Thesis

Chronic endometritis and endometrial microbiome in infertility patients

A literature review and retrospective data analysis

submitted by

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under the supervision of

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Graz, 02.04.2024

Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 02.04.2024 Christina Raid, m.p.

1 Acknowledgements

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Last but not least, I would like to thank Paul for always believing in me and encouraging me to leave my comfort zone and outgrow my limits.

2 Zusammenfassung

Hintergrund: Chronische Endometritis (CE) ist eine persistierende Entzündung der Gebärmutterschleimhaut, welche sich hauptsächlich durch eine Plasmazellinfiltration des endometrialen Stromas kennzeichnet. Im Rahmen der Kinderwunschbehandlung gilt das Vorliegen einer chronischen Endometritis als Risikofaktor für gehäufte Fehlgeburten und Implantationsversagen. In den letzten Jahren konnten Forscher*innen den Einfluss des uterinen Mikrobioms auf Fruchtbarkeit und diverse Erkrankungen des Uterus aufzeigen. Mögliche Zusammenhänge zwischen einer Dysbalance des Mikrobioms und chronischer Endometritis werden stark vermutet.

Ziele dieser Arbeit: Ziel dieser Arbeit ist einerseits eine Zusammenfassung der aktuellen Literatur zum Thema chronische Endometritis und endometriales Mikrobiom zu erstellen. Andererseits soll ein Überblick über gesammelte Daten der Kinderwunschpatientinnen, die sich in Abklärung an der Abteilung für Geburtshilfe der Medizinischen Universität Graz befanden, gegeben werden. Somit können mögliche Zusammenhänge zwischen Mikrobiom, chronischer Endometritis und unerfülltem Kinderwunsch dargestellt werden.

Material und Methoden: Es wurde eine narrative Zusammenfassung der aktuellen Literatur zum Thema chronische Endometritis und des Einflusses des Mikrobioms auf das Endometrium erstellt. Dafür wurde die medizinische Datenbank PubMed hauptsächlich nach systematischen Übersichtsarbeiten und randomisierten kontrollierten Studien, welche von 2018 bis 2023 publiziert wurden, durchsucht. Im Rahmen der retrospektiven Studie wurden Daten zu Frauen, die eine Diagnostik bezüglich chronischer Endometritis erhielten, erfasst. Mit Hilfe des Krankenhausinformationssystems "openMEDOCS" konnten weitere klinische Informationen über die Patientinnen eingeholt werden. Um die gesammelten Daten statistisch auszuwerten und einen Überblick zu schaffen wurde das Programm Excel von Microsoft verwendet.

Ergebnisse: 99 Frauen im Alter von 18 bis 45 Jahren wurden in diese Studie aufgenommen. Bei 19 dieser Patientinnen (19,2 %) wurde eine chronische Endometritis diagnostiziert. Im Kollektiv der Frauen mit diagnostizierter chronischer Endometritis, hatten 13 (68%) bereits

ein Abortgeschehen oder wiederholte Implantationsversagen in der Vorgeschichte. Weiters stellte sich heraus, dass 80% der CE-Fälle, bei welchen einen Mikrobiomanalyse durchgeführt wurde, ein nicht-*Lactobacillus*-dominantes Mikrobiom vorweisen. Bei sechs von sieben Frauen konnte die chronische Endometritis durch Antibiotikagabe geheilt werden. Zwei Frauen wurden darauffolgend schwanger.

Schlussfolgerung: In dieser Studie wurde die Auswirkung von chronischer Endometritis auf Spontanaborte und RIF hervorgehoben und der effektive Nutzen einer Antibiotikabehandlung aufgezeigt. Das Mikrobiom der CE-Patientinnen stellte sich als divers und nicht-*Lactobacillus*-dominant (<90%) heraus. Dies könnte mit niedrigeren Schwangerschaftserfolgen in Zusammenhang stehen.

3 Abstract

Background: Chronic endometritis (CE) is a persistent inflammation of the endometrium, mainly characterized by the infiltration of plasma cells into the endometrial stroma. In context with infertility issues CE is known to be a risk factor for recurrent implantation failure and repeated abortions. The uterine microbiome has become increasingly relevant in the past years since researchers have reported its influence on infertility and uterine-related diseases. An association between a disbalance of the endometrial microbiome and chronic endometritis is strongly assumed.

Objective: The goal of this thesis is to summarize current literature on the topic chronic endometritis and uterine microbiome and to give an overview of collected data on infertility patients treated at the Department of Obstetrics of the Medical University of Graz to demonstrate possible connections between chronic endometritis, the endometrial microbiota and infertility issues.

Material and methods: A narrative summary of the recent literature on the topic chronic endometritis and the influence of the uterine microbiome was conducted. The medical data bank PubMed was mainly searched for systematic reviews and randomized controlled trials published from 2018 to 2023. In the context of the retrospective study data on women, which underwent diagnostic procedures to identify chronic endometritis was collected. Using the hospital information system 'openMEDOCS' further clinical information was obtained. Microsoft Excel was used to statistically evaluate and give an overview of all the collected data.

Results: 99 women were included in this study. 19 patients (19,2%) were diagnosed with chronic endometritis. 13 women (68%) out of the CE patients had already experienced abortion or RIF. Furthermore 80% of CE cases, which were analyzed by molecular techniques, had a non-*Lactobacillus*-dominated (<90%) endometrial microbiome. Six out of seven women were cured after antibiotic treatment (85,7%) and two patients subsequently achieved a spontaneous conceptus.

Conclusion: This study highlighted the impact of chronic endometritis on spontaneous aborts and RIF and demonstrated the effects of antibiotic treatment, which seemingly

improves reproductive outcomes. Concerning the microbial composition within CE patients, a more diverse and non-*Lactobacillus* dominant microbiome is to be found. This decrease in dominance of *Lactobacillus* spp. may be linked to poor reproductive outcomes.

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4 Index of Abbreviations

ART- assisted reproductive techniques

AUB- abnormal uterine bleeding

CE- chronic endometritis

DNA- deoxyribonucleic acid

e.g.- for example

ESPC- endometrial stromal plasma cell

EW- endometrial waves

H&E- hematoxylin and eosin

HPF- high -power field

Ig- immunoglobulin

IHC- immunohistochemistry

IL-1β- Interleukin1β

IL-6- Interleukin-6

IM- intramuscular

IUD- intrauterine device

IVF- in vitro fertilization

LBR- live birth rate

mi-RNA- microRNA

mRNA- messengerRNA

mTOR - mammalian target of rapamycin

NK cells- natural killer cells

NLR- NOD-like receptor

PAMP- pathogen-associated molecular pattern

PCOS- polycystic ovary syndrome

PID- pelvic inflammatory disease

PO- per os (through oral route)

PR- pregnancy rate

PRP- platelet-rich plasma

PRR- pattern recognition receptor

RCT- randomized controlled trials

RIF- recurrent implantation failure

RM- recurrent miscarriage

RNA- ribonucleic acid

rRNA- ribosomal ribonucleic acid

RPL- recurrent pregnancy loss

RT- PCR - reverse transcription polymerase chain reaction

STD- sexually transmitted disease

TLR- toll-like receptor

TNF-α- tumor necrosis factor-α

UGT- upper genital tract

uNK cells- uterine natural killer cells

WOI- window of Implantation

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7 Introduction

7.1 Endometrium and fertility

The key to successful implantation and placentation of a fertilized oocyte is a healthy endometrium. Therefore, the endometrium undergoes different phases depending on hormonal influences including progesterone and estrogen along with the influence of immune cells and their products. The transformation of the endometrium starts in the mid-to-late luteal phase when a spontaneous decidualization takes place. Whether there is a conceptus present or not is not important for this process. To achieve a receptive environment, the cytoskeleton of the endometrium needs to remodel in order to allow stromal fibroblasts to become bigger and rounder. Around day 19 to 24 of the menstrual cycle, about six to ten days after the ovulation has taken place, the decidualization is complete and the endometrium is receptive for implantation. This time frame of about two to four days is called ‘the window of implantation’ (WOI). To ensure a successful attachment of the blastocyst onto the luminal epithelium there has to be a way of communication between them. There are different adhesion molecules, like integrins, L-selectin ligands and oligosaccharides involved in this interaction. If the selection, apposition and attachment as shown in Figure 1 have been correctly performed, the implantation can take place. Invasive growth of the blastocyst and differentiated trophoblastic cells will start the placenta formation. Inadequacies in placental vascularization that occur at this stage are irreparable and can be the cause of conditions like pre-eclampsia and intrauterine growth restriction. Important mechanisms that contribute to the correct migration of the trophoblast as well as physiological vascular remodeling may be controlled by the endometrium. Therefore, a successful placentation and subsequently a healthy pregnancy are rooted in the endometrium (1).

To fully understand how the endometrium can affect fertility it is necessary to take a closer look at the microbial structure and the physiological changes it goes through during a menstrual cycle.

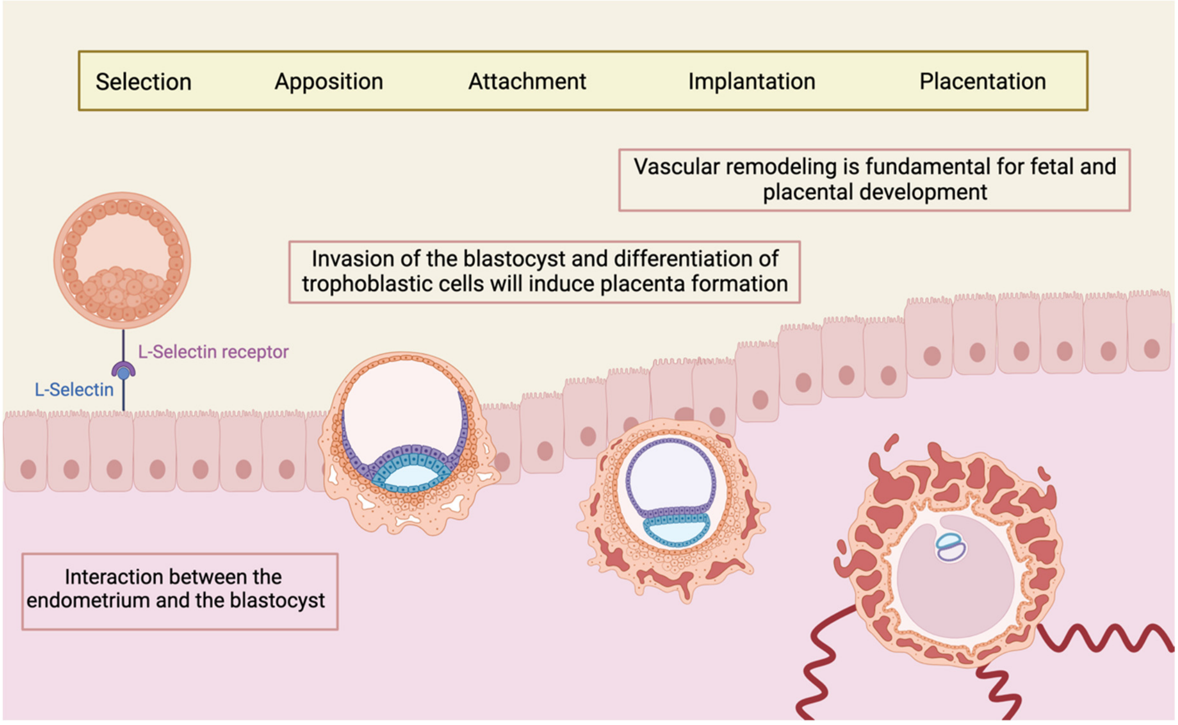


Figure 1- Blastocyst and endometrium interaction, modified by (1)

7.2 Microbiome in the female reproductive tract

Vaginal microbiota has long been the focus of research on the female reproductive system whereas colonization of microorganisms in the upper genital tract (UGT) was mostly linked to infectious diseases. Despite the common perception that the uterine cavity is sterile, endometrial samples, obtained within hysterectomies, showed that there is bacterial colonization and a specific endometrial microbiome without any connection to infectious events (2). To explain the existence of microorganism in the UGT, the most reasonable hypothesis states an ascension of bacteria from the vagina. This assumption has been supported by the discovery of *G. vaginalis* in polymicrobial biofilm on the endometrium and fallopian tubes of women who suffer from bacterial vaginosis (3). Nonetheless the hematogenous dissemination of oral, gastrointestinal or respiratory microorganisms has also been demonstrated by previous research (4). Similarities between oral and placental microbiomes in pregnant women and an increased number of cervical *Lactobacillus iners* within obese reproductive-age women with dysbiosis of the digestive system are supporting this theory (5,6).

To understand the UGT microbiota even better, the development of very sensitive molecular tools, particularly next-generation sequencing, has made a huge impact. It was shown that from the vagina to the ovaries, the microbial composition changes. Starting with 99,97% of *Lactobacillus* as the dominating species in the vaginal microbiome, the endometrial microbiome consists of a variety of bacteria, such as *Lactobacillus, Pseudomonas, Acinetobacter, Vagococcus* and many more as shown Figure 2. A clear decrease of the *Lactobacillus* population along the female reproductive tract is visible (7).

Summarizing the results of different studies, the vaginal and endometrial microbiome are similar but they do differ. Either in the prevailing bacterial taxa or in the relative abundance in which they are present (2). Furthermore, the uterine microbiome is exposed to the influence of the menstrual cycle, which also affects the variety of bacteria.

