Ninety-four percent and 96 % of EBs were taken during an ovulatory cycle without medication in Groups A and B, respectively. Three and one diagnostic cycles were treated with rec-FSH for ovulation in Groups A and B, respectively. None of the recorded cycles received hMG or hormone replacement treatment. The group comparison showed no significant difference in these aspects.

During the 9-month follow-up period, there were four spontaneous conceptions in Group A and two in Group B. Three and four IVF ($p = 0.34$), 37 and 24 ICSI ($p = 0.79$), and five and three FET ($p = 0.87$) treatments were undertaken in Groups A and B, respectively. A mean of 1.58 (SD 0.88) and 1.23 (SD 0.85) ETs per patient were documented ($p = 0.25$). The mean recorded sonographic endometrial thickness at ovulation induction before ET was 9.86 (SD 2.05) mm in Group A and 10.39 (SD 1.55) mm in Group B ($p = 0.23$). The morphology of transferred embryo groups did not differ significantly between the groups, with proportions of 40 % and 28 % for grade A, 51 % and 60 % for grade B, and 9 % and 12 % for grade C morphology for Groups A and B, respectively (Table 4).

Fifty-three percent of patients in Group A and 54 % of patients in Group B became pregnant ($p = 0.89$). A total of 88 % and 89 % in Groups A and B, respectively, had a live birth ($p = 0.89$), and 11 % of patients in both groups had a miscarriage ($p = 0.99$). Seven twin births were seen in Group A and three were seen in Group B ($p = 0.48$). One and two ectopic pregnancies were documented in Groups A and B, respectively ($p = 0.35$) (Table 4).

Table 1
Demographic characteristics of subgroup analysis.

	Group A: no CE detected ($n = 49$)	Group B: CE detected and treated ($n = 33$)	p-value[a]
Age	35.04 ± 3.24	35.48 ± 3.53	0.65
BMI	23.79 ± 4.91	24.03 ± 4.62	0.97
Years of infertility	7.31 ± 2.63	6.55 ± 2.21	0.18
Previous pregnancies	26 (53 %)	18 (54 %)	0.63
Previous births	10 (20 %)	9 (27 %)	0.23
AMH	3.99 ± 3.98	2.88 ± 2.37	0.23
Endometriosis	5 (10 %)	7 (21 %)	0.17
Unilateral or bilateral tubal occlusion	11 (22 %)	7 (21 %)	0.89
Thrombophilic factors	27 (55 %)	17 (51 %)	0.75
Autoantibodies	5 (10 %)	7 (21 %)	0.16
Diabetes mellitus	2 (4 %)	0	0.24
Diagnosed insulin resistance	12 (24 %)	7 (21 %)	0.73
PCOS	10 (20 %)	3 (9 %)	0.17
Hypothyroidism	26 (53 %)	17 (51 %)	0.89
Nicotine consumption	4 (8 %)	0	0.09
Thyroxine intake	24 (48 %)	18 (54 %)	0.62
Metformin intake	5 (10 %)	2 (6 %)	0.51
Andrological subfertility	34 (69 %)	29 (81 %)	0.21

CE, chronic endometritis; BMI, body mass index; AMH, anti-Müllerian hormone; PCOS, polycystic ovary syndrome.
[a] Two-sided p-value.

Table 2
Characteristics of pre-cycles with number and morphology of transferred embryos.

	Group A: no CE detected ($n = 49$)	Group B: CE detected and treated ($n = 33$)	p-value[a]
IVF	10 (7 %)	14 (13 %)	0.76
ICSI	130 (85 %)	82 (79 %)	0.71
FET	13 (8 %)	7 (7 %)	0.64
Mean value of transferred embryos	5.78 ± 2.51	6.03 ± 2.39	0.77
Morphology of transferred embryos			
A	2.71 ± 1.80	2.91 ± 1.10	0.23
B	2.55 ± 1.72	2.64 ± 2.52	0.66
C	0.51 ± 0.65	0.48 ± 0.75	0.61

CE, chronic endometritis; IVF, in-vitro fertilization; ICSI, intracytoplasmic sperm injection; FET, frozen embryo transfer.
[a]Two-sided p-value.

Table 3
Cycle type and day of endometrial biopsy.

	Group A: no CE detected ($n = 49$)	Group B: CE detected and treated ($n = 33$)	p-value[a]
Ovulatory cycle	46 (94 %)	32 (96 %)	0.52
Cycle with rec-FSH stimulation	3 (6 %)	1 (3 %)	0.51
Cycle day of endometrial biopsy	15.08 ± 4.97	13.65 ± 5.26	0.11

CE, chronic endometritis; rec-FSH recombinant follicle-stimulating hormone.
[a] Two-sided p-value.

Table 4
Results of the subgroup analysis during the follow-up period.

