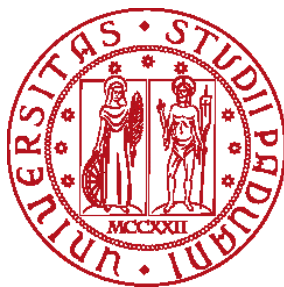# UNIVERSITA' DEGLI STUDI DI PADOVA

DIPARTIMENTO DI SALUTE DELLA DONNA E DEL BAMBINO

Corso di Dottorato in Medicina dello Sviluppo e Scienze della Programmazione Sanitaria

TESI DI DOTTORATO

CHRONIC ENDOMETRITIS: A SUBTLE DISEASE UNDERLYING DEFECTIVE ENDOMETRIAL RECEPTIVITY AND ENDOMETRIAL PROLIFERATIVE DISORDERS. A MULTIFACETED RESEARCH PROGRAM.

COORDINATORE: Ch.mo Prof. Guido Ambrosini, MD, PhD

SUPERVISORE: Ch.mo Prof. Alessandra Andrisani, MD, PhD

CANDIDATO: Dott. Amerigo Vitagliano

Capsule:

MUM-1 immunohistochemistry may represent a novel, promising technique for the diagnosis of chronic endometritis. Chronic endometrial inflammation may play a role in in reproductive failure, as well as in the development of endometrial polyps in young women.

ABSTRACT

Chronic endometritis (CE) is a chronic inflammatory disorder of the endometrium characterized by the infiltration of plasma cells within endometrial stromal compartment.

The diagnosis of CE is challenging. Clinical examination and transvaginal ultrasound are nonspecific for CE. Immunohistochemistry for the plasmacyte marker CD-138 is currently the most reliable and time-saving diagnostic method for CE. However, this technique is not exempt from limitations. Endometrial epithelial cells constitutively express CD-138 on the basolateral sides of their plasma membrane, potentially leading to misdiagnosis. Therefore, further staining techniques for CE would be useful.

In a recent study, we found a dysregulation of several genes involved in cell cycle regulation in the endometrium of women with CE, with a dominance of proliferative and anti-apoptotic activity. CE may therefore represent a substrate for the development of endometrial proliferative lesions, including endometrial polyps (EPs). Different studies have investigated the correlations between CE and EPs, with controversial results. A summary of evidence on this topic is still missing.

Moreover, CE may impair the endometrial receptivity towards the embryo, resulting in infertility and recurrent pregnancy loss. Unfortunately, the exact mechanisms underlying CE-related infertility are still unknown. An electron microscopy study investigating any alterations in the morphological markers of endometrial receptivity in CE is still missing.

Over that backround, our multifaceted research program had the following objectives: i) to evaluate the accuracy and reliability of a novel immunohistochemical marker (Multiple Myeloma Antigen-1) for the diagnosis of CE; ii) to summarize current evidence on the correlation between CE and EPs; iii) to evaluate whether CE induces morphological changes in

the mid-secretory endometrium (as assessed by transmission electron microscopy [TEM]) that may account for defective receptivity.

i) We found that immunohistochemistry for MUM-1 and CD-138 had similar accuracy for detecting endometrial stromal plasma cells. Notably, MUM-1 showed higher reliability in the paired comparison of the individual samples than CD-138. Therefore, MUM-1 immunohistochemistry may represents a novel, promising add-on technique for improving the diagnostic process in women with CE.

ii) Our systematic literature review found high prevalence of CE in pre-menopausal women suffering from EPs. The risk of CE was higher in women with EPs compared to women with a non-polypoid endometrium, as well as in those with three or more EPs compared to those with a single EP. Available evidence in pre-menopausal women suggests that CE and EPs may have a dependent relationship and may represent two consequent steps of a common pathological process.

iii) TEM analysis revealed lower expression of pinopodes and higher expression of microvilli and cilia in the mid-secretory endometrium of women with CE compared to controls. These endometrial features in CE may account for defective endometrial receptivity. As our study is still ongoing, full results will be required to draw firm conclusions regarding the expression of endometrial organoids in the mid-secretory endometrium of women with CE.

Background and rationale

Endometrial physiology in women of reproductive age

Endometrium is the innermost layer of the uterus, whose physiological functions are preparation for implantation, maintenance of pregnancy (if implantation occurs), and menstruation in the absence of pregnancy (1, 2).

Histologically the endometrium consists of a simple comprises a simple columnar epithelium forming numerous tubular glands, supported by a thick vascular stroma (1, 3). The stroma hosts fibroblast-like cells and a fluctuating traffic of recruited innate immune cells (4). Additionally, the basal layer host an endometrial adult stem cell population whose role is to restore endometrial integrity following menstruation (5, 6).

During the human menstrual cycle, the endometrium undergoes cyclical histological changes under the stimulus of circulating sex hormones. During the early endometrial cycle (i.e. "proliferative phase"), the endocrine environment is characterized by estradiol rise, and the endometrial tissue undergoes progressive proliferation until the time of ovulation (1, 2, 7). Following ovulation, progesterone production by the corpus luteum is responsible of endometrial secretory changes (i.e. "secretory phase"), namely the endometrial glands become elongated, tortuous and boosted in their secretions, spiral arterioles proliferate and the acellular extracellular matrix increases in volume (4, 8). About seven days after ovulation, the "pre-decidual" endometrium is physiologically ready for embryo implantation. This phase is defined as "implantation window" (WOI) or "receptive phase", and lasts for about two to four days. In specific endometrial areas, the local realease of chemokines and growth factors will attract the blastocyst to landing platforms known as "pinopodes" (9, 10). These structures have the role to extend over the tips of microvilli expressing the repellent mucin-1, with the aim of favouring the embryo adhesion on endometrial surface (1, 11). At this step, several adhesion molecules including integrins and cadherins are expressed in order to facilitate the adhesiveness between the blastocyst and the endometrium. Specific clusters of endometrial genes are expressed/silenced during this phase (12). Host immune response towards the embryo is down-regulated, and the local immune scenario is dominated by NK-cells, dendritic cells and macrophages (13). If pregnancy does not occur, the corpus luteum undergoes regressive changes causing the decline in serum progesterone and estradiol concentrations. This phenomenon starts the onset of menstruation as a result of ischemic processes, in which the functional layer of the endometrium is broken down, shed, and subsequently restored (14).

Endometrial factor infertility

Endometrial factor infertility (EFI) refers to the inability of the endometrium to accept a good quality embryo due to structural or functional defects (15). Since the endometrium is the layer of the uterus intimately in contact with the embryo, EFI is often a misnomer referred to uterine obstacles to conception in general. The EFI is diagnosed indirectly, when sustained implantation does not occur despite multiple transfers of good quality embryos. A precise definition of EFI is still lacking, and therefore the prevalence of this condition cannot be precisely estimated. Until recent times, it was believed that implantation failure was mainly caused by defective endometrial receptivity (in up to two-thirds of cases) (16, 17). Nevertheless, the recent introduction of pre-implantation genetic testing for aneuploidies (PGTA; through adjusting for the majority of embryonic causes of implantation failure) has allowed the understanding that the endometrium directly impedes implantation of a chromosomally normal embryo in no more than 50% of cases (18). The remaining cases of implantation failures are of embryonic origin.

The paramount importance of a functional endometrium and, more generally of a healthy uterus, was highlighted by the results of a recent study of surrogacy programs. When compared to intended parent recipients, gestational carriers showed significantly higher live birth rates after the transfer of donor oocyte embryos (19). Such a considerable difference in the perinatal outcomes between groups remained stable when comparing all the age subgroups (from <35 to 51-53 years old patients). The study concluded that a "healthy uterus" was a prerequisite for sustained embryo implantation.