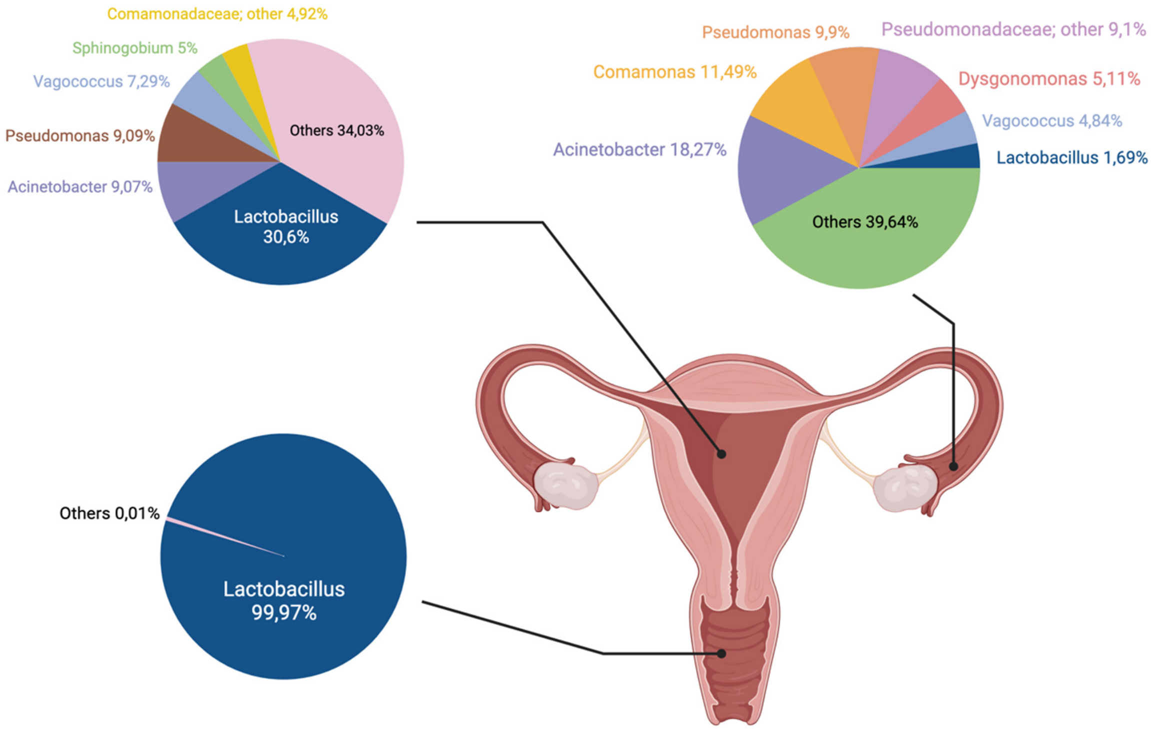


Figure 2- Microbiome in the female reproductive tract- decrease of *Lactobacillus in the UGT, modified by(7)*

7.3 Uterine microbiome during the menstrual cycle

To demonstrate the changes the uterine microbiome undergoes in a menstrual cycle and prove possible intra-cycle variations, that occur in patients with recurrent implantation failure (RIF) or recurrent miscarriage (RM), Vomstein et al. examined samples taken within uterine flushing during follicular, ovulatory and luteal phase. The prospective study included 20 healthy control patients, 20 RIF- and 20 RM- patients. The study showed that the control group experienced a significant decrease in the variety of bacterial species around the ovulation and during the luteal phase, implying a more consistent dispersion of the microbiota. In contrast to that, RF- and RM- patients did not display a loss of diversity during the menstrual cycle. It even appeared that the two groups have a higher degree of taxonomic similarity. Concluding, an increase in variety could be the result or cause of a micro-environment that is more prone to pregnancy failures (8).

7.4 Chronic endometritis

During the menstrual cycle the endometrium undergoes a physiological infiltration of immunocompetent cells. Macrophages, natural killer cells and T lymphocyte subsets invade the endometrium and fluctuate in their density and variety depending on the menstrual phase. These periodical variations in leukocyte subpopulations are believed to contribute to the tissue remodeling which is needed to achieve endometrial receptivity and therefore a successful implantation of the blastocyst (9). Endometritis is described as an infection or inflammation of the endometrium and is histopathologically classified into acute and chronic endometritis. Histologically, acute endometritis presents itself with micro- abscesses and an invasion of neutrophils in the superficial layers of the endometrium and clinically with acute symptoms. Chronic endometritis is a silent condition which is often discovered while examining patients with secondary amenorrhea or infertility issues. Both conditions have been linked to poor reproductive outcomes, whereas chronic endometritis has a more negative effect (10).

7.4.1 Definition and histopathology of chronic endometritis

Chronic endometritis, a continuous inflammation of the endometrium, is mainly characterized by the infiltration of plasma cells (endometrial stromal plasma cells- ESPCs) into the endometrial stroma. As mentioned before, chronic endometritis is a silent disease with no or poor symptomatology such as spotting, pelvic discomfort and leucorrhoea. Hypomenorrhoea, secondary amenorrhoea and infertility can be other possible concerns of the patient (10). As mentioned by Kushnir et al., 45% of infertile patients, particularly those suffering from RIF, were diagnosed with chronic endometritis (6). Depending on the individual patient profile and biopsy method, the prevalence of CE varies from 0.2% to 46%. Due to the difficulties in the diagnostic process, the prevalence of CE is often underestimated. At present, there are no universally standardized criteria or diagnostic guidelines for this condition (10). According to Kitaya et al., 2011&2018, the consensus among specialists implies, that the presence of multiple ESPCs is the most specific and sensitive indicator (9,11). Besides the invasion of ESPCs, histopathological characteristics of CE are a superficial endometrial lining oedema, a significantly increased amount of stromal cell density and a dissociated development of the stroma and epithelium (10).

7.4.2 Risk factors

There are several different risk factors that are linked to CE. An already long known fact is that the insertion of an intrauterine device (IUD) can cause CE. This seems to be independent from short- or long term-use, the inflammatory response seems to be the same and can remain even after IUD removal (12). Multiparity, atypical uterine bleeding and other gynecological symptoms, as well as characteristics regarding obstetric history, such as cesarean section, abortion, miscarriages, preterm delivery, ectopic pregnancy, and molar pregnancy are reported risk factors for CE (11). In addition to that, gynecological conditions such as bacterial vaginosis, endometrial polyps and endometriosis are associated with CE (13–15).

7.4.3 Etiology

The idea that the uterine cavity was sterile under physiological conditions has been generally believed for almost 100 years. This theory is based on the concept that the cervical mucosal system is an impermeable barrier to bacterial ascent from the vagina. But since the existence of microorganisms in the uterine cavity has been proven, these bacteria are believed to be the main cause of chronic endometritis (16). This is supported by the fact that antibiotic treatment is an effective therapy for patients with CE (17). Additionally, it has been shown that the uterine mucus plug is not a complete block for bacterial ascension and the uterine peristaltic pump enables the translocation of particles within minutes from the vagina to the uterus (18,19). This explains why acute endometritis and PID (pelvic inflammatory disease) are caused by microorganisms, such as Chlamydia trachomatis and Neisseria gonorrhoeae, ascending from the vagina into the UGT (20). Researchers thought that this would also be the case in CE, however it was reported that different microorganisms, for example Streptococcus spp., Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Staphylococcus spp., were predominant within CE patients (21). Thus, the question where these bacteria come from arises. The existence of a uterine microbiome has also been documented within animal models. Specific bacterial species, like Fusobacterium, colonizes cow and mouse uteri and have been reported to spread through the hematogenous route (bloodstream) (22,23). Epithelial barrier breach (for example gingivitis and leaky barrier) enhances hematogenous transmission of oral or gut microorganisms. Allowing the invasion of local, mucosal bacteria of the gastrointestinal tract and the oral cavity into distal mucosal sites (24). Additionally, microbiota in the peritoneum were reported by recent studies (7). It is probable that the fallopian tube serves as a pathway for peritoneal microorganisms to reach the uterus. But there is further research required to determine the source and progression of colonized bacteria that cause CE (24). Another hypothesis that tried to enlighten the etiology of CE, stated that CE could be an autoimmune condition. Because CE is defined by plasma cell infiltrates, which are present in almost all organ autoimmune responses, this could be a possible explanation. However, subsequent studies by Kushnir et al. demonstrated that CE does not have a significant autoimmune component. Notwithstanding the fact that these outcomes need further investigation, for now, the concept of CE as an autoimmune-driven condition cannot be endured (25). Concluding that the main immune trigger most likely happens to be infection (26).

7.4.4 Pathophysiology

As mentioned above, inflammation seems to be the main activator for chronic endometritis. This endometrial inflammation causes a modified release of cytokines and chemokines, which in turn effect leukocyte population recruitment. These alterations affect the uterine contractility, endometrial function in decidualization, receptivity and vascularization (26).

7.4.4.1 Cellular modification

As previously described, the endometrium experiences a physiological inflammation process throughout the menstrual cycle. The invasion of immune cells varies depending on the menstrual phase (27). However, there are major variations concerning the leukocyte quantity, arrangement and distribution in patients with CE. Especially B lymphocytes, which normally constitute <1% of the leukocyte population, increase their quantity and invade the basal layer and even the functional layer of the endometrium. Additionally, B lymphocytes infiltrate into the lumen of glands by passing through the glandular epithelium. Kitaya and Yasuo demonstrated that this abnormal extravasation of B cells in CE, is associated with the modified expression of adhesion molecule E-selectin and chemokines CXCL1 and CXCL13 (28). Another subset of leukocytes, the uNK (uterine natural killer) cells, are believed to contribute to proper decidualization by supporting adequate vascular remodeling. Moreover, these cells are most likely involved in immunomodulation. In CE endometrium, a decrease in uNK cells was demonstrated. The percentage of $CD56^{+}/CD16^{-}$ and of $CD56^{bright}/CD16^{-}$ natural killer (NK) cells happens to be lower compared to patients without CE, while the percentage of $CD3^{+}$ cells was higher. Within the T-cell population there is a significant increase in CD4 cells in CE endometrium, whereas CD8 cells remain the same as in patients without CE. This could probably have a negative effect on implantation (29).

7.4.4.2 Biochemical changes

In menstrual effluents of CE patients proinflammatory cytokines such as IL-6, IL-1β (Interleukin-6, Interleukin-1β), and TNF-α (tumor necrosis factor-α) are significantly increased(30). TNF-α exposure is known to promote the production of local estrogen in endometrial glandular cells. This is most likely associated with the development of micropolyps and polypoid endometrium (31). ESPC, which are a histological characteristic for CE, produce a quantity of different immunoglobulin (Ig) subclasses, especially IgG2. These high levels of mucosal antibodies may impair the implantation process(32). Furthermore, women with CE have altered expression in different genes such as inflammatory response (CCL4, IL11), proliferation (IGF1, IGFBP1) and apoptosis (BCL2, BAX, CASP8). A study showed that within women with chronic endometritis IL11, CCL4, IGF1 and CASP8 were downregulated, IGFBP1, BCL2 and BAX were upregulated. This could affect reduced endometrial receptivity and endometrial hyperplastic lesions (33).
Additionally, there is growing interest in the significance of mi-RNA (microRNA) control in CE. MiRNAs are signaling proteins, transferred by exosomes, which have the potential to modulate the expression of mRNAs (messenger RNA). Exosomes operate as intercellular communication media and are secreted by the endometrial epithelium and released into the uterine cavity. A recent study analyzed mi-RNA compositions of exosomes in cows and stated that the expression of miRNAs was significantly different in cows with CE (34). Finally, it is believed that within CE the process of autophagy, especially concerning microtubule-associated protein 1A/1B-light chain 3 (LC3-II protein) and the cellular metabolism regulator mTOR (mammalian target of rapamycin), is altered and therefore can affect endometrial cell commitment and endometrial decidualization (26).

7.4.4.3 Altered uterine contractility

During the menstrual cycle the uterus shows different contractility patterns. Uterine motility is described by endometrial waves (EW), which originate in the myometrium and are most likely influenced by ovarian steroid levels. In a study by Pinto et al., CE patients showed altered patterns of EW, in both the periovulatory and midluteal phases. This abnormality in contractility may impair the implantation process and explain symptoms, typically for CE, such as pain, abnormal uterine bleeding (AUB) and infertility (35).

7.4.5 Diagnostics

As described earlier, most women with chronic endometritis have little to no symptoms. Moreover, the absence of specific ultrasound markers makes it impossible to detect CE within an ultrasound examination. That is why CE sometimes gets neglected within gynecological examinations and is often diagnosed by coincidence when taking a closer look to better understand infertility issues, abnormal uterine bleeding or chronic pelvic pain (26). The classic diagnostic techniques that are used to identify CE are hysteroscopy, microbial culture and the histopathological analysis of endometrial samples. Furthermore, molecular microbiology technology seems to be a new diagnostic tool, which helps to understand the endometrial microbiome and its influence on CE even further (36).

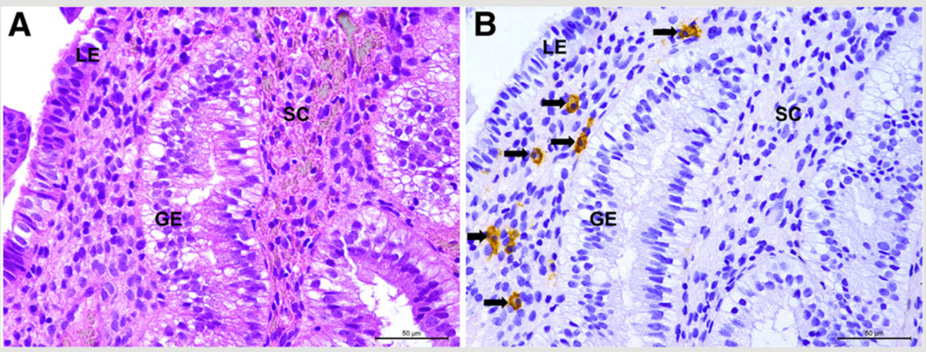
7.4.5.1 Hysteroscopy

This diagnostic modality allows the investigator to visualize certain endometrial changes such as polyps, focal hyperemia, endometrial strawberry aspect and stromal edema (37). These modifications can suggest chronic endometritis, but to rely on the accuracy of hysteroscopic findings, a subsequent endometrial biopsy and histological verification of the sample are the most common procedures. To only use hysteroscopy without taking a biopsy to confirm CE is still up for debate (36,38).

7.4.5.2 Histopathology

Speaking of examining endometrial samples, the universally accepted gold standard to diagnose CE is the histopathological detection of plasma cells in endometrial stroma.
To identify plasma cells, the endometrium sample is either stained with hematoxylin and eosin (H&E), which can be difficult, subjective and time-consuming- or the more reliable method of immunohistochemistry (IHC) is used. IHC makes it possible to detect the plasma cell marker syndecan-1 (CD138), which is a transmembrane heparan sulfate proteoglycan and plays a role in cell-cell and cell-matrix adhesion. Its expression is commonly perceived on mature epithelial cells and the cell membrane of plasma cells (36). Chronic endometritis is sometimes overlooked by pathologists because the characteristics of plasma cells get masked by stromal cell proliferation or numerous stromal mitoses typically happening in CE.

To solve this problem, IHC CD138 staining has the advantage of not only detecting typical round plasma cells, but also identifying atypical ones (39). Furthermore, this method reduced the number of false-positive outcomes and has proven to be an objective plasma cell identification with increased intra-observer and inter-observer agreement concerning the diagnosis(40). If you compare the two methods, staining with H&E and IHC CD138, the latter shows an increased sensitivity in plasma cell identification. A problem with this diagnostic tool is that researchers now need to determine a certain amount of plasma cells per tissue sample, which is sufficient for the diagnosis of CE. When observing recent publications, a wide range of used histopathological diagnostic criteria is evident (36). To address the issue of redefining CE, McQueen et al. compared the prevalence of CE among women with RPL (recurrent pregnancy loss) and the control group, applying different histopathological definitions. The authors concluded that the highest diagnostic sensitivity and specificity when defining CE happens to be at the presence of one or more plasma cells per 10 HPFs (high-power field) in the setting of endometrial stromal changes (41). In contrary to that Liu et al. stated that it may lead to an overdiagnosis of CE, when rating the presence of one plasma cells as pathological. It was proven that plasma cells can physiologically occur in samples of healthy women without association to upper genital tract inflammation. Therefore Liu et al. approached different references ranges, like three plasma cells per section or 2 plasma cells per ten randomly chosen HPFs to prevent an overestimated prevalence of CE (42,43). The result of a survey, that assessed the applied diagnostic criteria of pathologists in clinical practice, represents that there is no worldwide consensus concerning the histopathological definition of CE (44). Therefore, and because it plays an important role as a verification method for new diagnostic methods, it would be important to clarify the criteria (36).