	Group A: no CE detected (n=49)	Group B: CE detected and treated (n=33)	p-value[a]
Spontaneous conceptions	4 (8%)	2 (6%)	0.72
IVF	3 (5%)	4 (13%)	0.34
ICSI	37 (72%)	24 (77%)	0.79
FET	5 (13%)	3 (10%)	0.87
Number of embryos per patient	3.11 ± 2.47	2.42 ± 1.06	0.44
Embryo transfer per patient	1.58 ± 1.09	1.23 ± 0.85	0.25
Morphology of transferred embryos			
A	56 (40%)	21 (28%)	0.33
B	72 (51%)	45 (60%)	0.72
C	12 (9%)	9 (12%)	0.79
Endometrial thickness at OI	9.86 ± 2.05	10.39 ± 1.55	0.23
Clinical pregnancy rate	53% (26/49)	54% (18/33)	0.89
Birth rate	88% (23/26)	89% (16/18)	0.89
Percentage of twin births	30% 7/23)	19% (3/16)	0.48
Miscarriage rate	11% (3/26)	11% (2/18)	0.99
Percentage of ectopic gravidities	2% (1/49)	6% (2/33)	0.35

CE, chronic endometritis; IVF, in-vitro fertilization; ICSI, intracytoplasmic sperm injection; FET, frozen embryo transfer; ET, embryo transfer; OI, ovulation induction.
[a]Two-sided p-value.

Discussion

Using the cut-off of at least five PCs per 10 HPFs, this study found an incidence of CE of 40.2 %. This observation correlates with previously published data reporting high incidence of CE in patients with RIF [6,25–31]. However, other findings indicate that the detection of fewer than five PCs has a negative impact on embryo implantation [32,33].

With an incidence of approximately 25 %, even in patients without fertility disorders, CE seems to represent a widespread phenomenon, with links to endometrial polyps, endometrial hyperplasia and atypical bleeding observed [34].

There is still no consensus on the exact cycle time for EB to diagnose CE, but the late proliferation phase has been discussed. To the authors' knowledge, most studies of CE in patients with RIF have not provided accurate information on this issue. In some cases, a time window of several days after the completion of menstruation has been indicated. However, the late proliferation and early to middle secretion phases have also been discussed [30,31,35–37]. In this analysis, sampling after cycle day 8 was the most reliable way to diagnose CE with at least five PCs per 10 HPFs.

Doxycycline achieved a high response rate of 88 % in the study cohort, which was comparable to that reported by Kitaya et al. [38] and Espinós et al. [39]. However, significantly lower response rates have also been described [29].

The present study found no significant differences between Groups A and B in terms of the specificities of applied treatments, clinical outcomes (such as pregnancy or birth rates), or other evaluated parameters [40] The results were comparable to those in other studies [14,30,31,35,38–42].

Limiting factors

Regarding the cycle day of EB, evaluation of the endometrial samples included originally showed significant differences. The retrospective study design led to this methodological bias, which affected the diagnosis of CE. Therefore, it is important to consider the appropriate phase when performing an EB in an ovulatory cycle.

Patients who underwent CE treatment (Group B) required fewer embryos to become pregnant than patients who were not diagnosed with CE (Group A) (1.23 for Group B vs 1.58 for Group A; p = n.s.) Here, it could be assumed that cases with four PCs or fewer could also impact embryo implantation [32,33]. Therefore, some cases of clinically relevant CE may not have been recorded. However, the study design was not sufficient to support this assumption.

As all patients with suspected CE underwent antibiotic treatment, it was not possible to compare these patients with patients who did not receive therapy, in contrast to the findings of Kato et al. and Gu et al. [35]. Meta-analyses comparing groups with CE without subsequent antibiotic treatment showed no difference in subsequent pregnancy rates, but were markedly heterogeneous and difficult to compare ($I^2 \leq$ 77 %) due to the inclusion criteria. For example, patients with only one ET or who underwent repeated miscarriage without assisted reproductive technology (ART) were included in these studies, which limited the significance of the control group [43].

Conclusion

In this retrospective case–control study, patients with RIF frequently (40 %) exhibited histological signs (at least five PCs per 10 HPFs in ES) of CE. The findings indicate that EB for detecting CE should be performed in the late follicular phase or after day 8 of an ovulatory cycle if a cut-off value of at least five PCs is used. Antibiotic treatment with doxycycline led to a reduction in PCs in over 80 % of cases. Persistent findings can be treated successfully with ciprofloxacin and metronidazole. After treatment of CE, the outcomes of patients were found to be comparable with those without CE. Therefore, an investigation to exclude CE with PCs in patients with RIF seems advisable before performing further ART.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Approval was granted by the Ethics Committee of the State Medical Association from Hessen (File No. 1497-evBO).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

CRediT authorship contribution statement

Michael Amrani: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Christoph Renné:** Resources, Investigation. **Viktoria Blaschke:** Resources, Investigation, Data curation. **Esther Schlautmann:** Resources, Investigation, Data curation. **Michael Schaffrath:** Project administration, Investigation.

Bartosz Linek: Project administration, Investigation. **Christine Skala:** Supervision, Project administration, Investigation, Formal analysis. **Markus Schepers:** Writing – review & editing, Validation, Supervision, Project administration. **Walburgis Brenner:** Writing – review & editing, Validation, Supervision, Formal analysis, Data curation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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