When considering the uterus in its totality, several congenital anomalies and acquired diseases may influence its physiological function and result in implantation failure (20). With regard to acquired diseases, the vast majority pertain to the endometrium (egs. Endometrial polyps, Asherman syndrome, endometritis) or somehow influence the endometrial physiology (egs. Myomas, adenomyosis). Therefore, at the end of the story, the endometrium represent a conjunction point of the vast majority of the uterine diseases with a potential influence on reproduction. Namely, endometrium is the other actor (besides the embryo) deserving a special attention by reproductive specialists.

Uterine microbiota: a new actor in reproduction

For more than one century, consensus was that a healthy uterine cavity was sterile. Uterine sterility was presumed to be maintained by the cervical plug providing an impermeable barrier to bacterial ascension (21). This theory has been called into question about thirty years ago. At that time, several studies demonstrated the presence of bacteria in endometrial specimens from healthy women by using traditional culture techniques. Additionally, during the same period, other studies found that some particles can translocate from the vagina to the uterus during

specific stages of the menstrual cycle. Thus, the theories about uterine sterility and cervical mucus impermeability were rapidly dismissed (22 , 23).

In the following years, a number of animal studies showed that a plethora of bacteria colonize the uterine cavity in physiologic conditions. Those bacteria can translocate to the uterus from the vagina through the cervical canal, from gastrointestinal mucosae through the hematogenous route, or from other sites during medical procedures (egs. Hysteroscopic procedures, endometrial curettage). In humans, the ascending bacteria from the vagina are assumed as the main contributors to uterine microbiome (24-26).

The exact constitution of uterine microbiome is still to be clarified. Until 2010, traditional culture techniques were applied in the attempt to characterize intrauterine bacterial communities. Unfortunately, the most of the bacteria are not colturable, and the results of the studies were therefore inconclusive. The application of Next Generation Sequencing (NGS) has enabled species-level quantification, potentially allowing for the determination of the full microbiome in both healthy and diseased hosts (27).

Recent studies showed that vaginal and uterine microbiome may be actively involved in human reproduction. The vaginal microbiota has been shown to be different in pregnant and non-pregnant women, suggesting a putative role of vaginal bacteria in the prevention of intrauterine infections during pregnancy (28). On the other side, bacterial vaginosis is a known risk factor for obstetric complications, including pregnancy loss and preterm birth. With respect to the uterine microbiome, Moreno et al found that a "healthy" microbiome was constituted by >90% Lactobacillus spp, similarly to vaginal microbiome (29). Notably, in their study on infertile women undergoing IVF, the presence of a non-Lactobacillus-dominated microbiota was associated with significant decrease in live birth rates after embryo transfer (58.8% vs 6.7%).

Although a certain influence of the uterine microbiome on embryo implantation process is plausible, it is uncertain whether routine assessment of endometrial microbiome may increase the success rates of IVF in infertile women. First, it is still unknown whether endometrial bacteria impede implantation directly by affecting embryo viability (egs. by modifying intrauterine ph or by releasing lipopolysaccharide) or indirectly by altering the endometrial receptivity. Second, endometrial samples are inevitably contaminated with vaginal and cervical bacteria, making the assessment of endometrial microbiome somehow biased in principle.

On the other hand, what is already known is that abnormal endometrial microbiome often co-exists with signs of endometrial inflammation at histopathology (30). This condition is also known as "chronic endometritis". Diversely from intrauterine microbiome, the endometrial histology may be of easier investigation and of more objective interpretation. Moreover,

histological endometrial inflammation may indirectly indicate the abnormal overgrowth of intrauterine bacteria, thus directing specific microbiological investigations in selected cases.

Chronic endometritis: the other side of the coin

Chronic endometritis (CE) is a case of a persistent endometrial inflammation caused by infectious agents including Enterococci, Streptococci, Staphylococci, Mycoplasma spp, Gardnerella vaginalis, Ureaplasma urealyticum, Chlamydia trachomatis e Neisseria gonorrhoeae (31, 32). This pathology is often overlooked by physicians due to a lack of specific symptoms which include abnormal uterine bleeding, dyspareunia, pelvic discomfort and leucorrhaea (33). The prevalence of CE is quite high among women with reproductive disorders, ranging between 13.95% and 57.55% in women with infertility, repeated implantation failures and recurrent pregnancy loss (34, 35). The causal link between CE and reproductive failure seems to be attributable to numerous factors, including the release of inflammatory mediators within the uterine cavity, endometrial vascular damage and an abnormal uterine contractility in the mid-secretory phase (all the potential mechanisms involved have been summarized in a recent systematic review published by our group in 2020) (36). All these factors may theoretically impede the accomplishment of a correct and sustained implantation of human embryos.

The up-regulation of numerous anti-apoptotic and pro-proliferative genes observed in CE (found in another of our recently published study) may potentially alter the complex communication between the embryo ad the endometrium (37). At the same time, such an aberrant gene expression may cause the gradual development of endometrial proliferative pathologies (benign lesions such as endometrial polyps and hyperplasia up to carcinogenic processes).

In women with repeated embryo implantation failures (≥2 failed embryo transfers), we recently demonstrated (35) that CE, when diagnosed and cured, improves the chances of embryo implantation (OR 3.24), clinical (OR 4.02) and ongoing (OR 6.81) pregnancy after IVF / ICSI procedures. Significant improvement in the reproductive prognosis after CE cure was also found, by our group, in women with unexplained infertility seeking for spontaneous pregnancy (38).

The diagnosis of CE is a challenging. Clinical examination and transvaginal ultrasound are nonspecific for CE. Hysteroscopy may be a helpful tool for raising the suspicion of CE. In our recent study entitled "Unified diagnostic criteria for chronic endometritis at fluid hysteroscopy: proposal and reliability evaluation through an international randomized-controlled observer study." (39), we found that the endometrium in CE is characterized by some macroscopic endometrial changes (micropolyps, stromal edema, "strawberry appearance", haemorrhagic

spots, focal or diffuse hyperemia) that can be recognized through hysteroscopy. However, as hysteroscopy is an operator-dependent technique, the current gold standard for the diagnosis of CE is histopathology showing the presence of plasma cells within endometrial stromal compartment (40). Identification of plasmacells by conventional tissue staining alone is difficult. Plasmacytes typically have a large cell body, high nuclei/cytoplasm ratio, basophilic cytoplasm, and nuclei with heterochromatin rearrangement referred as "spoke-wheel" or "clock-face" pattern (41). These morphological features of plasmacytes are not always evident under microscopic examination, as plasmacells often exhibit the appearance similar to stromal fibroblasts and mononuclear leukocytes that reside in the human endometrium (39).

Immunohistochemistry for the plasmacyte marker CD-138 (also known as syndecan-1, a transmembrane type heparan sulfate proteoglycan) is currently the most reliable and time-saving diagnostic method for CE (42). It was shown that CD-138 immunostaining is superior to conventional tissue staining using methyl green pyronin, hematoxylin, and eosin in CE detection (odds ratio 2.8; sensitivity, 100% versus 75%; specificity, 100% versus 65%) with less inter-observer (96% versus 68%) and intra-observer variability (93% versus 47%) (43). Despite the usefulness of CD-138 immunostaining in diagnosis of CE, some caution should be exercised in its practical use. Endometrial epithelial cells constitutively express CD-138 on the basolateral sides of their plasma membrane. As consequence, monoclonal antibodies targeting CD-138 on plasmacytes are also reactive to the epitope of this antigen expressed on endometrial epithelial cells, despite that staining intensity is generally weaker than endometrial stromal plasma cells (41-44). This immunohistochemical reaction may potentially lead to misdiagnosis and further staining techniques for CE would be useful.