Expression of plasma cells using (**A**) hematoxylin and eosin staining and (**B**) immunohistochemistry staining for syndecan-1 (CD138) in the same field of endometrial tissue from the same woman. Plasma cells are indicated by *arrows*. GE = glandular epithelium; LE = luminal epithelium; SC = stromal cell. Magnification = ×400; scale bar = 50 μm.

Figure 3- HE and IHC CD138 staining in CE patient, with permission to reprint of Tin-Chiu Li, M.D. (42)

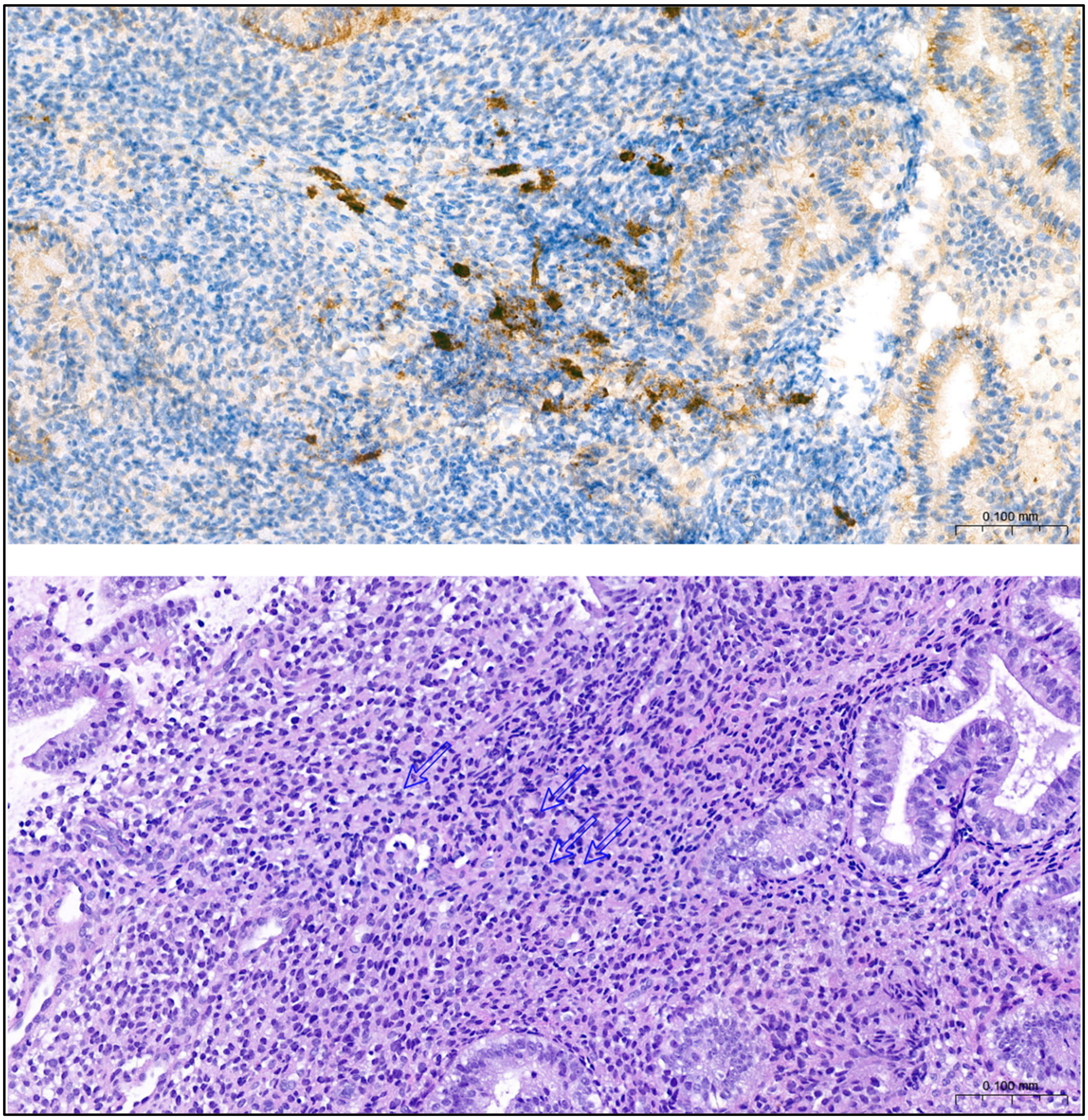


Figure 4- Comparison of positive CD138 staining and HE staining in a suspected chronic endometritis sample. Blue arrows within the HE staining pointing towards potential plasma cells. 400x magnification (40- lens)

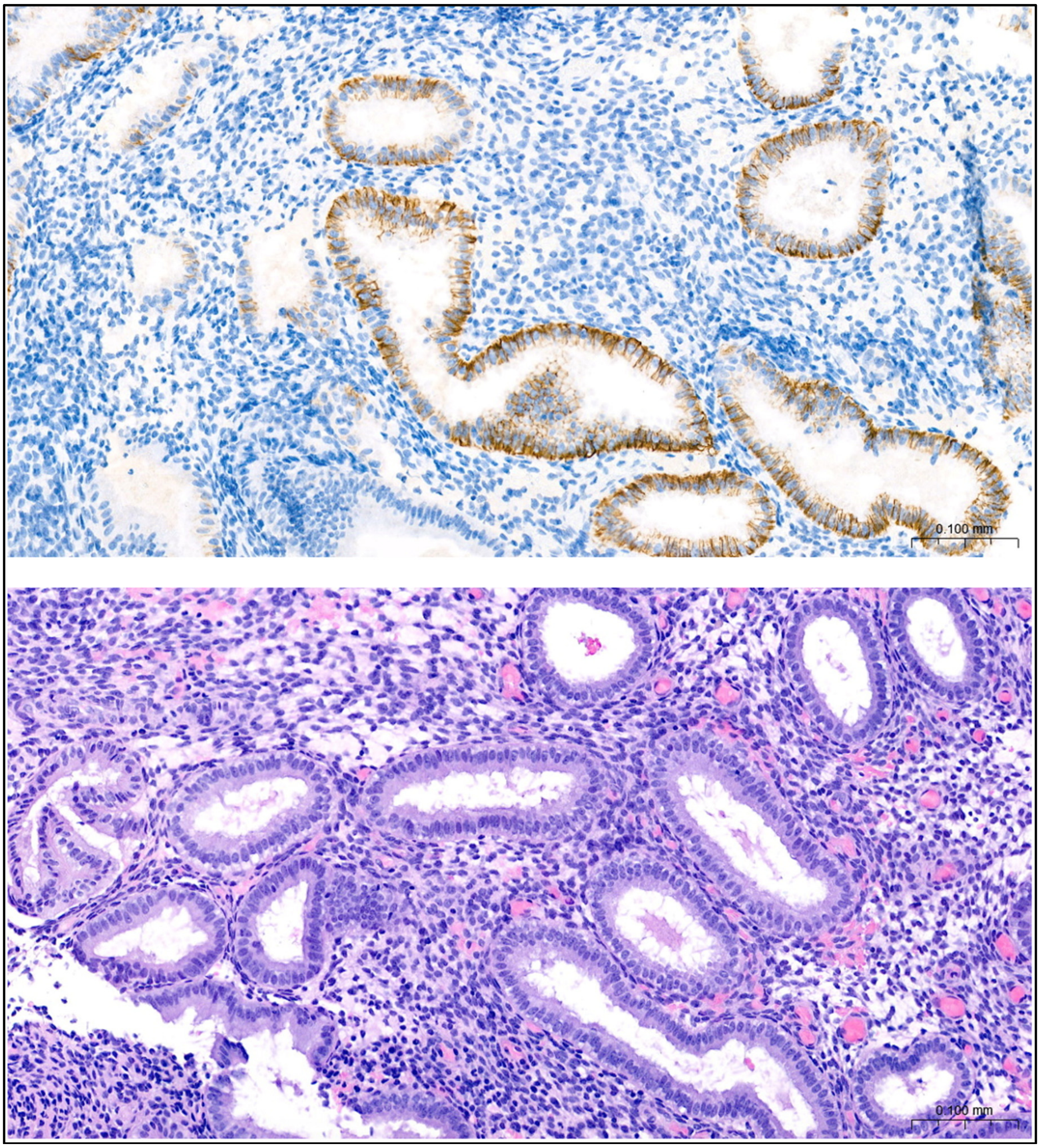


Figure 5- Negative CD138 staining and HE staining in non- chronic endometritis

The images were kindly provided by Ao. Univ.-Prof. Dr. med. univ. Peter Regitnig (Diagnostics & Research Institute for Pathology of the Medical University Graz).

7.4.5.3 Microbial culture

One of the most valuable tools within the diagnostic process of chronic endometritis is bacterial culture of the endometrial tissue. With this diagnostic technique an objective analysis of pathogens and a targeted antibiogram-guided treatment is possible (36). Microbial culture mainly detected the common bacteria *Streptococcus species, Escherichia coli, Enterococcus faecalis and Staphylococcus species, Mycoplasma/Ureaplasma species, Proteus species, Pseudomonas aeruginosa, Klebsiella pneumoniae, Gardnerella vaginalis, Corynebacterium species* and yeast within diagnosed CE patients. A significant disadvantage of this diagnostic method is that not all bacteria can be cultured under typical laboratory conditions (21). In cases of histopathologically verified CE, the reported rate of positive microbial culture ranges from 52-73% (21,45). It is a scientifically proven fact that under physiological conditions the uterine cavity is not sterile. Therefore, a negative bacterial culture is most likely traced back to method limitations (36).

7.4.5.4 Molecular techniques

Quantitative polymerase chain reaction and next-generation sequencing of the 16S RNA (ribonucleic acid) bacteria are molecular techniques which can identify low biomass uterine microbiota. This gained information about the endometrial microbiome made it possible to focus more on microbial and immune-cross talk as a modern concept behind the pathophysiology of CE and its influence on endometrial receptivity and fertility (36). A prospective pilot study by Moreno et al., which classified uterine microbiota into *Lactobacillus*-dominated (>90% *Lactobacillus spp.*) and non-*Lactobacillus*-dominated (<90% *Lactobacillus spp.* with >10% other bacteria), demonstrated that reproductive outcomes of patients undergoing IVF (in vitro fertilization) procedures were notably worse within individuals with a non-*Lactobacillus*-dominated endometrial microbiome (46). Another study by Moreno in collaboration with Cicinelli, stated that RT-PCR (Reverse transcription polymerase chain reaction) in comparison with all three classic diagnostic techniques (hysteroscopy, histology, microbial culture) had a sensitivity of 75%, 100% specificity and 77% accuracy. This revealed a chance to provide new diagnostic tools, while overcoming the bias of the classic methods (45). However, when analyzing low-biomass microbiota contamination must always be taken into consideration. The different handling steps can make it difficult to detect microbes only from the sampling site.

To minimize data misrepresentation a special DNA (deoxyribonucleic acid) isolation kit for low microbial biomass samples should be used (1).

7.4.6 The influence of uterine microbiota on the endometrium

The effect of microorganisms on our overall health is becoming more and more evident. Microbiota influence our physiology, metabolism and immune system by interacting with our cells through the production of bioactive molecules. The regulating function of our immune system has a significant effect on our health and shapes our response to disease. Especially the gut microbiome and its various effects, for example on blood pressure or even motoric skills, have been researched extensively (47–50). But what about the uterine microbiome? The endometrium appears to be an immunologically suited niche for microorganisms, thus these bacteria have an impact on inflammatory and immune response, influencing fertility and pregnancy (47,51). In the following I will describe the different mechanisms in which uterine microorganisms may interact with the endometrium, to better understand their role in CE.

7.4.6.1 Pattern recognition receptors (PRRs)

The innate immune system identifies pathogens through pathogen-associated molecular patterns (PAMPs) with the help of pattern recognition receptors (PRRs). PRRs, such as toll-like receptors (TLRs) or NOD-like receptors (NLRs), are expressed by genital epithelial cells and can recognize many different pathogens and subsequently initiate an inflammatory cascade which should trigger an adaptive immune response (1,52) (Figure 6). For example, to protect the female reproductive tract against sexually transmitted diseases (STDs) PRRs form the first line of defense and start a potent inflammatory response upon receptor recognition. PRRs are believed to be some sort of communication medium between the host and the microbiota (1,53). Furthermore, it is probable that TLRs and NLRs influence the periconceptual regulation system as they play a major part in the cytokine induction cascade (54).

7.4.6.2 Defending pathogens

To protect itself from pathogens, the host maintains the colonization of commensal bacteria. There are different mechanisms that microbiota use to defend themselves and the host against harmful invaders. 'Colonization resistance' is a concept, that describes improved adaptation for the niche by residential microorganisms compared to a pathogenic species (1).

Commensal bacteria compete against invading pathogens by using nutrients of their habitat efficiently. They consume limited resources in their environment, which are needed by competing pathogenic invaders for growth, and therefore cause the starvation of them (55). Moreover, symbionts defend their niche through the production of small molecules with bacteriostatic or bactericidal activity (56) as shown in Figure 6. Additionally, commensals can induce TLR upregulation by continuous receptor stimulation, which is beneficial for first line defense against pathogens (57). To summarize, bacterial colonization within the endometrium may also protect against uterine infections such as CE (1).

7.4.6.3 Epithelial barrier

To provide a safe colonization of bacteria without significant pathogenic barrier breach, the epithelium must remain intact. If that is the case, the epithelium represents a physical barrier and epithelial cells can show a response towards bacterial ligands. However, bacteria can also be beneficial for a healthy barrier development. For example, commensal bacteria in the uterus may aid in the remodeling required for a receptive endometrium. Dysbiosis within the process of remodeling and preparing for the blastocyst reception most likely plays a huge role in RIF (1). The barrier function of the endometrial epithelium, which involves tight junctions, changes over the menstruation cycle. This is beneficial for trophoblastic invasion and implantation (58,59). The barrier changes, which are controlled by nutrients and cytokines, allow a higher permeability for barrier breach by microbiota. This can result in an environment that is pro-inflammatory and therefore stimulates mucosal cells to secrete cytokines which benefit the peri-implantation period (1).

7.4.6.4 Pathophysiological effects

On the other hand, it was proposed that there are also negative effects of uterine microbes on the endometrium and therefore can lead to diseases and infertility. The presence of microorganisms could affect the genomic stability of uterine epithelium by modulation of transcription factors and other genomic and epigenetic alterations. It could also disrupt the integrity of the epithelial barrier and result in a suppression or overgrowth of certain bacteria species, triggered by TLR activation and secretion of various metabolites (16) (Figure 6).

In conclusion, it is important to mention that in order to provide an environment for successful implantation, there has to be the right balance between tolerance and reactivity by the host towards bacteria. It is possible to imagine that the presence of a certain microbial coexistence does not harm the conceptus and may even play an important role in processes during the peri-implantation period and pregnancy. But that is only achieved when there is no pathologic pro-inflammatory response against the uterine microbiome (1,60).