Multiple myeloma antigen 1 (MUM-1) is a protein which is normally expressed in plasma cells, activated B and T cells. Studies showed that MUM1 protein is required at several stages of B-cell development, including in the differentiation of mature B cell into antibody-secreting plasma cells (45, 46). Immunohistochemistry for MUM1 has been widely used for the diagnosis of lymphomas and plasma cell tumors, but has never been applied for the diagnosis of CE.

Over that backround, our multifaceted research program had the following objectives: i) to evaluate the accuracy and reliability of a novel immunohistochemical marker (Multiple Myeloma Antigen-1) for the diagnosis of CE; ii) to summarize current evidence on the correlation between CE and endometrial polyps; iii) to evaluate whether CE induces pathological changes in the mid-secretory endometrium (as assessed by transmission microscopy) that may account for defective receptivity.

Materials and methods

Materials and methods of each stand-alone study of the PhD project are reported separately.

-I: Accuracy and reliability of MUM-1 immunostaining for the identification of endometrial plasma cells in chronic endometritis: head to head comparison with CD-138. A multi-centre study.

Study design

This was an observational multi-centre study conducted at three centers (hysteroscopy units of the University of Bari, Padua and Barcelona) from August 2017 to January 2020. The study received local ethical approval. All patients gave their written consent for the anonymous use of their clinical data for research purpose.

Participants

We included patients referred to our hysteroscopy services due to infertility (i.e. the failure to achieve pregnancy after twelve months or more of unprotected sexual intercourse), recurrent miscarriage (i.e. two or more consecutive miscarriages), abnormal uterine bleeding, suspicion of endometrial polyps or myomas.

Exclusion criteria were: post-menopausal age, diagnosis of placental remnants, endometrial cancer or atypical hyperplasia, previous diagnosis of tuberculosis, hormonal or steroid treatment whitin three months or during the study period, any antibiotic treatments whitin three months or during the study period.

Hysteroscopy and endometrial sampling

All women underwent diagnostic mini-hysteroscopy in the follicular phase of menstrual cycle. Mini-hysteroscopy was performed using a lens-based 2.7 mm OD mini-telescope, 105° angle of visual field equipped with a 3.5 mm OD single-flow diagnostic sheath (Karl Storz, Tuttlingen, Germany). In order to minimize the risk of iatrogenic contamination of endometrial cavity, all examinations were performed after placing vaginal speculum and cleaning external uterine ostium with a gauze soaked in iodine solution.

Saline was employed to distend the uterine cavity at a pressure generated by simple drop from a bag suspended 1 m above the patient. A 300 W light source with a xenon bulb and a HD digital camera (Karl Storz, Tuttlingen, Germany) were used.

The exploration of the uterine cavity consisted of a panoramic view of the cavity followed by a thorough evaluation of the endometrial mucosa as previously described (39). Diagnosis of CE was based on criteria previously published (38). All hysteroscopies were performed by one author for each centre (A.V, E.C. and S.H.). After hysteroscopy, an endometrial biopsy using a 3 mm Novak's curette connected to a 20 ml syringe was performed. The obtained tissue samples were placed in formalin for histological and immunohystochemical examinations.

Histology and Immunohistochemistry

Endometrial samples were fixed in neutral formaline and later embedded in paraffin. Five microsections were stained with hematoxiline-eosine. Histological diagnosis of CE was based on criteria described in literature. Five-micrometer sections were cut and incubated, separately, with mouse anti-human monoclonal CD-138 and MUM-1 antibodies. The clones of anti-CD-138 and anti-MUM-1 monoclonal antibodies used in our study were MI15 Cell Marque (Biocare Medical, Concord, CA) and MRQ-8 Cell Marque (Sigma-Aldrich, St. Louis, USA). CD138- positive plasma cells were identified in the stroma. The biopsies were graded as "negative" for CE if there was less than one plasma cell identified per 20 high-power fields (HPFs) and ''positive'' when there was one or more plasma cell identified per 20 HPFs. Initially, histopathological and immunohistochemical analyses were completed by a single pathologist for each centre and served for the assessment of the primary and tertiary endpoints of our study. In a subset of patients, the immunohistochemical analyses with CD-138 and MUM-1 were repeated by two additional pathologists, who were unaware of their respective evaluations. The results of their observations were included in the inter-observer agreement analyses.

Data collection

Clinical data were collected in our clinical registers by two Authors for each centre. Data included patients' general features (age, BMI, parity), medical history (general health status, pharmacological treatments), the indication for hysteroscopy, and the results of each hysteroscopic, histological and immunohistochemical examination with CD-138 and MUM-1. In case of missing data, the same authors contacted patients by telephone to obtain complete information.

Study Outcomes

Primary outcome was to evaluate the accuracy of MUM-1 immunohistochemistry, as compared with CD-138 immunohistochemistry for the diagnosis of CE. Secondary outcome was to assess the inter-observer reliability of MUM-1 and CD-138 immunohistochemistry for the detection of plasma cells. Tertiary outcome was to compare the number of plasma cells identified through using MUM-1 versus CD-138.

Statistical analysis

Statistical analysis was performed by using SPSS v.22.0 (IBM Corp., Armonk, NY, USA).. Continuous variables were reported as mean with standard deviation (SD), whereas qualitative variables were presented as absolute frequencies and percentages. Comparisons between categorical variables were tested by using contingency tables and chi-square test or Fisher's test when necessary. Comparisons between normally distributed continuous variables were made by using Student's t-test. A value of $p<0.05$ was considered as statistically significant. Receiver operating characteristic (ROC) curves were elaborated to assess the performance of CD-138 and MUM-1 immunohystochemistry to detect CE (by using the combination of histological and hysteroscopic diagnoses as reference standard). The agreement was statistically assessed between the two immunohistochemical techniques (i.e. CD-138 and MUM-1 immunostaining), as well as between the evaluation of three pathologists for each single technique (i.e. separately for CD-138 and MUM-1 immunostaining). The measure of inter-observer agreement was expressed as the intraclass correlation coefficient (ICC). An ICC of 0.20 was considered as poor agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 substantial agreement, while a value of 0.81–1.00 was considered as perfect agreement. The ICC was calculated by using a linear mixed model.

-II: Association between endometrial polyps and chronic endometritis: is it time for a paradigm shift in the pathophysiology of endometrial polyps in pre-menopausal women? Results of a systematic review and meta-analysis.

Study design

This is a systematic review of published data. Review was reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (47).

As it was a review of published data, institutional review board approval was not required.

Search strategy

Electronic databases (Sciencedirect, Medline, Scopus, Embase, the Cochrane library, Clinicaltrials.gov, EU Clinical Trials Register and World Health Organization Inter-national Clinical Trials Registry) were searched until 30th September 2021 (without date restriction).

Key search terms were: chronic endometritis OR endometrial inflammation OR endometrial plasma cells AND endometrial polyps OR endometrial proliferation OR endometrial lesions OR polypoid endometrium OR endometrial polyposis. The electronic search and the eligibility of the studies were independently assessed by two of the authors (A.V., R.C.).