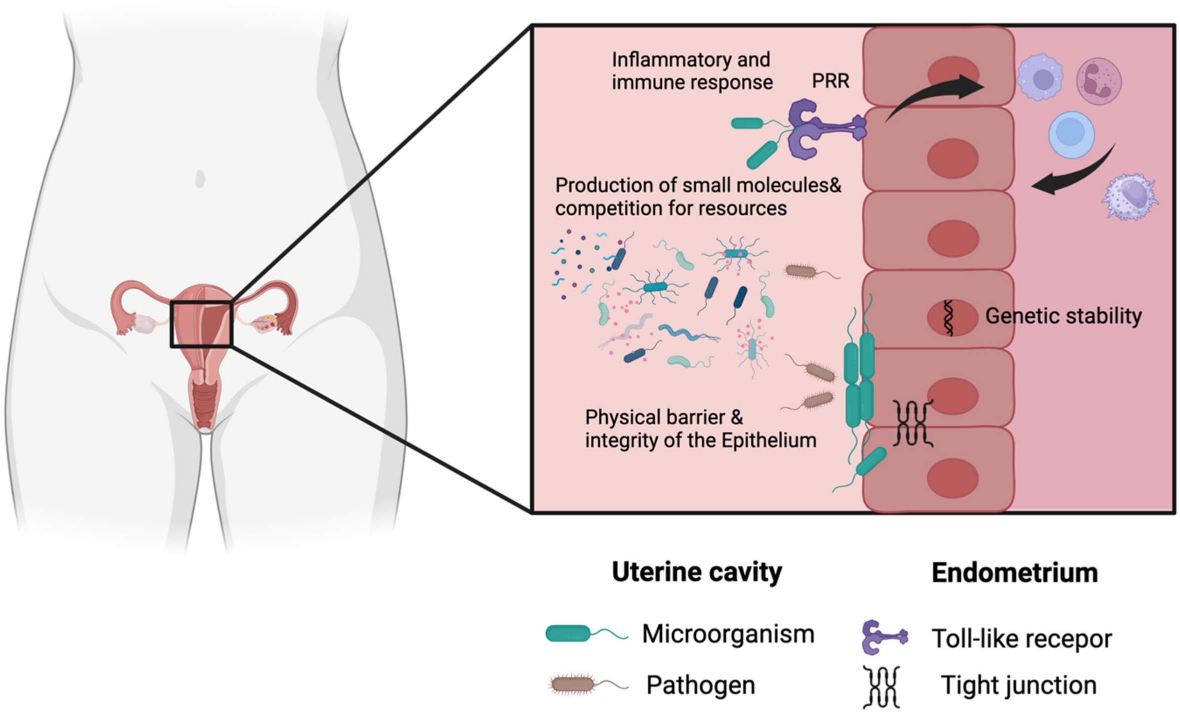


Figure 6- Uterine microbiome and interactions with the endometrium, modified by(47)

7.4.7 Therapy of chronic endometritis and its effect on reproductive outcome

If a CE case is confirmed, there are different oral antibiotics that can be used, depending on microbial culture or gram stain findings. For now, there is no defined antibiotic regimen that must be followed, but various antibiotics and dosages have been tried. As first line therapy doxycycline, 100mg twice per day PO (per os) or 200mg once a day, for 14 days is suggested, as second line therapy ciprofloxacin and metronidazole 500mg PO once a day for 14 days or ofloxacin 400mg and metronidazole 500mg PO once a day for two weeks is recommended (10,61). Cicinelli et. al took a closer look at the microbiologic profiles of infertile patients with chronic endometritis and prescribed specific antibiotics for each individual case. Gram-negative bacteria were treated with ciprofloxacin 500 mg twice a day for 10 days, Gram-positive bacteria with amoxicillin and clavulanate 1g twice a day for 8 days. Josamycin 1g twice a day for 12 days was prescribed when treating Mycoplasma and *U. urealyticum.* In case of negative cultures Ceftriaxone 250 mg IM (intramuscular) in a single dose plus Doxycycline 100 mg PO with Metronidazole 500 mg PO twice a day for two weeks was employed. The success of antibiotic therapy was controlled by subsequent hysteroscopy with biopsy. If signs of CE persisted after the first treatment, the antibiotic regimen was repeated up to three times. In 75% of diagnosed CE patients the therapy was successful and there were no remaining signs of CE, which implies adequate effectiveness of oral antibiotic treatment (17).

7.4.7.1 Improving reproductive outcomes

Untreated CE has been linked to poor obstetric outcomes and decreasing the success rates of both spontaneous conception and IVF cycles. In a study by Cicinelli et. al, it was demonstrated that clinical pregnancy rate (PR) and live birth rate (LBR) were significantly increased in IVF patients that successfully underwent antibiotic treatment, comparing to patients with persisting CE(17). Furthermore, Vitagliano et al. and Cheng et al., both reported that antibiotic therapy may improve IVF outcomes in CE patients, after confirming that CE was cured by a control biopsy before proceeding with IVF (62,63).

7.4.7.2 New treatments

An alternative option to treat CE, particularly in patients who do not respond to antibiotics, are anti-inflammatory drugs and progestins. These therapy options still need more investigation since available data is not sufficient to prove their safety and efficacy yet (64). Intrauterine infusions with autologous platelet-rich plasma (PRP) could also be potential alternative treatments for CE patients. It was reported that they can improve CE, due to their antimicrobial and anti-inflammatory characteristics (65). Furthermore low- intensity intravascular laser irradiation of blood and microbiota regulators such as pro- and pre-biotic administration and microbiota transplants, represent new treatment methods which focus on systematic and local effects and regulate the immunological and inflammatory response (47,64,66).

8 Material and methods

In this thesis a literature review and a retrospective data analysis were combined. The main goal of the retrospective study was to collect data on women, which underwent diagnostic procedures to identify chronic endometritis at the Department of Obstetrics of the Medical University of Graz, in the time frame 01.01.2022 to 01.03.2023. Various clinical information, the histological evaluation as well as the analysis of the microbiome were noted and statistically evaluated. The hypothesis of this study states: Women who experience recurrent implantation failure and repeated abortions are more likely to have chronic endometritis.

8.1 Literature review

For this literature review a narrative summary of the recent literature on the topic chronic endometritis and the influence of the uterine microbiome on the endometrium was compiled. The medical data bank PubMed was mainly searched for systematic reviews and randomized controlled trials (RCT) over the last five years. The searching terms included:

- “Chronic endometritis” AND “microbiome” –13 results
- “Endometrium” AND “Fertility”- 932 results
- “Female reproductive tract” AND “microbiome”- 538 results
- “CD138” AND “CE”- 76 results
- “Chronic endometritis” AND “pathophysiology”- 3 Results
- “Chronic endometritis” AND “therapy”- 13 results
- "Endometritis/classification"[Mesh] OR "Endometritis/physiopathology"[Mesh]- 1 result

Additional filters like article type (reviews, systematic reviews and RCTs) or time frame (not over 5 years) were set.

8.2 Retrospective data analysis

Due to working with sensible data, approval for this retrospective data analysis was obtained from the ethics committee of the Medical University Graz (35-210 ex 22/23).
In the following flow chart, the entire process from the selection of patients, the diagnostic procedures to the collection and analysis of the data is visualized. (Figure 7)

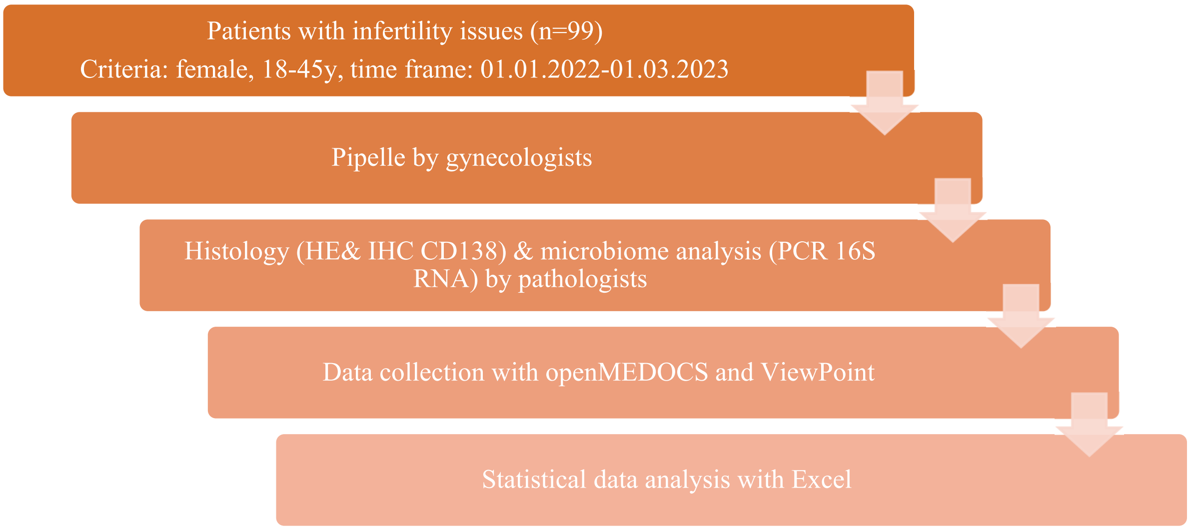


Figure 7- Flow chart- entire process

8.2.1 Patient collective and diagnostics

Patients included in this retrospective data analysis are women with infertility issues, within the minimum age of 18 years to maximum 45 years. The data was collected in the time frame 01.01.2022 to 01.03.2023 when patients underwent diagnostic procedures due to their infertility issues at the Department of Obstetrics of the Medical University of Graz. 99 patients were examined and endometrial biopsy via pipelle was taken. Seven additional control biopsies to ensure successful antibiotic treatment were done. A pipelle is a biopsy device that is inserted into the uterus until it reaches the fundus to retrieve an endometrial sample for histological workup (Figure 8, Figure 9). Additionally, some of the patients also had their bloodwork done to determine the hormone levels of estradiol and progesterone. The endometrial samples were analyzed by pathologists at the Institute of Pathology of the Medical University of Graz. On the one hand, the endometrial tissue was histologically evaluated by immunohistochemistry with plasma cell marker syndecan-1 (CD138) to identify samples with three or more plasma cells.

As of three plasma cells within an endometrial sample, the Institute of Pathology in Graz declares the diagnosis ‘chronic endometritis’. On the other hand, PCR with the variable 16S rRNA (ribosomal ribonucleic acid) gene region was used to investigate the endometrial microbiome. The results were noted in an Excel sheet.

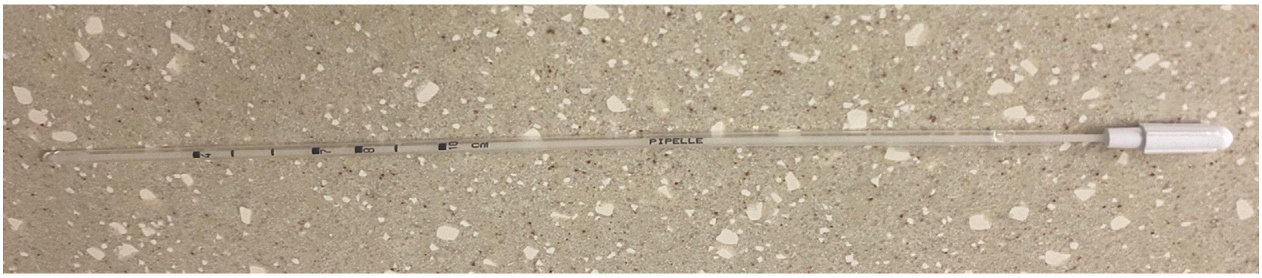


Figure 8- Photo of pipelle

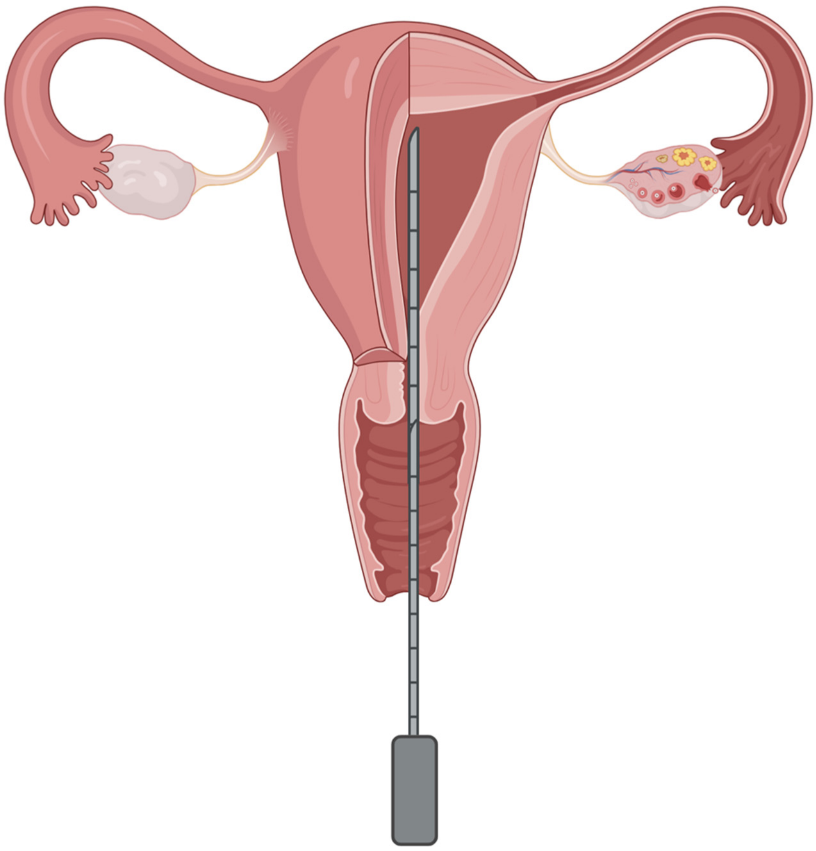


Figure 9- Schematic of inserted pipelle

8.2.2 Collection of data

With the help of the electronic hospital information system 'openMEDOCS' and the GE HealthCare software 'View Point' further information about each individual patient was collected. Existing medical reports were scanned for medical history, menstrual cycle day and laboratory results on the day of the biopsy (estradiol and progesterone), endometrial layer thickness and the presence of a corpus luteum in sonography, diagnosis after fertility workup and subsequent reproductive outcomes. The data was processed using pseudonyms. The information was added to the already existing Excel spread sheet.

8.2.3 Statistical analysis

To statistically evaluate the data the program Microsoft Excel was used. Numeric data were described by arithmetic mean value and standard deviation or by median, first and third quartile, depending on the distribution of the data. Categorical data were presented by absolute and relative quantities and summarized using tables. For graphical depiction pie charts and boxplots were used.