Inclusion criteria

All the studies assessing the association between CE and EPs were evaluated. All studies (experimental and observational) reported in English language were eligible. Meeting abstracts were also eligible for inclusion. CE was defined according to the criteria applied in the original articles. Studies evaluating other types of endometrial inflammation (such as acute, subacute or tubercular endometritis) were excluded.

Study Selection and Data Extraction

Two authors (A.V., M.C.) independently assessed the inclusion criteria and study selection. Disagreements were discussed with a third reviewer (E.C.).

Data extraction was performed by two independent investigators (R.C., C.M.S.). Data extracted included study features (design, setting, objectives, main findings), population characteristics (age, inclusion criteria, pre-menopausal/post-menopausal, infertile/fertile), and the criteria for CE diagnosis. When studies involved a control group considered negligible for the endpoints of the review, inherent data was not extracted. A manual search of reference lists of studies was performed to avoid missing relevant publications. One Author (E.C.) reviewed the selection and data extraction process. The results were then com-pared, and any disagreement discussed and resolved by consensus.

Study outcomes

The primary outcome of this study was to evaluate the prevalence of CE in women with EPs. Secondary outcome was to determine the prevalence of CD-138 positive EPs among EPs.

Tertiary outcomes were: to compare the prevalence of CE in women with EPs versus women with a non-polypoid endometrium; to compare the prevalence of CE in women with a single EP versus women with multiple EPs.

Data Synthesis and Analysis

We reported all descriptive characteristics of study including study design, study aim, year of publication, study setting, type and number of patients, criteria and techniques for achieving CE diagnosis as well as study results. Data analysis was completed by two Authors (A.V., M.N.). For the primary and secondary outcomes, statistical analysis was performed using MedCalc 16.4.3 (Ostend, Belgium). Proportion method was performed, and forest plot was derived for meta-analysis. The proportion of patients was analyzed at a 95% confidence interval (CI). For the tertiary outcomes, the analysis was conducted by using Review Manager version 5.3 (Nordic Cochrane Centre, Cochrane Collaboration, Denmark). The study outcomes were expressed using odds ratio (OR) with 95 % confidence interval (95 % CI). P value lower than 0.05 were statistically significant. The I^2 statistics was used to assess heterogeneity. Degree of heterogeneity was considered as low when I^2 was < 30%, moderate if between 30% and 50%, and high if I^2 was >50%. Random-effects model (DerSimonian and Laird method) was applied to meta-analyses. Subgroup and sensitivity analysis were also planned in order to explore the sources of heterogeneity across studies (when at least three studies when included in meta-analysis).

-III: Whether chronic endometritis modifies the endometrial interface for embryo implantation at transmission electron microscopy: a case-control study. (Ongoing study)

Study design and participants

This is an observational study conducted at the IVF Unit of the University of Padua from January 2020 (still ongoing). We included patients referred to our IVF Unit due to infertility, defined as the failure of achieving an ongoing pregnancy after twelve months of sexual intercourse.

All the patients who received any antibiotic treatments during the three months before the infertility consultation were excluded. Patients were included regardless of the number of previous IVF attempts. Only women with regular menstrual periods were included.

Hysteroscopy and endometrial sampling

Hysteroscopy and endometrial sampling were performed with the same approach described in the section 2. The biopsies were graded as "positive" for CE if there was ≥5 plasma cells identified per 20 high-power fields (HPFs). Women with "mild CE" (namely 1-4 plasma cells per 20 HPF) were excluded.

In the following menstrual cycle, all the patients underwent a second endometrial biopsy (without hysteroscopy) in the mid luteal phase of the cycle by using a Novak curette. The day for performing endometrial biopsy was calculated as follows: Mean length of the menstrual cycle during the last six months (days) – 7 days. Tissue samples were then divided in two aliquots. The first aliquot underwent histological analysis in order to confirm the mid-secretory endometrial dating (based on Noyes criteria) (4). The second aliquot was used for evaluation at transmission electron microscopy (TEM).

Study Outcome

The aim of the study is to evaluate any differences in the morphology of mid-secretory endometrium in women with severe CE compared to women without CE.

Descriptive analysis

For TEM analysis, endometrial fragments obtained from CE women and controls were fixed in cold Karnovsky's fixative and maintained at 5 °C for 2 hours. Tissue was washed and fixed in 1% buffered osmium tetroxide for 2 hours. After a repeat wash with cacodylate buffer, tissue was dehydrated with alcohol and incubated in propilene oxide. All tissue was embedded in Araldite resin for at least two days. Ultrathin sections (600 µm) were obtained at ultramicrotome, mounted on copper grids and stained with lead citrate and uranyl acetate. Subsequently, they were observed and photographed at TEM. Particular attention was pointed on cellular organoids (pinopodes, clia and microvilli) in order to evaluate any differences between groups.

Results

The reporting of the results of the PhD project follows the same order applied in the methods section.

-I: Accuracy and reliability of MUM-1 immunostaining for the identification of endometrial plasma cells in chronic endometritis: head to head comparison with CD-138. A multi-centre study.

Descriptive results

The demographic characteristics of the study population are displayed in **Table 1**.

Age, years (mean ± SD)	39.12 ± 4.55
Parity (mean ± SD)	0.86 ± 1.21
BMI (Kg/m²)	23.85 ± 3.17
Indications for hysteroscopy, n (%)	
Infertility	92 (47.67%)
Repeated miscarriage	37 (19.17%)
Abnormal uterine bleeding	25 (12.95%)
Suspicion of uterine polyps or myomas	39 (20.21%)
CE at hysteroscopy, n (%)	76 (39.40%)
CE at histology, n (%)	54 (28.00%)
CE at immunohistochemistry with CD-138, n (%)	71 (36.80%)
CE at immunohistochemistry with MUM-1, n (%)	65 (33.70%)

Table 1: Baseline characteristics of patients included in the study. (n=193)

Values are given as mean ± Standard Deviation or number (%) as appropriate. CE: Chronic endometritis

261 patients were initially assessed for eligibility. After the exclusion of 68 women, a total number of 193 patients were included in the study. Of these, n=92 (47.67%) suffered from infertility and n=37 (19.17%) had a history of recurrent miscarriages. Remaining patients were referred to our centers due to abnormal uterine bleeding (n=25, 12.95%) or the suspicion of endometrial polyps or myomas (n=39, 20.21%).

Hysteroscopy identified CE in 76 women (39.40%), while istology diagnosed CE in 54 women (28.00%). Cumulatively, hysteroscopy and histology diagnosed CE in 84 patients (43.52%), of whom n=46 were positive to both techniques (23.83%). CD-138 immunostaining was positive for CE in 71 cases (36.80%) and MUM-1 immunostaining was positive for CE in 65 cases (33.70%). The total number of patients in whom CD-138 or MUM-1 immunostaining were positive was 76 (39.40%). Both CD-138 and MUM-1 revealed the presence of plasma cells in n=60 patients (31.09%). At least one technique among histology, hysteroscopy, CD-138 and MUM-1 immunohistochemistry was positive in 97 cases (50.26%), with high prevalence in

women with recurrent miscarriage (n=26/37, 70.27%) and infertility (n=53/92, 57.61%). Notably, results of CD-138 and MUM-1 did not match in 16 cases (**Table 2**).

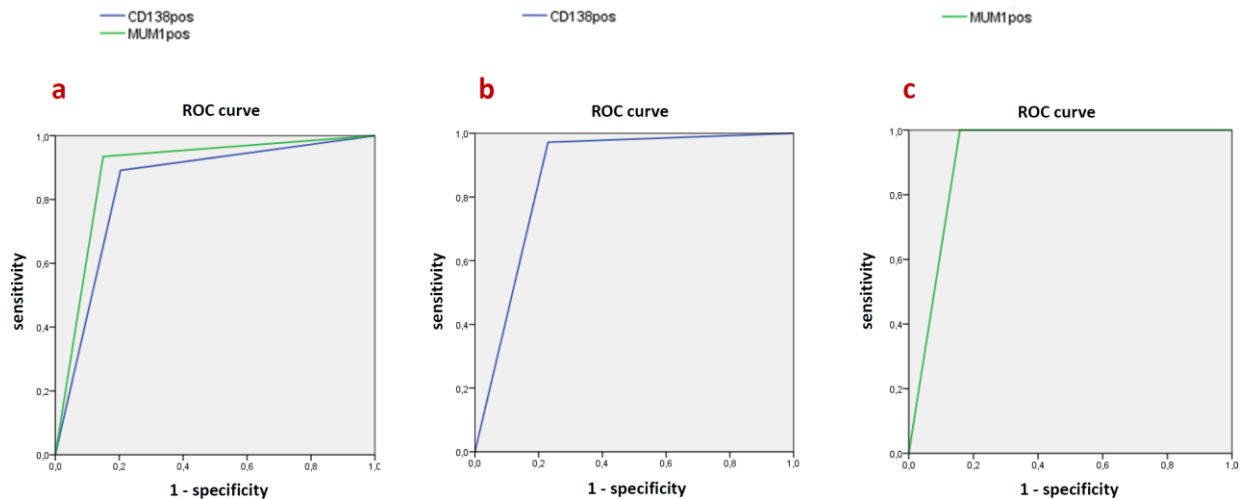|		Chronic endometritis (histology plus hysteroscopy)		Total
		Positive	Negative	
Chronic endometritis at CD-138 immunostaining	Positive	58	15	73
	Negative	4	92	96
	Total	62	107	169

Table 2: 2 x 2 table. Immunohistochemical staining with CD-138 as compared with reference standard (hystology plus hysteroscopy)

CD-138 and MUM-1: diagnostic accuracy and inter class correlation

Using hysteroscopy plus histology as reference standard, sensitivity and specificity of CD-138 and MUM-1 were respectively 89.13%, 79.59% versus 93.48% and 85.03%. The overall diagnostic accuracy of MUM-1 and CD-138 were similar (AUC=0.893 vs AUC= 0.844; **Figure 1a**).

Using a combination of hysteroscopy, histology and CD-138 immunohistochemistry as reference standard, sensitivity and specificity of MUM-1 were 100% and 84.21%. The overall diagnostic accuracy of MUM-1 was AUC= 0.921 (**Figure 1b**). Conversely, using hysteroscopy plus histology plus MUM-1 as refence standard, sensitivity and specificity of CD-138 were 95.35% and 80.00%. %. The overall diagnostic accuracy of CD-138 was AUC= 0.877 (**Figure 1c).** The intercorrelation coefficient for single measurements between CD-138 and MUM-1 was high (ICC=0.831, 0.761-0.881, 95%CI). Importantly, MUM-1 showed similar but higher closeness of agreement with histology (ICC=0.800 [0.743-0.846, 95%CI]) and hysteroscopy (ICC=0.592 [0.492-0.677, 95%CI]) as compared to those observed for CD-138 immunohistochemistry (ICC=0.696 [0.615-0.762, 95%CI] and ICC=0.463 [0.344-0.567, 95%CI] with histology and hysteroscopy, respectively).

Figure 1 (a-c): ROC analyses: a) diagnostic accuracy of MUM-1 and CD-138 immunohistochemistry for chronic endometritis using histology plus hysteroscopy as reference standard; b) diagnostic accuracy of CD-138 immunohistochemistry for chronic endometritis using a combination of MUM-1 immunohistochemistry, histology and hysteroscopy as reference standard; c) diagnostic accuracy of MUM-1 immunohistochemistry for chronic endometritis using a combination of CD-138 immunohistochemistry, histology and hysteroscopy as reference standard.

Inter-observer reliability of CD-138 and MUM-1 immunohistochemistry

The inter-observer variability between three pathologists was assessed for 86 patients. Although the agreement was fair with respect to both techniques, MUM-1 showed higher inter-observer agreement than CD-138 (ICC=0.902 [0.843-0.943, 95% CI] versus ICC=0.809 [0.712-0.881, 95% CI]).

*Number of plasma cells detected by CD-138 and MUM-1 immunochemistry*

Among those women in whom MUM-1 and CD-138 were both positive for CE, MUM-1 allowed the detection of higher number of plasma cells/20hpf than CD-138 (6.50 [SD 4.80] vs 5.05 [SD 3.37]; p=0.017).

Analysis of cases where CD-138 and MUM-1 immunohistochemistry provided conflicting results

The results of CD-138 and MUM-1 staining did not match in 16 cases (Table 2), of whom in 5 women CE was revealed by MUM-1 but not by CD-138. Notably, in all those cases the total

number of plasma cells counted was ≤3. In a single patient, both histology and hysteroscopy were negative. In the remaining cases, at least one technique between histology and hysteroscopy was positive.

Differently, in the 11 cases for which CD-138 showed immunoreactivity but MUM-1 did not, both hysteroscopy and histology were negative. In all those cases, the total number of plasma cells counted was ≤3 (**Figure 2**).
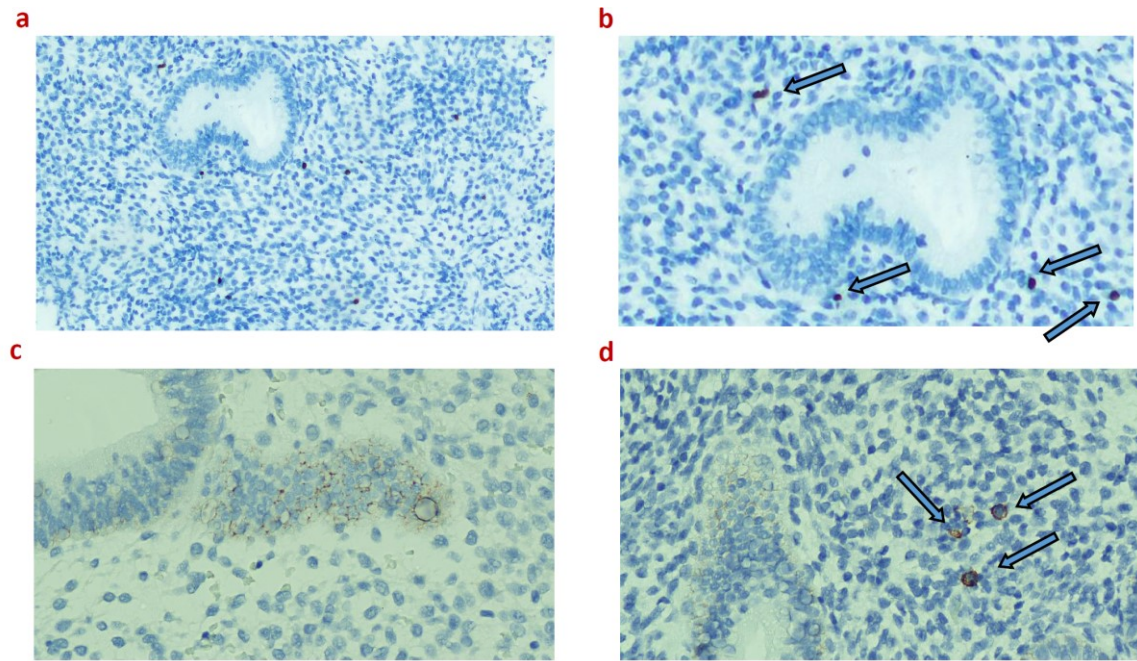

Figure 2 (a-d): MUM-1 (figures 2a, b) and CD-138 stains (figures 2c, d) demonstrating the presence of plasma cells within endometrial stroma (blue arrows). Note the endometrial glandular/surface reaction to CD-138 antibodies (figure 2c) but not to MUM-1 antibodies (figure 2a).