9 Results

9.1 Statistical analysis- descriptive statistics

The collected data were analyzed with the spreadsheet editor program Microsoft Excel. As mentioned before, numeric data were presented by arithmetic mean value and standard deviation or median and first and third quartile, depending on their distribution (normally distributed or not). This was tested by plotting a histogram and visually checking if the data is following a normally distribution curve. Categorical data were described as absolute and relative quantities. Graphs and tables were also created with the program Excel.

9.1.1 Patient collective

99 women (n=99) within the age range of 18 to 45 years were included in this study. From six women a control biopsy was taken to ensure a successful treatment. One woman was examined three times because of too little sample material and persisting CE despite antibiotic therapy. The following analysis includes the 99 women as well as the control biopsies. Consequently, the data of 107 cases were examined.

The mean value concerning the age of the patients is 34,3 ±4,4 years. Within the group of patients diagnosed with CE, the mean value is 34,8 ±4,8 years. 19 out of 99 patients (19,2%) were diagnosed with CE for the first time. 72 patients (72,7%) did not show signs of CE and eight patients (8,1%) were excluded because their samples could not be assessed adequately. Table 1 provides a brief overview of the patient collective with a graphical description following in Figure 10.

Patient collective (n = 99)		
Diagnosis	*Absolute*	*Relative*
CE	19	19,2%
no CE	72	72,7%
not assessable	8	8,1%

Table 1- Absolute and relative quantities of the patient collective

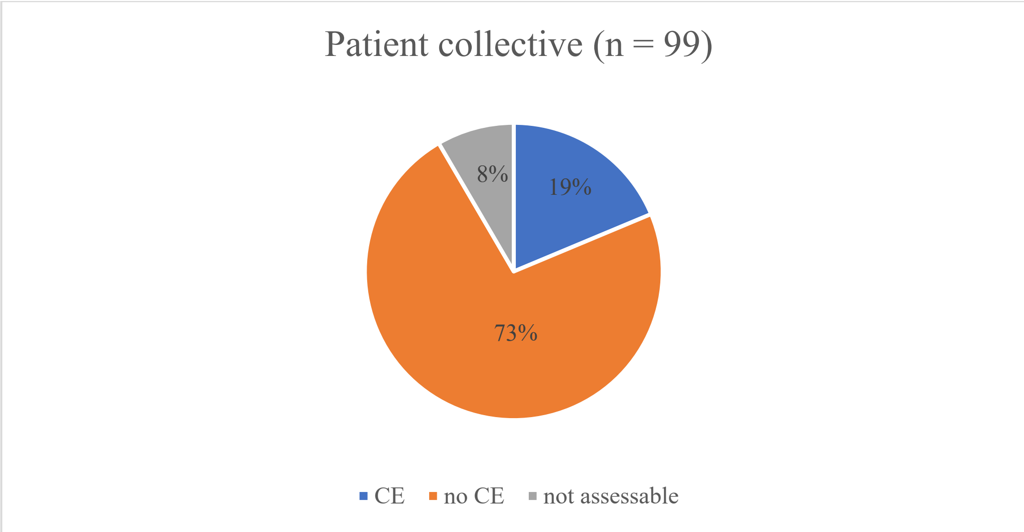


Figure 10- Patient collective overview

9.1.2 Medical indications

Patients were classified according to three different indications: Recurrent implantation failure (RIF), subfertility and spontaneous abortion. The indication 'RIF' includes patients with two or more unsuccessful embryo transfers, indication 'spontaneous abortion' includes women with one or more spontaneous aborts and 'subfertility' including the remaining causes for pregnancy failure. All in all, 99 patients were considered and one patient had no further classification (n=98). The most common indication happened to be spontaneous abortion with 41 patients (42%), secondly subfertility with 30 patients (31%) and the third common was RIF with 27 patients (27%).

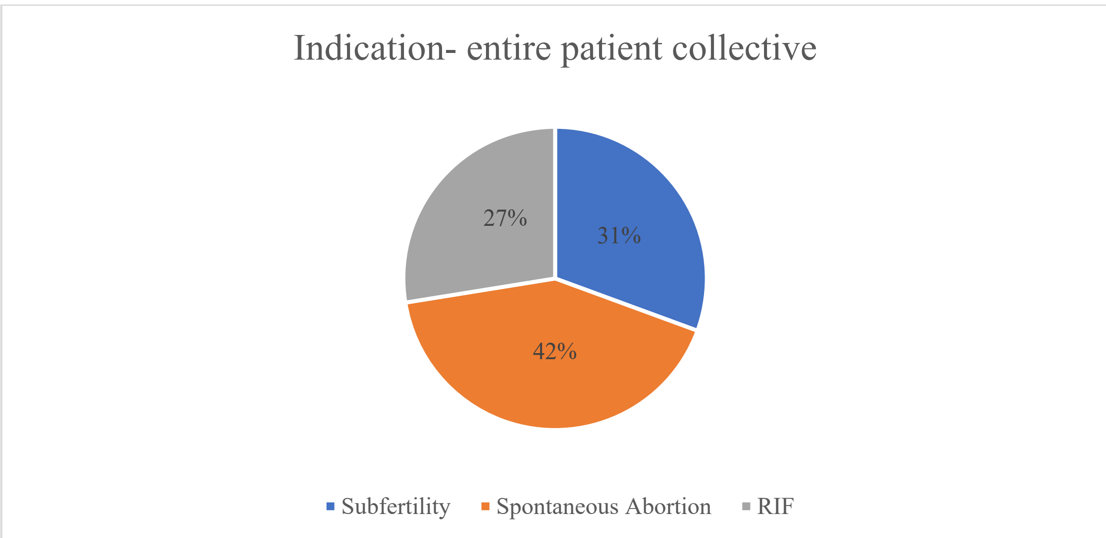


Figure 11- Indication, entire patient collective

9.1.3 CE patients- indication

Within the patients that were diagnosed with CE (n=19), spontaneous abortion was the most frequent indication including nine patients (47%), followed by subfertility with six patients (32%) and RIF with 4 patients (21%).

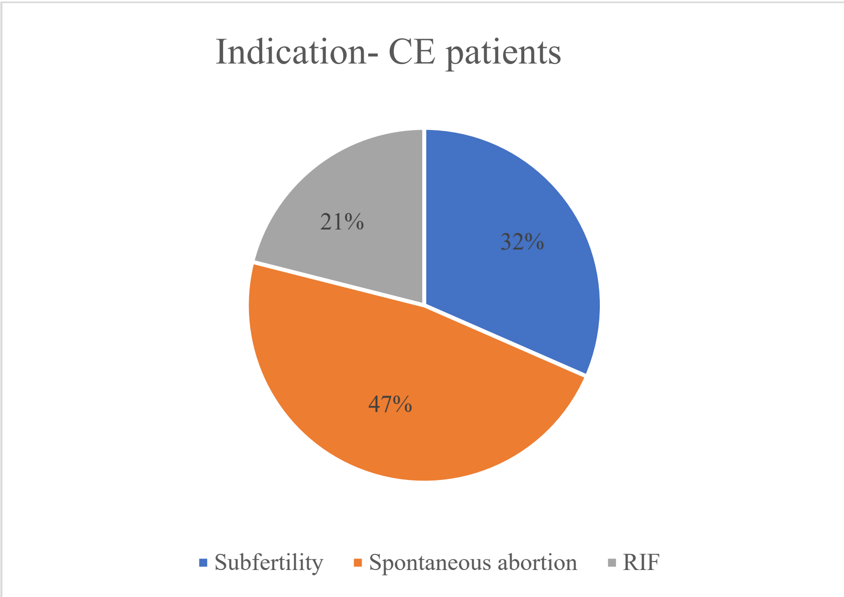


Figure 12- Indication, CE patients

9.1.4 Pregnancies per patient

In the entire collective n= 98 (one patient was not included), the number of pregnancies per patient varied from 0 to 7. The Median amounts to one pregnancy per patient, the first quartile is zero (25% of the patients) and the third quartile two (75%). The minimum and maximum values are zero and five. The numbers six and seven classified as outliers as shown in the boxplot below (Figure 13Figure 13).

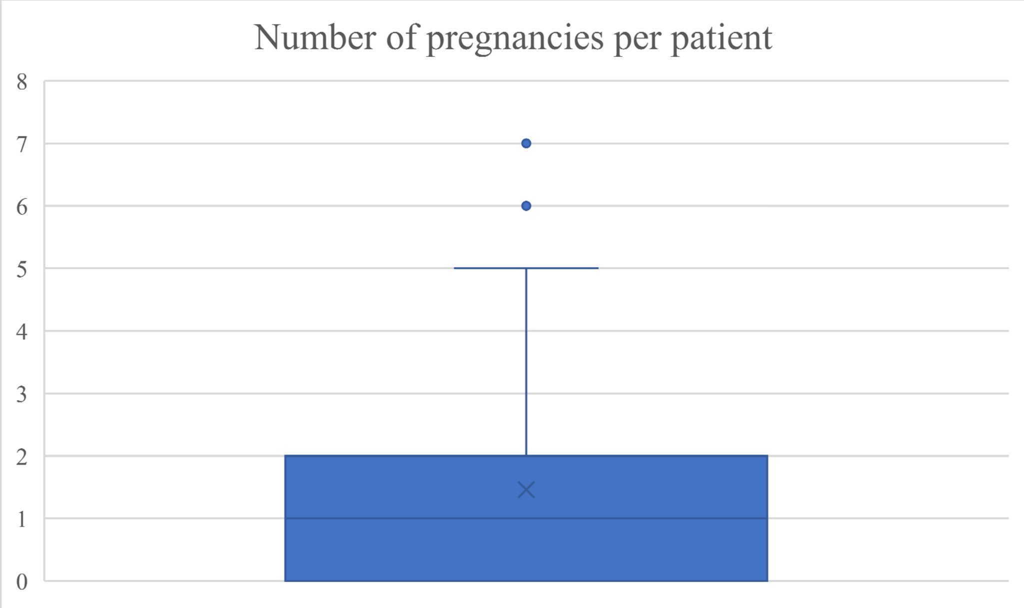


Figure 13- Number of pregnancies per patient

9.1.5 Pregnancies- abortions, births

62 out of 98 patients (one woman was not included because of missing information) were already pregnant once (63,3%). Out of these 62 patients, 55 women (88,7%) had already had at least one abortion.

9.1.5.1 CE patients

In the collective “CE patients” (n=19), 12 women were already pregnant once out of which 10 have already had an abortion (83,3%) in the past.

9.1.5.2 Non-CE patients

In the group classified as “no CE” (n=72), 44 patients had previously been pregnant at least once with 40 of them having experienced at least one abortion (91%).

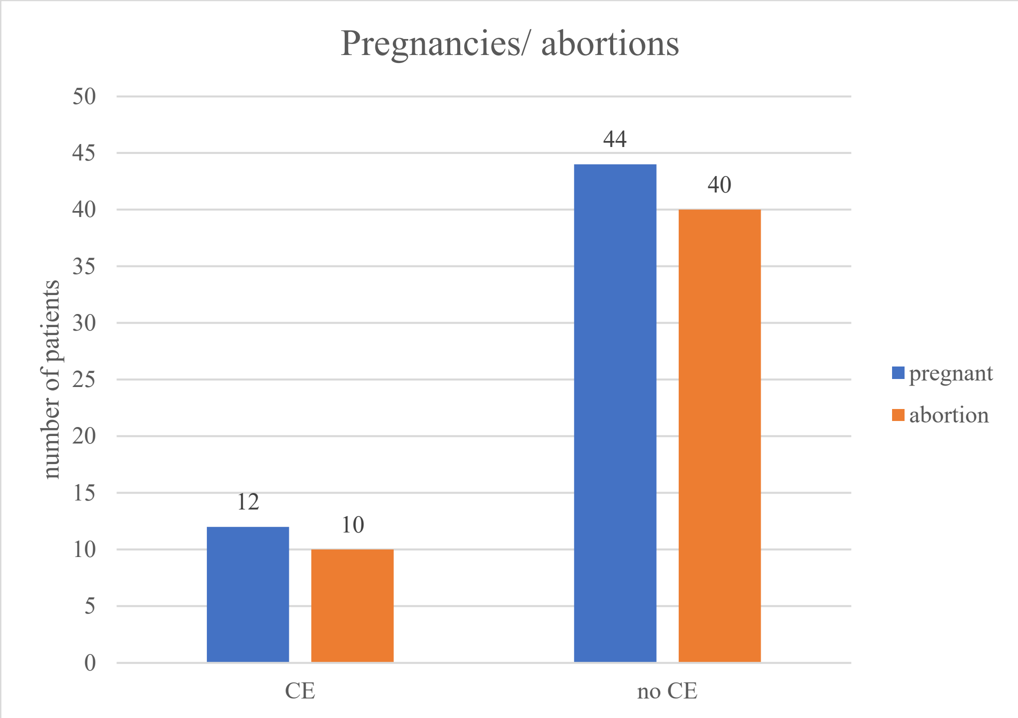


Figure 14- Pregnancies/ abortions

In Figure 14 the two groups “CE” and “no CE” are depicted in a column chart to visualize the share of patients that already had at least one abortion.

9.1.5.3 Abortion rate within the CE group

When adding up all pregnancies of women, before their diagnosis of CE, within this group, there were 37 pregnancies of which 26 were abortions (70,3%) and 11 resulted in births 29,7%).

9.1.5.4 Abortion rate within the non-CE group

In this group 96 pregnancies were counted in total. 72 of them were abortions (75,0%) and the remaining 24 (25,0%) births.

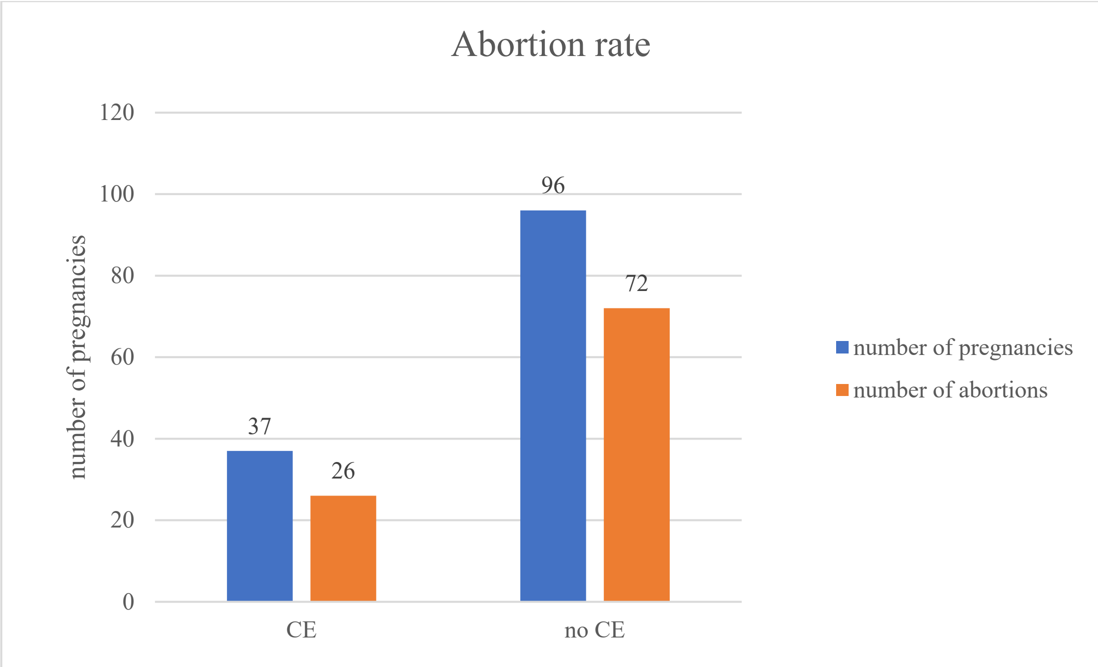


Figure 15- Abortion rate

9.1.6 Menstrual cycle day

Endometrial samples via pipelle were collected around day 18 to 27 of the menstrual cycle. As shown in Figure 16 the Median is day 20, the first quartile and therefore 25% were collected on day 19 and 75%, the third quartile, on day 21. Day 18 and day 24 represent the minimum and maximum numbers and day 27 the outlier.