-II: Association between endometrial polyps and chronic endometritis: is it time for a paradigm shift in the pathophysiology of endometrial polyps in pre-menopausal women? Results of a systematic review and meta-analysis.

Study Selection

The literature search initially identified 1264 articles, after removing duplicates. The titles of these manuscripts were screened, resulting in 75 studies potentially eligible for inclusion. Of the manuscripts identified, 61 studies were excluded after the evaluation of the abstracts and 14 studies were further evaluated. Six studies were excluded after the evaluation of full text (48-53).

Finally, a total number of eight studies were included in this present review (54-60). Study flow diagram is displayed in **Figure 3**.
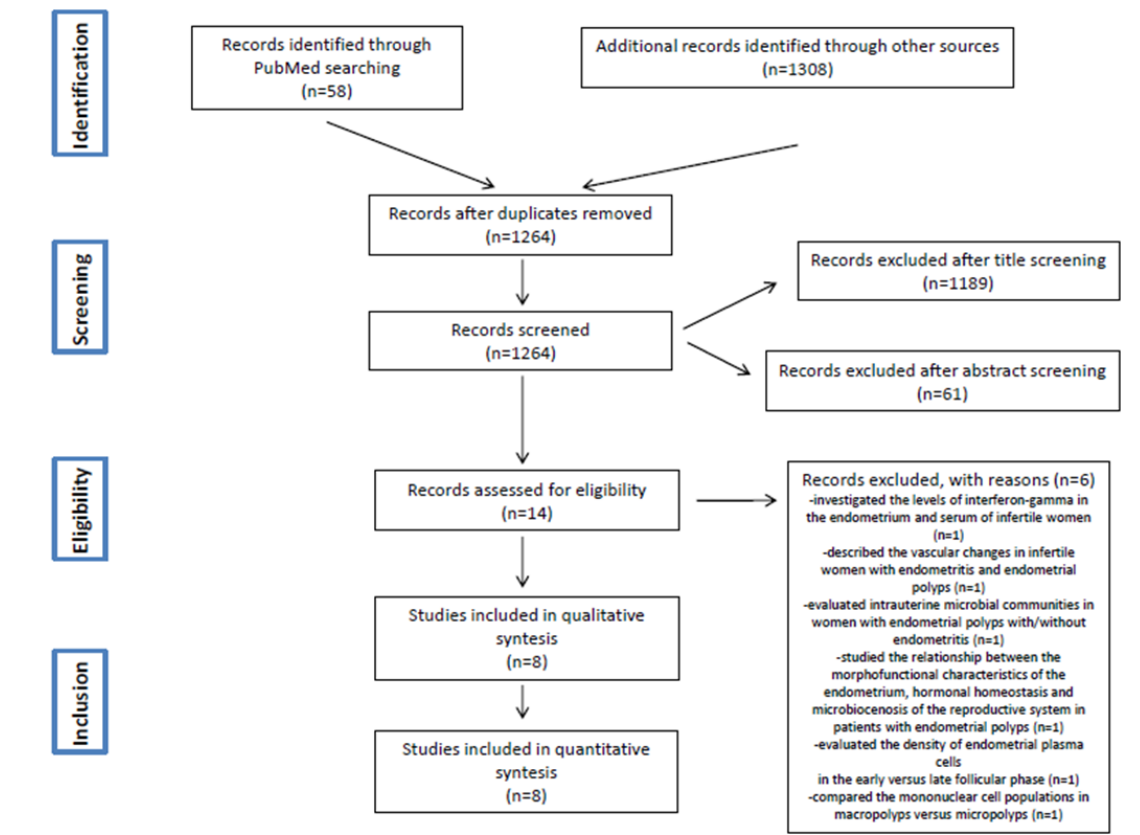
Figure 3. PRISMA Flow-Diagram.

Included studies

All the studies were monocentric. Five were retrospective cohort or case-control studies. Two were cross-sectional studies. Sklyarova et al 2020 did not report any information about the design of their study (55). Main characteristics of included studies, patients and study aims are summarized in **Table 3-4**.

Study ID	Study design	Country	Patients (number)	Women characteristics	CE definition	Study outcomes
Song et al 2018 (57)	Retrospective cohort study	China	1551	Premenopausal women with abnormal uterine bleeding or reproductive failure.	≥1 CD-138 positive plasma cell per 10 HPF	to examine the prevalence of chronic endometritis in a consecutive series of endometrial biopsies, and to identify confounding variables that may affect the prevalence of chronic endometritis.
Cicinelli et al 2019 (60)	Retrospective case-control study	Italy	480	Premenopausal women with AUB. **Group A:** n=240 women with EPs (diagnosed at hysteroscopy and histology) **Group B:** included 240 patients without evidence of EPs at hysteroscopy.	>1 CD-138 positive plasma cell per 10 HPF	to investigate the correlation between endometrial polyps (EPs) and chronic endometritis (CE).
Volodarsky-Perel et al	Retrospective cohort	Canada	277	Patients undergoing hysteroscopic	≥1 plasma cells per	1) to evaluate the prevalence of CE in

2019 (61)				polipectomy	10 HPF	infertile
				Group A: Infertile (n=137)		women with EPs compared with infertile women with an EPs.
				Group B: Fertile (n=140)		2) to investigate the prevalence of CE in women with primary infertility compared with those with secondary infertility.
Inaba et al 2020 (56)	Retrospective case-control study	Japan	40	4 groups of 10 patients each by the shape of the polyp (sessile type or pedunculated type) and Dienogest treatment prior to the operation.	>5 CD138-positive cells per 10 HPF	to investigate the effects of Dienogest on the proliferation and inflammation of endometrial polyps.
Sklyarova et al 2020 (55)	na	Ukraine	133	Reproductive age women with reproductive health disorders

Group I: 30 patients

with recurrent pregnancy loss | na | to analyze the incidence of chronic endometritis in women of reproductive age with reproductive health disorders. |

				Group II: 47 women with primary infertility		
				Group III: 36 women who had a polyp or endometrial polyps detected during routine ultrasound.		
				Control group: 20 women		
Guo et al 2021 (59)	Cross-sectional study	China	277	Premenopausal patients who have undergone hysteroscopic inspection with gynecologic conditions for different reasons	≥5 CD138-positive cells in 10 HPF	To determine whether single endometrial polyp (EP) or multiple EP (polyp number ≥ 6) are associated with CE
				Group A: single EP: n=82		
				Group B: ≥ 6		

Author	Study design	Country	N	Population	CE definition	Objective
				EPs: n=92 **Control group:** n=103		
Kuroda et al 2021 (54)	Cross-sectional study	Japan	222	Infertile patients undergoing hysteroscopic polipectomy **-Group A**: women with CE who received doxicicline after polypectomy: n=62 **-Group B**: women with CE who did not receive doxicicline after polypectomy**: n=160.**	≥5 CD138-positive cells in 10 HPF	To compare the therapeutic effects of hysteroscopic polypectomy with and without doxycycline treatment on CE.
Nomiyama et al 2021 (58)	Retrospective cohort study	Japan	245	Women with a suspicion of EPs undergone diagnostic hysteroscopy **Group 1**: 38 patients with	≥10 CD138-positive cells in 20 HPF	To determine the prevalence of CE in groups 1, 2, and 3

CD138 + EPs

Group 2: 31 patients with CD138- EPs

Group 3: no EPs

Table 3. General Features of included studies.