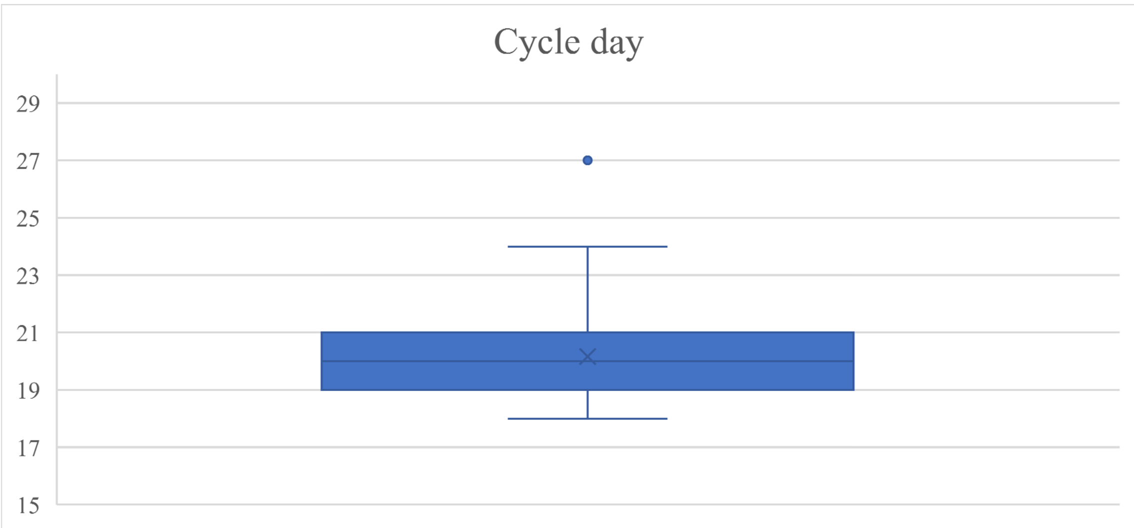


Figure 16- Cycle day

9.1.7 Hormones- estradiol and progesterone

On the day of the endometrial sample taking, some patients also had blood drawn to determine the levels of the hormones estradiol [pg/mL] and progesterone [ng/mL]. This included 49 out of 107 cases. In the following two figures (Figure 17 and Figure 18) estradiol and progesterone are presented in relation to the menstrual cycle day on which the hormone levels were assessed.

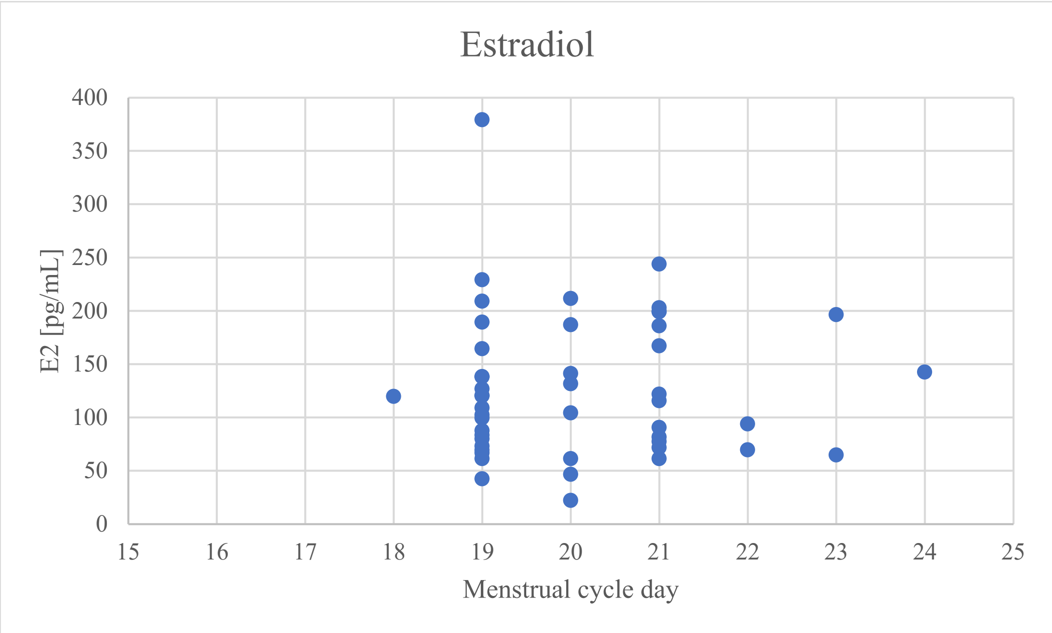


Figure 17- Estradiol in relation to cycle day

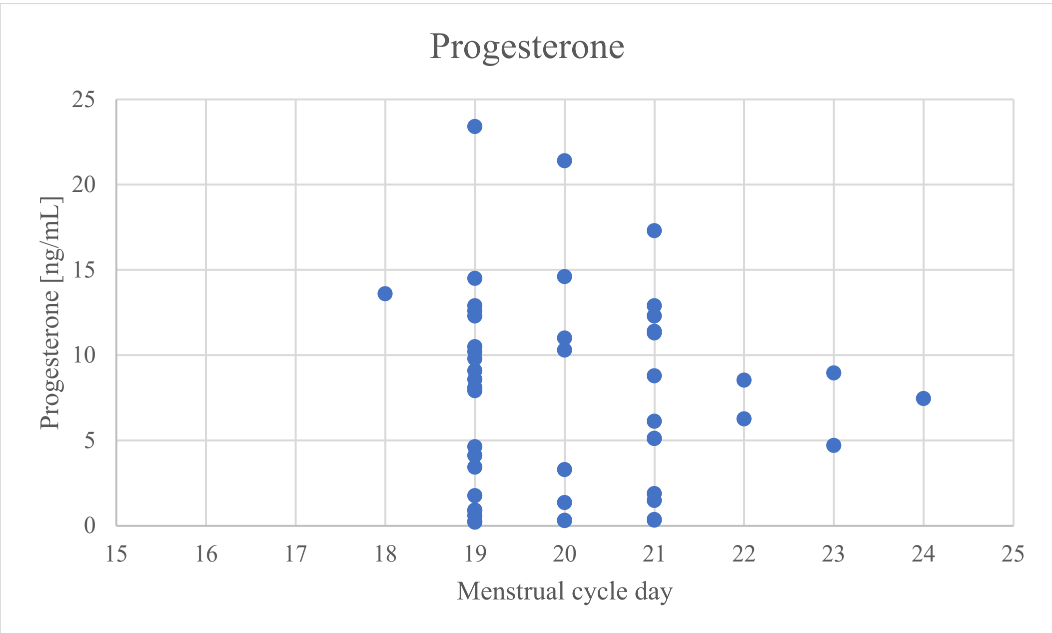


Figure 18- Progesterone in relation to cycle day

9.1.7.1 Hormones in the CE group

There were eight patients with laboratory values in this group (n=8). The Median of estradiol happens to be 102,6 [pg/mL], the first and third quartiles are 72,6 and 153,7 [pg/mL]. For progesterone the Median is 4,2 [ng/mL] and first and third quartiles are 0,78 and 9 [ng/mL].

Hormone levels- group CE			
	1. Quartile	Median	2. Quartile
Estradiol [pg/mL],	72,6	102,6	153,7
Progesterone [ng/mL]	0,78	4,2	9

Table 2- Hormone levels- CE patients

9.1.7.2 Hormones in the non-CE group

The hormone levels of 36 patients were determined (n=36). Estradiol shows a Median of 119,7 [pg/mL] and first and third quartile values of 80,7 and 176,5 [pg/mL]. The Median of the hormone level progesterone is 8,6 [ng/mL] with first and third quartiles at 4,4 and 12,5 [ng/mL].

Hormone levels- group no CE			
	1. Quartile	Median	2. Quartile
Estradiol [pg/mL],	80,7	119,7	176,5
Progesterone [ng/mL]	4,4	8,6	12,5

Table 3- Hormone levels- no CE patients

9.1.8 Ovulatory cycle

To determine if there was an ovulatory cycle present, progesterone had to be higher than 2 [ng/mL]. There were 49 cases with assessed progesterone levels, out of which 36 had an ovulatory cycle. The remaining 13 did not have an ovulation within this cycle.

9.1.8.1 CE patients

Five out of 20 CE cases had a proven ovulatory cycle. 12 times, no blood sample was taken and three samples showed a progesterone level under 2 [ng/mL].

9.1.9 Diagnosis after evaluation

After evaluation at the Department of obstetrics, every patient was classified by their diagnosis. There were 98 patients included (n=98). The most common diagnosis was recurrent miscarriage with 36,7% (36 patients). In the following table (Table 4) the different diagnosis, such as miscarriage, RIF, subfertility (including primary/secondary sterility and tubal factor) and PCOS (Polycystic ovary syndrome) and their distribution amongst the patient collective are shown. *Male factor* (*) was an additional diagnosis in 26 cases.

Diagnosis after evaluation (n=98)		
	Absolute	*Relative*
Miscarriage	36	36,7%
RIF	25	25,5%
Subfertility	32	32,7%
PCOS	4	4,1%
* *Male factor*	*27*	*27,6%*

*Table 4- Diagnosis after evaluation, *additional diagnosis of the male partner*

9.1.10 Histological analysis

Endometrial samples were histologically examined with IHC CD138 at the Institute of Pathology of the Medical University Graz. The diagnosis of the condition CE depends on the quantity of ESPCs (endometrial stromal plasma cell) detected within the sample. At the Institute of Pathology in Graz the threshold value for the diagnosis CE is three or more plasma cells per section. In cases with the diagnosis CE (n=20), the Median of plasma cells found by pathologists was eight cells, the first quartile was three cells and the third quartile 15 cells. Minimum and maximum were three and 21 cells, with two outliers at 40 and 75 cells.

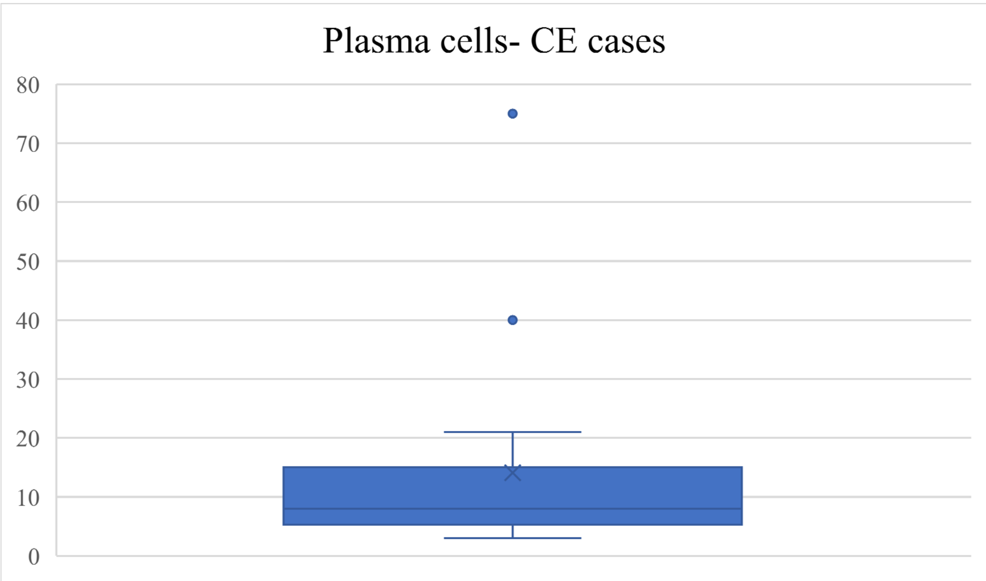


Figure 19- ESPCs in CE cases

9.1.11 Analysis of the microbiome

To identify the distribution of bacteria species within patients, pathologists used PCR with the variable 16S rRNA gene region.

9.1.11.1 CE patients

Out of 20 CE cases (n=20), five had no microbial specification. In 12 samples *Lactobacillus sp*. was found to be the most common bacterial taxa. In three occasions *Bifidobacteriaceae* happened to be the most common bacteria.

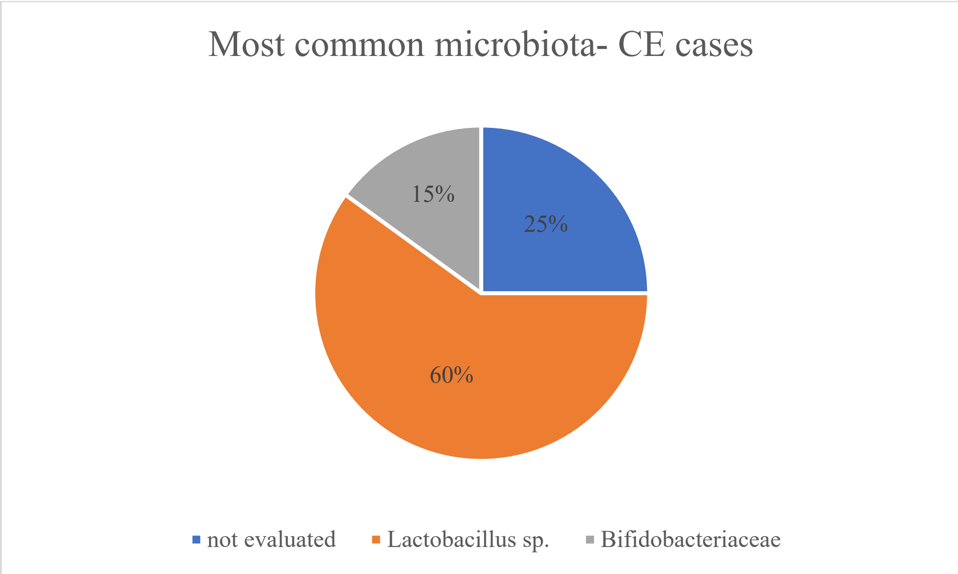


Figure 20- Most common microbiota- CE cases

To visualize the microbiotic distribution regarding the different indications (RIF, subfertility and spontaneous abortion) the following Table 5 was created.