Study ID	Main findings
Song et al 2018 (57)	The prevalence of CE was increased in women with recurrent implantation failure, abnormal uterine bleeding and endometrial hyperplasia compared with those without the respective conditions, and also significantly higher in the proliferative stage of menstrual cycle compared with the luteal phase. Women with EPs had a global prevalence of CE of 28.7%. ©
Cicinelli et al 2019 (60)	-EPs were commonly associated with CE in the premenopausal women suffering from AUB (64.1%). -Moreover, the majority of EPs were positive for CD-138 staining (76.7%), suggesting a possible hidden association between chronic inflammation and EPs. ©
Volodarsky-Perel et al 2019 (61)	-The prevalence of CE in the group of infertile women was significantly higher than that in the control group (22.6% vs 8.6%; p = 001). - Women with primary infertility and those with secondary infertility showed no difference in CE prevalence. ©
Inaba et al 2020 (56)	-Dienogest prescription prior to hysteroscopic surgery of EPs has inhibitory effects on cellular proliferation. -Patients with EPs might be significantly complicated by CE. 80% of EPs showed CD-138 positivity. ©
Sklyarova et al 2020 (55)	1. In patients with habitual miscarriage, primary infertility, and women in the planning of pregnancy and endometrial polyps, a high frequency of bacterial vaginosis and recurrent inflammatory diseases of the lower parts of the reproductive system chronic endometritis was noted (p>0.01). 2. In immunohistochemical examination of the endometrium, CE was diagnosed in 80% of patients with habitual miscarriage, in 55% of women with primary infertility and in 61% of women when planning pregnancy and EPs (p>0.01). ©

Guo et al 2021 (59)	Multiple EPs were positively associated with CE among reproductive-aged women (58.7%) compared to single EP (28%) and controls (29.1%), suggesting a possible hidden etiopathogenetic link between chronic inflammation and multiple EPs. ©
Kuroda et al 2021 (54)	92.6% of women with EPs had CE. Most CE patients with endometrial polyps had been cured by polypectomy without doxycycline (88.8% vs 58.1%). Clinical pregnancy rate within 6 months was higher in women who did not receive antibiotics (63.2% vs 43.8%). ©
Nomiyama et al 2021 (58)	Infertile patients with EPs have higher prevalence of CE compared to those without EPs. Women with CD-138 positive EPs have higher rate of CE compared to those with CD-138 negative EPs and those without EPs (68.4% vs 32.2% vs 28.3%).

Table 4. Main Findings of included studies.

*Study in abstract form   © Full text studies.

CE: chronic endometritis   EPs: Endometrial polyps.

Patients

The total number of patients evaluated was 3225. Studies included patients with re-productive issues (infertility, recurrent miscarriage), AUB or miscellaneous populations undergoing hysteroscopic procedures.

Diagnosis of CE

In all studies, the diagnosis of CE was based on the demonstration of endometrial plasma cells by using CD-138 immunohistochemistry. Diagnostic criteria for CE varied among studies. The most common criteria applied were the detection of ≥5 plasma cells in 10 HPF (54, 55, 56, 59) and ≥1 plasma cells in 10 HPF (57, 60, 61) by using CD-138 immunostaining (See Table 1).

Synthesis of results

Primary outcome: Prevalence of CE in women with EPs

Meta-analysis on the prevalence of CE in women with EPs was conducted based on data from seven studies (54, 55, 57-61). A total number of 1248 patients with EPs were included. The prevalence of CE ranged from 28.71% in the study by Song et al (57) to 92.59% in the study by Kuroda et al (54). The pooled proportion was 51.35% (95% CI, 27.24%–75.13%; I^2 = 98.72%) (**Figure 4**).
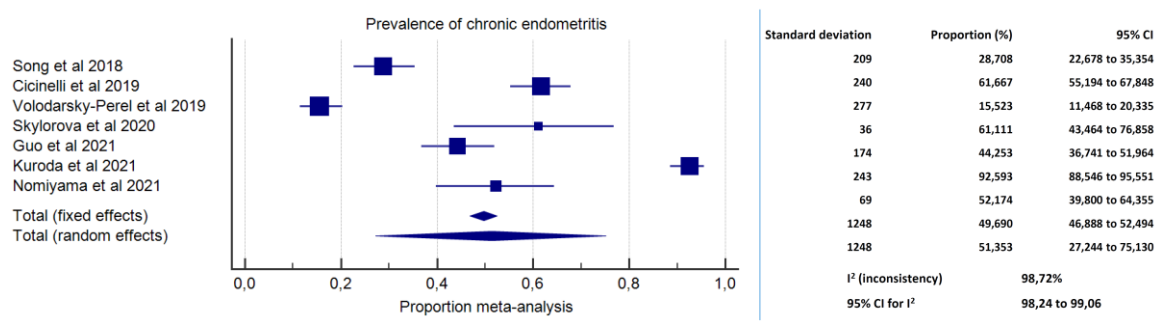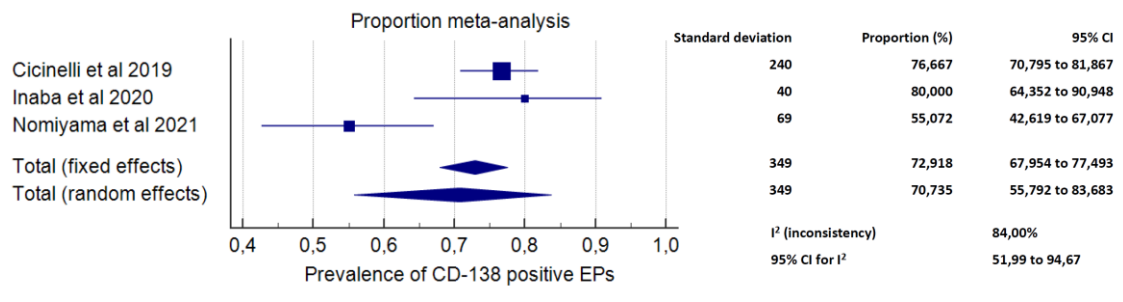

Figure 4. Forest Plot. Prevalence of chronic endometritis in pre-menopausal women with endometrial polyps.

Secondary outcome: prevalence of CD-138 positive EPs among EPs

Meta-analysis included data from three studies. A total number of 349 EPs were analysed (56, 58, 60). The prevalence of CD-138 positive EPs among EPs ranged from 55.07% in the study

by Nomiyama et al (58) to 80.00% in the study by Inaba et al. (56). The pooled proportion was 70.73% (95% CI, 55.73%–83.68%; I^2 = 84.00%) (**Figure 5**)

Figure 5. Forest plot. Proportion of CD-138 immunoreactive endometrial polyps.