Microbiota – CE patients (different indications) n=15		
	Most common bacteria	
Indication	*Lactobacillus sp.*	*Bifidobacteriaceae*
Spontaneous abortion (n=7)	6	1
RIF (n=4)	*3*	1
Subfertility (n=4)	*3*	1

Table 5- Microbiota – CE patients (different indications)

When dividing the CE cases with an analyzed microbiome (n=15) into two groups, *Lactobacillus*-dominated (>90%) and non-*Lactobacillus*-dominated (<90%), there are 12 samples with a non-*Lactobacillus*-dominated microbiome (80%) and three samples (20%) with a *Lactobacillus* colonization over 90%.

Furthermore, gram negative bacteria were identified. *Flavobacterium* in seven and *Prevotella* in 11 samples. Additionally, *Actinomyces* (one case), *Ureplasma spp*. (four cases), *Streptococcus* (four patients) and *Staphylococcus* (six cases) were present.

9.1.11.2 Non-CE patients

Within this group (n=78) 61 samples were analyzed regarding their endometrial microbiome. 17 samples could not be assessed adequately. The most common bacterial taxa within this group was *Lactobacillus sp*. in 59 cases. In one sample *Coriobacteriacae* and in one *Enhydrobacter* were the most common bacteria.

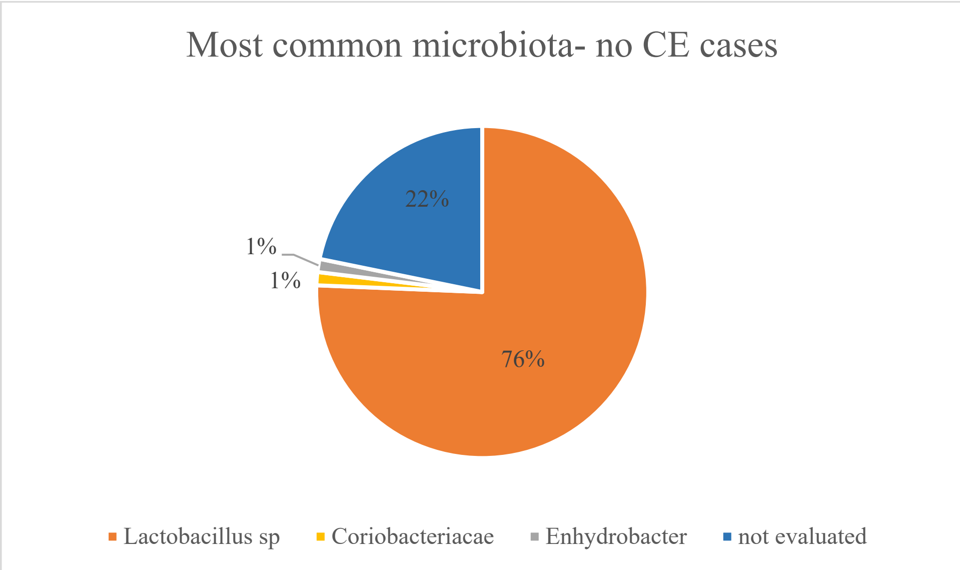


Figure 21- Most common microbiota- no CE cases

Moreover, 38 out of 61 specimens (62%) presented a *Lactobacillus*-dominated (>90%) and 23 samples (38%) a non-*Lactobacillus*-dominated (<90%) microbiome.

9.1.12 Follow up

The entire patient collective was followed up on their further developments considering pregnancies. Spontaneous conceptus, conceptus after ART (assisted reproductive techniques) or aborts were noted as shown in Table 6. If patients were diagnosed with CE, they were treated with antibiotics. To ensure a successful treatment a subsequent endometrium biopsy was made and their further reproductive outcomes were followed up.

9.1.12.1 CE patients

Out of 19 women (n=19), two had a spontaneous conceptus and one woman had a conceptus through IVF. One patient had a missed abortion and one a biochemical pregnancy.

9.1.12.2 Non-CE patients

In the group "no CE" (n=72), four patients had a spontaneous conceptus and 10 patients had a conceptus after IVF. One patient had a biochemical pregnancy and another one had an ectopic pregnancy.

Follow up				
	CE n=19		no CE n=72	
	Absolute	*Relative*	*Absolute*	*Relative*
Conceptus IVF	1	5,3%	10	13,9%
Conceptus spontaneous	2	10,5%	4	5,6%
Abort	2	10,5%	2	2,8%
no events	14	73,7%	56	77,8%

Table 6- Follow up

9.1.12.3 Treatment and control biopsy- CE patients

Seven women with diagnosed CE, which were treated with the antibiotic doxycycline for 14 days, had a control biopsy taken to ensure the successful therapy. Six patients (85,7%) showed no signs of CE after the treatment. One patient (14,3%) still experienced persisting CE, even after being treated with antibiotic therapy two times (doxycycline and ciprofloxacin). Two patients with a successful antibiotic treatment of CE achieved a spontaneous conceptus.

Treatment and control biopsy- CE patients		
	Control biopsy after treatment (n=**7**)	Pregnancy after treatment (n=**2**)
CE cured after treatment	**6** (85,7%)	**2**
CE persisting after treatment	1 (14,3%)	**0**

Table 7-Treatment and control biopsy- CE patients

10 Discussion

Chronic endometritis is known to be a risk factor for recurrent implantation failure and repeated abortions. Therefore, it is more common in women with infertility issues. In this study, 99 women with infertility issues were examined of which 19 patients were diagnosed with CE. Within these 19 patients, 47% have already had a spontaneous abortion and 21% experienced RIF. The abortion rate among CE patients amounts to 70,3%. Comparing this percentage to the abortion rate of the normal population worldwide, which Quenby et al. stated with 15,3% of all recognized pregnancies (67), it represents the impact of CE on reproductive outcomes.

10.1 Progesterone

Since progesterone plays a very important role in achieving and maintaining a successful pregnancy, hormone levels of the study population were determined on the day of the endometrium biopsy. A difference in progesterone levels between CE patients and no CE patients was evident. The Median of the progesterone level in the no CE patient group was about 50% higher than in the CE patients collective. This finding may suggest that an inadequate secretion of progesterone can be causative for infertility issues within CE patients. When scanning recent literature for progesterone supplementation within women with spontaneous aborts, opinions are divided. Saccone et al. proposed a benefit for women with spontaneous aborts through progesterone intake during the early pregnancy, whereas Coomarasamy et al. stated no positive effect of a supplementation (68,69). However, progesterone supplementation in RIF patients is known to be effective and supports IVF protocols and increases birth rates (70).

10.2 Endometrial microbiome

The uterine cavity is colonized by a diverse microbiome with *Lactobacillus* as the most common bacteria. However, studies proved that individuals with CE have a slightly different microbiome, which shows a lower abundance of *Lactobacillus* and a higher frequency of Actinobacteriota (e.g., *Cutibacterium* and *Bifidobacteriacae)* and Cyanobacteria (71). When identifying the most common bacteria within the patient collective, the non- CE group reached 76% of the *Lactobacillus* species and the CE group 60%. But when classifying the study population into non-*Lactobacillus*-dominated (<90%) and *Lactobacillus*-dominated (<90%) endometrial microbiome, the distribution differs.

80% of CE cases had a non-*Lactobacillus*-dominated (<90%) endometrial microbiome, whereas the no CE patients presented a *Lactobacillus*-dominated (>90%) microbiome in 62%. Furthermore, a prospective pilot study demonstrated that reproductive outcomes of patients undergoing IVF (in vitro fertilization) procedures were notably worse within individuals with a non-*Lactobacillus*-dominated endometrial microbiome. In summary it can be said that a non-*Lactobacillu*s dominated microbiome may be linked to implantation failure and pregnancy loss (46).

10.3 Antibiotic therapy and alternative treatments

Chronic endometritis is treated with antibiotics. Currently there is no consensus regarding the most optimal antibiotic therapy. In clinical practice, the responsible microorganism for causing the inflammation in CE is usually not detected. Consequently, broad- spectrum antibiotics are prescribed, potentially leading to recurrent infections after treatment and eliminating microbiota that are "off- target" and beneficial for the microenvironment. Hence the right antibiotic should be chosen based on the identified pathogen within the microbial sample (72). It was proposed by Kyono et al. that in addition to antibiotic therapy, intake of pre- and/ or probiotics could be useful to establish a *Lactobacillus*- dominant microbiome, which in return could benefit the implantation process (73). As an alternative for orally antibiotic treatment, a case study by Sfakianoudis et al. showed that intrauterine antibiotic infusions within CE patients were highly successful and even improved reproductive outcomes in the three patients that were assessed (74). Furthermore, microbiotic transplants are a potential new option for restoring a balanced uterine microbiome and therefore various uterine related diseases. But this method still needs more research and testing within RCTs(47).

10.4 Reproductive outcome after treatment

To ensure therapeutic success, a control biopsy has to be done. A meta-analysis by Pirtea et al. showed that in 87,9% of confirmed CE cases, antibiotic treatment is successful (27). A recently published prospective RCT even stated that the CE cure rate, after the recommended two weeks broad- spectrum antibiotic therapy, reaches up to 89,8%. In comparison to the spontaneous cure rate of 12,7%, the antibiotic therapy seems promising (75). But does this treatment also effect reproductive outcomes? Several studies state that successful antibiotic therapy could improve reproductive outcomes. McQueen et al. demonstrated in an observational prospective study that antibiotic treatment among recurrent pregnancy loss patients increased live birth rate up to 49% (76). Cicinelli et al. published a retrospective study in which live birth rates in cured CE patients were significantly higher than in patients with persisting CE or even in women without CE (77). In line, Kitaya et al. showed that RIF patients with cured CE had a higher live birth rate than RIF patients without CE and therefore no antibiotic treatment (78). But it is important to mention that the previously stated studies are retrospective or prospective observational studies. RCT studies would be essential to demonstrate the real impact of antibiotic therapy on reproductive outcome in CE patients (64).

Within the study population, six out of seven women (85,7%) showed no sign of CE in the control biopsy after antibiotic treatment. Two patients that were successfully treated, achieved a spontaneous conceptus (33,3%). These results are in line with the previously mentioned literature.

11 Conclusion

In summary, this study highlighted the impact of chronic endometritis on spontaneous aborts and RIF and demonstrated the effects of antibiotic treatment, which seemingly improves reproductive outcomes. Concerning the microbial composition of the endometrium, this retrospective data analysis showed that within CE patients a more diverse and non-*Lactobacillus* dominant (<90%) microbiome is to be found. This decrease in dominance of *Lactobacillus spp.* may be linked to poor reproductive outcomes.

12 References

1. Benner M, Ferwerda G, Joosten I, Van Der Molen RG. How uterine microbiota might be responsible for a receptive, fertile endometrium. Human Reproduction Update. 2018 Jul 1;24(4):393–415.
2. Moreno I, Simon C. Relevance of assessing the uterine microbiota in infertility. Fertility and Sterility. 2018 Aug;110(3):337–43.
3. Swidsinski A, Verstraelen H, Loening-Baucke V, Swidsinski S, Mendling W, Halwani Z. Presence of a Polymicrobial Endometrial Biofilm in Patients with Bacterial Vaginosis. Ratner AJ, editor. PLoS ONE. 2013 Jan 8;8(1):e53997.
4. Solt I. The human microbiome and the great obstetrical syndromes: A new frontier in maternal–fetal medicine. Best Practice & Research Clinical Obstetrics & Gynaecology. 2015 Feb;29(2):165–75.
5. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The Placenta Harbors a Unique Microbiome. Sci Transl Med [Internet]. 2014 May 21 [cited 2023 Oct 12];6(237). Available from: https://www.science.org/doi/10.1126/scitranslmed.3008599
6. Oh HY, Seo SS, Kong JS, Lee JK, Kim MK. Association between Obesity and Cervical Microflora Dominated by Lactobacillus iners in Korean Women. Munson E, editor. J Clin Microbiol. 2015 Oct;53(10):3304–9.
7. Chen C, Song X, Wei W, Zhong H, Dai J, Lan Z, et al. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. Nat Commun. 2017 Oct 17;8(1):875.
8. Vomstein K, Reider S, Böttcher B, Watschinger C, Kyvelidou C, Tilg H, et al. Uterine microbiota plasticity during the menstrual cycle: Differences between healthy controls and patients with recurrent miscarriage or implantation failure. Journal of Reproductive Immunology. 2022 Jun;151:103634.
9. Kitaya K, Takeuchi T, Mizuta S, Matsubayashi H, Ishikawa T. Endometritis: new time, new concepts. Fertility and Sterility. 2018 Aug;110(3):344–50.
10. Singh N, Sethi A. Endometritis - Diagnosis,Treatment and its impact on fertility - A Scoping Review. JBRA [Internet]. 2022 [cited 2023 Oct 3]; Available from: https://www.jbra.com.br/trab/pub/download_trabalho.php?fileSource=/var/www/vhosts/jbra.com.br/media/trab/arq_3532&fileName=22%20-%201858%20-%20Endometritis.pdf&id_trabalho=1280
11. Kitaya K, Yasuo T. Immunohistochemistrical and Clinicopathological Characterization of Chronic Endometritis: CHARACTERIZATION OF CHRONIC ENDOMETRITIS. American Journal of Reproductive Immunology. 2011 Nov;66(5):410–5.
12. Moyer DL, Mishell DR, Bell J. Reactions of human endometrium to the intrauterine device. American Journal of Obstetrics and Gynecology. 1970 Mar;106(6):799–809.
13. Peipert JF, Montagno AB, Cooper AS, Sung CJ. Bacterial vaginosis as a risk factor for upper genital tract infection. American Journal of Obstetrics and Gynecology. 1997 Nov;177(5):1184–7.
14. Carvalho FM, Aguiar FN, Tomioka R, De Oliveira RM, Frantz N, Ueno J. Functional endometrial polyps in infertile asymptomatic patients: a possible evolution of vascular changes secondary to endometritis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2013 Sep;170(1):152–6.
15. Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, et al. The Association between Endometriosis and Chronic Endometritis. Critchley HOD, editor. PLoS ONE. 2014 Feb 18;9(2):e88354.

16. Baker JM, Chase DM, Herbst-Kralovetz MM. Uterine Microbiota: Residents, Tourists, or Invaders? Front Immunol. 2018 Mar 2;9:208.
17. Cicinelli E, Matteo M, Tinelli R, Lepera A, Alfonso R, Indraccolo U, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Human Reproduction. 2015 Feb 1;30(2):323–30.
18. Hansen LK, Becher N, Bastholm S, Glavind J, Ramsing M, Kim CJ, et al. The cervical mucus plug inhibits, but does not block, the passage of ascending bacteria from the vagina during pregnancy. Acta Obstet Gynecol Scand. 2014 Jan;93(1):102–8.
19. Zervomanolakis I, Ott HW, Hadziomerovic D, Mattle V, Seeber BE, Virgolini I, et al. Physiology of Upward Transport in the Human Female Genital Tract. Annals of the New York Academy of Sciences. 2007 Apr;1101(1):1–20.
20. Miguel RDV, Chivukula M, Krishnamurti U, Amortegui AJ, Kant JA, Sweet RL, et al. Limitations of the criteria used to diagnose histologic endometritis in epidemiologic pelvic inflammatory disease research. Pathology - Research and Practice. 2011 Nov;207(11):680–5.
21. Cicinelli E, De Ziegler D, Nicoletti R, Colafiglio G, Saliani N, Resta L, et al. Chronic endometritis: correlation among hysteroscopic, histologic, and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies. Fertility and Sterility. 2008 Mar;89(3):677–84.
22. Santos TMA, Bicalho RC. Diversity and Succession of Bacterial Communities in the Uterine Fluid of Postpartum Metritic, Endometritic and Healthy Dairy Cows. Ravel J, editor. PLoS ONE. 2012 Dec 27;7(12):e53048.
23. Han YW, Redline RW, Li M, Yin L, Hill GB, McCormick TS. *Fusobacterium nucleatum* Induces Premature and Term Stillbirths in Pregnant Mice: Implication of Oral Bacteria in Preterm Birth. Infect Immun. 2004 Apr;72(4):2272–9.
24. Kimura F, Takebayashi A, Ishida M, Nakamura A, Kitazawa J, Morimune A, et al. Review: Chronic endometritis and its effect on reproduction. J of Obstet and Gynaecol. 2019 May;45(5):951–60.
25. Kushnir VA, Solouki S, Sarig-Meth T, Vega MG, Albertini DF, Darmon SK, et al. Systemic Inflammation and Autoimmunity in Women with Chronic Endometritis. American J Rep Immunol. 2016 Jun;75(6):672–7.
26. Buzzaccarini G, Vitagliano A, Andrisani A, Santarsiero CM, Cicinelli R, Nardelli C, et al. Chronic endometritis and altered embryo implantation: a unified pathophysiological theory from a literature systematic review. J Assist Reprod Genet. 2020 Dec;37(12):2897–911.
27. Pirtea P, Cicinelli E, De Nola R, De Ziegler D, Ayoubi JM. Endometrial causes of recurrent pregnancy losses: endometriosis, adenomyosis, and chronic endometritis. Fertility and Sterility. 2021 Mar;115(3):546–60.
28. Kitaya K, Yasuo T. Aberrant expression of selectin E, CXCL1, and CXCL13 in chronic endometritis. Modern Pathology. 2010 Aug;23(8):1136–46.
29. Matteo M, Cicinelli E, Greco P, Massenzio F, Baldini D, Falagario T, et al. ORIGINAL ARTICLE: Abnormal Pattern of Lymphocyte Subpopulations in the Endometrium of Infertile Women with Chronic Endometritis: UNK CELLS IN WOMEN WITH CHRONIC ENDOMETRITIS. American Journal of Reproductive Immunology. 2009 Apr 1;61(5):322–9.
30. Tortorella C, Piazzolla G, Matteo M, Pinto V, Tinelli R, Sabbà C, et al. Interleukin-6, interleukin-1β, and tumor necrosis factor α in menstrual effluents as biomarkers of chronic endometritis. Fertility and Sterility. 2014 Jan;101(1):242–7.
31. Salama SA, Kamel MW, Diaz-Arrastia CR, Xu X, Veenstra TD, Salih S, et al. Effect of Tumor Necrosis Factor-α on Estrogen Metabolism and Endometrial Cells:

Potential Physiological and Pathological Relevance. The Journal of Clinical Endocrinology & Metabolism. 2009 Jan 1;94(1):285–93.
32. Kitaya K, Tada Y, Hayashi T, Taguchi S, Funabiki M, Nakamura Y. Comprehensive Endometrial Immunoglobulin Subclass Analysis in Infertile Women Suffering from Repeated Implantation Failure with or without Chronic Endometritis. American J Rep Immunol. 2014 Oct;72(4):386–91.
33. Di Pietro C, Cicinelli E, Guglielmino MR, Ragusa M, Farina M, Palumbo MA, et al. Altered Transcriptional Regulation of Cytokines, Growth Factors, and Apoptotic Proteins in the Endometrium of Infertile Women with Chronic Endometritis. Am J Reprod Immunol. 2013 May;69(5):509–17.
34. Wang X, Tian F, Chen C, Feng Y, Sheng X, Guo Y, et al. Exosome-derived uterine microRNAs isolated from cows with endometritis impede blastocyst development. Reproductive Biology. 2019 Jun;19(2):204–9.
35. Pinto V, Matteo M, Tinelli R, Mitola PC, De Ziegler D, Cicinelli E. Altered uterine contractility in women with chronic endometritis. Fertility and Sterility. 2015 Apr;103(4):1049–52.
36. Klimaszyk K, Svarre Nielsen H, Wender-Ozegowska E, Kedzia M. Chronic endometritis — is it time to clarify diagnostic criteria? Ginekol Pol. 2023 Feb 28;94(2):152–7.
37. Cicinelli E, Resta L, Nicoletti R, Zappimbulso V, Tartagni M, Saliani N. Endometrial micropolyps at fluid hysteroscopy suggest the existence of chronic endometritis. Human Reproduction. 2005 May 1;20(5):1386–9.
38. Song D, Li TC, Zhang Y, Feng X, Xia E, Huang X, et al. Correlation between hysteroscopy findings and chronic endometritis. Fertility and Sterility. 2019 Apr;111(4):772–9.
39. Kannar V, Lingaiah HKM, Sunita V. Evaluation of Endometrium for Chronic Endometritis by Using Syndecan-1 in Abnormal Uterine Bleeding. J Lab Physicians. 2012 Jul;4(02):069–73.
40. Kitaya K, Yasuo T. Inter-observer and intra-observer variability in immunohistochemical detection of endometrial stromal plasmacytes in chronic endometritis. Experimental and Therapeutic Medicine. 2013 Feb;5(2):485–8.
41. McQueen DB, Maniar KP, Hutchinson A, Confino R, Bernardi L, Pavone ME. Redefining chronic endometritis: the importance of endometrial stromal changes. Fertility and Sterility. 2021 Sep;116(3):855–61.
42. Liu Y, Chen X, Huang J, Wang CC, Yu MY, Laird S, et al. Comparison of the prevalence of chronic endometritis as determined by means of different diagnostic methods in women with and without reproductive failure. Fertility and Sterility. 2018 May;109(5):832–9.
43. Achilles SL, Amortegui AJ, Wiesenfeld HC. Endometrial Plasma Cells: Do They Indicate Subclinical Pelvic Inflammatory Disease? Sexually Transmitted Diseases. 2005 Mar;32(3):185–8.
44. Margulies SL, Dhingra I, Flores V, Hecht JL, Fadare O, Pal L, et al. The Diagnostic Criteria for Chronic Endometritis: A Survey of Pathologists. International Journal of Gynecological Pathology. 2021 Nov;40(6):556–62.
45. Moreno I, Cicinelli E, Garcia-Grau I, Gonzalez-Monfort M, Bau D, Vilella F, et al. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. American Journal of Obstetrics and Gynecology. 2018 Jun;218(6):602.e1-602.e16.
46. Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazán J, et al. Evidence that the endometrial microbiota has an effect on implantation

success or failure. American Journal of Obstetrics and Gynecology. 2016 Dec;215(6):684–703.
47. Molina N, Sola-Leyva A, Saez-Lara M, Plaza-Diaz J, Tubić-Pavlović A, Romero B, et al. New Opportunities for Endometrial Health by Modifying Uterine Microbial Composition: Present or Future? Biomolecules. 2020 Apr 11;10(4):593.
48. Marques FZ, Mackay CR, Kaye DM. Beyond gut feelings: how the gut microbiota regulates blood pressure. Nat Rev Cardiol. 2018 Jan;15(1):20–32.
49. Acuña I, Cerdó T, Ruiz A, Torres-Espínola FJ, López-Moreno A, Aguilera M, et al. Infant Gut Microbiota Associated with Fine Motor Skills. Nutrients. 2021 May 14;13(5):1673.
50. Power ML, Quaglieri C, Schulkin J. Reproductive Microbiomes: A New Thread in the Microbial Network. Reprod Sci. 2017 Nov;24(11):1482–92.
51. Agostinis C, Mangogna A, Bossi F, Ricci G, Kishore U, Bulla R. Uterine Immunity and Microbiota: A Shifting Paradigm. Front Immunol. 2019 Oct 17;10:2387.
52. Nguyen PV, Kafka JK, Ferreira VH, Roth K, Kaushic C. Innate and adaptive immune responses in male and female reproductive tracts in homeostasis and following HIV infection. Cell Mol Immunol. 2014 Sep;11(5):410–27.
53. Wira CR, Grant-Tschudy KS, Crane-Godreau MA. Epithelial Cells in the Female Reproductive Tract: a Central Role as Sentinels of Immune Protection. American J Rep Immunol. 2005 Feb;53(2):65–76.
54. Robertson SA, Chin PY, Glynn DJ, Thompson JG. Peri-Conceptual Cytokines – Setting the Trajectory for Embryo Implantation, Pregnancy and Beyond. American J Rep Immunol. 2011 Jul;66(s1):2–10.
55. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol. 2013 Jul;14(7):685–90.
56. Ubeda C, Djukovic A, Isaac S. Roles of the intestinal microbiota in pathogen protection. Clin & Trans Imm. 2017 Feb;6(2):e128.
57. Vaishnava S, Behrendt CL, Ismail AS, Eckmann L, Hooper LV. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc Natl Acad Sci USA. 2008 Dec 30;105(52):20858–63.
58. Murphy CR, Rogers PAW, Hosie MJ, Leeton J, Beaton L. Tight Junctions of Human Uterine Epithelial Cells Change during the Menstrual Cycle: A Morphometric Study. Cells Tissues Organs. 1992;144(1):36–8.
59. Buck VU, Windoffer R, Leube RE, Classen-Linke I. Redistribution of adhering junctions in human endometrial epithelial cells during the implantation window of the menstrual cycle. Histochem Cell Biol. 2012 Jun;137(6):777–90.
60. Espinoza J, Erez O, Romero R. Preconceptional antibiotic treatment to prevent preterm birth in women with a previous preterm delivery. American Journal of Obstetrics and Gynecology. 2006 Mar;194(3):630–7.
61. Deutsche Gesellschaft für Gynäkologie und Geburtshilfe e.V. (DGGG). S2k-Leitlinie Diagnostik und Therapie von Frauen mit wiederholten Spontanaborten [Internet]. 2022. Available from: https://register.awmf.org/de/leitlinien/detail/015-050
62. Vitagliano A, Saccardi C, Noventa M, Di Spiezio Sardo A, Saccone G, Cicinelli E, et al. Effects of chronic endometritis therapy on in vitro fertilization outcome in women with repeated implantation failure: a systematic review and meta-analysis. Fertility and Sterility. 2018 Jul;110(1):103-112.e1.
63. Cheng X, Huang Z, Xiao Z, Bai Y. Does antibiotic therapy for chronic endometritis improve clinical outcomes of patients with recurrent implantation failure in subsequent IVF cycles? A systematic review and meta-analysis. J Assist Reprod Genet. 2022 Aug;39(8):1797–813.

64. Espinós JJ, Fabregues F, Fontes J, García-Velasco JA, Llácer J, Requena A, et al. Impact of chronic endometritis in infertility: a SWOT analysis. Reproductive BioMedicine Online. 2021 May;42(5):939–51.
65. Sfakianoudis K, Simopoulou M, Nitsos N, Lazaros L, Rapani A, Pantou A, et al. Successful Implantation and Live Birth Following Autologous Platelet-rich Plasma Treatment for a Patient with Recurrent Implantation Failure and Chronic Endometritis. In Vivo. 2019;33(2):515–21.
66. Konoplya AA, Gavrish SA, Konoplya AI, Loktionov AL. The application of intravascular laser irradiation of blood for the correction of the immune disturbances in patients presenting with chronic endometritis. Vopr kurortol fizioter lech fiz kul't. 2016;93(5):19.
67. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, et al. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. The Lancet. 2021 May;397(10285):1658–67.
68. Coomarasamy A, Williams H, Truchanowicz E, Seed PT, Small R, Quenby S, et al. A Randomized Trial of Progesterone in Women with Recurrent Miscarriages. N Engl J Med. 2015 Nov 26;373(22):2141–8.
69. Saccone G, Schoen C, Franasiak JM, Scott RT, Berghella V. Supplementation with progestogens in the first trimester of pregnancy to prevent miscarriage in women with unexplained recurrent miscarriage: a systematic review and meta-analysis of randomized, controlled trials. Fertility and Sterility. 2017 Feb;107(2):430-438.e3.
70. Bashiri A, Halper KI, Orvieto R. Recurrent Implantation Failure-update overview on etiology, diagnosis, treatment and future directions. Reprod Biol Endocrinol. 2018 Dec;16(1):121.
71. Chen Q, Zhang X, Hu Q, Zhang W, Xie Y, Wei W. The alteration of intrauterine microbiota in chronic endometritis patients based on 16S rRNA sequencing analysis. Ann Clin Microbiol Antimicrob. 2023 Jan 12;22(1):4.
72. Liu J, Liu ZA, Liu Y, Cheng L, Yan L. Impact of antibiotic treatment for chronic endometritis on pregnancy outcomes in women with reproductive failures (RIF and RPL): A systematic review and meta-analysis. Front Med. 2022 Nov 3;9:980511.
73. Kyono K, Hashimoto T, Kikuchi S, Nagai Y, Sakuraba Y. A pilot study and case reports on endometrial microbiota and pregnancy outcome: An analysis using 16S rRNA gene sequencing among IVF patients, and trial therapeutic intervention for dysbiotic endometrium. Reprod Medicine & Biology. 2019 Jan;18(1):72–82.
74. Sfakianoudis K, Simopoulou M, Nikas Y, Rapani A, Nitsos N, Pierouli K, et al. Efficient treatment of chronic endometritis through a novel approach of intrauterine antibiotic infusion: a case series. BMC Women's Health. 2018 Dec;18(1):197.
75. Song D, He Y, Wang Y, Liu Z, Xia E, Huang X, et al. Impact of antibiotic therapy on the rate of negative test results for chronic endometritis: a prospective randomized control trial. Fertility and Sterility. 2021 Jun;115(6):1549–56.
76. McQueen DB, Bernardi LA, Stephenson MD. Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. Fertility and Sterility. 2014 Apr;101(4):1026–30.
77. Cicinelli E, Matteo M, Trojano G, Mitola PC, Tinelli R, Vitagliano A, et al. Chronic endometritis in patients with unexplained infertility: Prevalence and effects of antibiotic treatment on spontaneous conception. American J Rep Immunol. 2018 Jan;79(1):e12782.
78. Kitaya K, Matsubayashi H, Takaya Y, Nishiyama R, Yamaguchi K, Takeuchi T, et al. Live birth rate following oral antibiotic treatment for chronic endometritis in infertile

women with repeated implantation failure. American J Rep Immunol. 2017 Nov;78(5):e12719.