Tertiary outcomes: Prevalence of CE in women with EPs compared to women with a non-polypoid endometrium

Data on 872 women (n=483 with EPs and n=389 with a non-polypoid endometrium) from three studies (58-60) showed a significantly higher prevalence of CE in women with EPs as compared to women with a non-polypoid endometrium (OR 3.07, 95% CI 1.59-5.95, I^2=77%, p=0.0008; **Figure 6**).
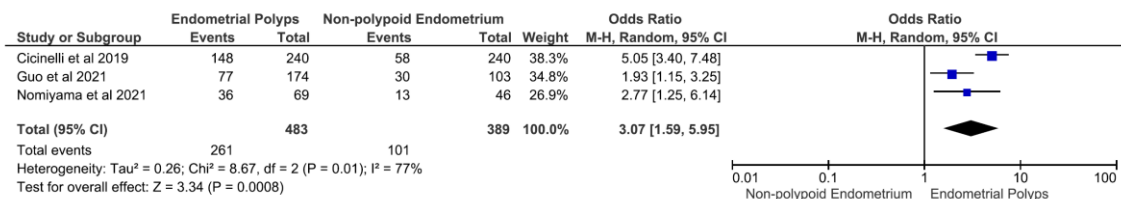
Figure 6. Forest plot. Women with endometrial polyps versus women with a non-polypoid endometrium: prevalence of chronic endometritis.

Prevalence of CE in Women with a Single EP Versus Women with Multiple EPs

Meta-analysis was not feasible for this outcome. Data on 174 women (n = 92 with multiple EPs [≥3]; n = 82 with a single EP) from a single study (59) revealed a significantly higher prevalence of CE in women with multiple EPs compared to women with a single EP (OR 3.43, 95% CI 1.83–6.46, p = 0.0001; data not shown).

Investigation of Sources of Heterogeneity across Studies

Sub-analyses were feasible for the primary outcome of our study. Splitting the analyses based on the diagnostic criteria for CE did not reduce the statistical heterogeneity. Meta-analysis of

studies in which CE was diagnosed with ≥5 plasma cells in 10 HPF (n = 486 patients) (54, 55, 56, 59) found a pooled prevalence of CE of 65.54% (95% CI 27.03%–94.77%; I^2 = 98.62%; data not shown). Differently, meta-analysis of studies in which the diagnosis of CE was based on the identification of ≥1 plasma cells in 20 HPF (n = 706 patients) (57, 60, 61) found a pooled prevalence of CE of 34.25% (95% CI 10.60–63.18%; I^2 = 98.46%; data not shown). Secondary analyses based on population characteristics were not feasible.

*-III: Whether chronic endometritis modifies the endometrial interface for embryo implantation at transmission electron microscopy: a case-control study. (Ongoing study)*

For descriptive purposes, the results about four patients will be reported (n=2 with CE and n=2 controls).

In all four patients the histological dating was appropriate (mid-secretory) according to Noyes criteria (4). In women without CE, the microscopic features of the endometrium at TEM were similar to those described in literature about physiological endometrium.
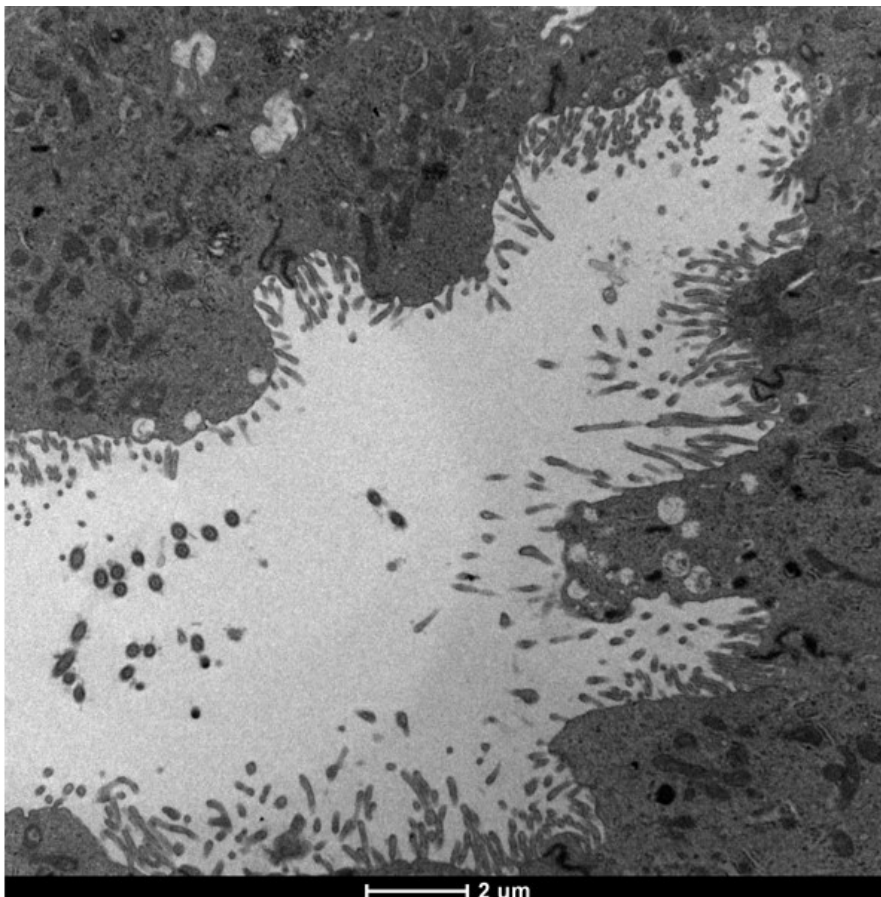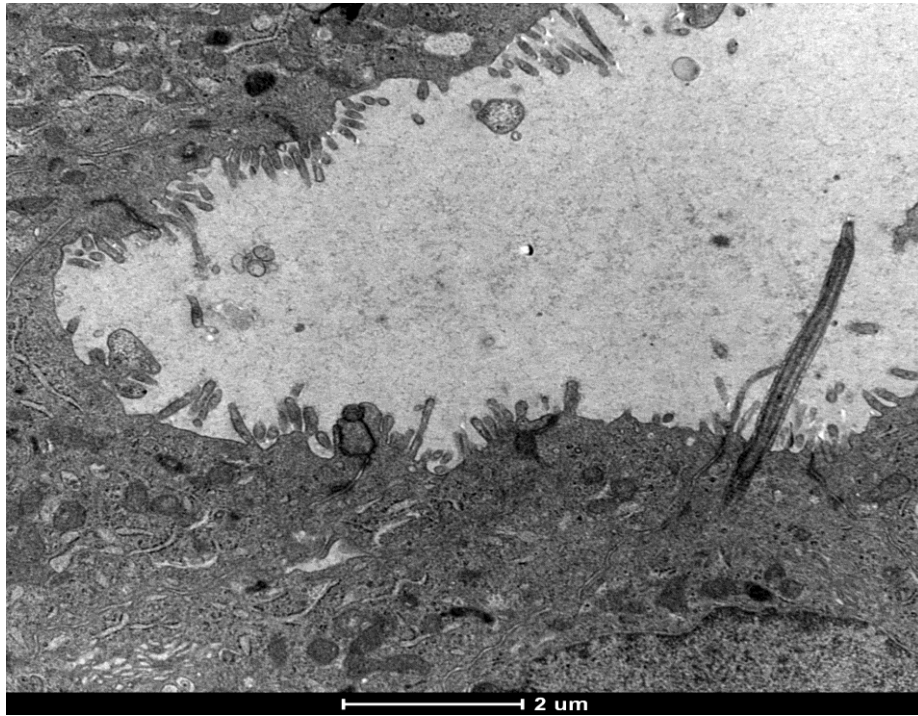

Figure 7. Transmission electron microscopy: Endometrium without chronic endometritis (Patient 1)
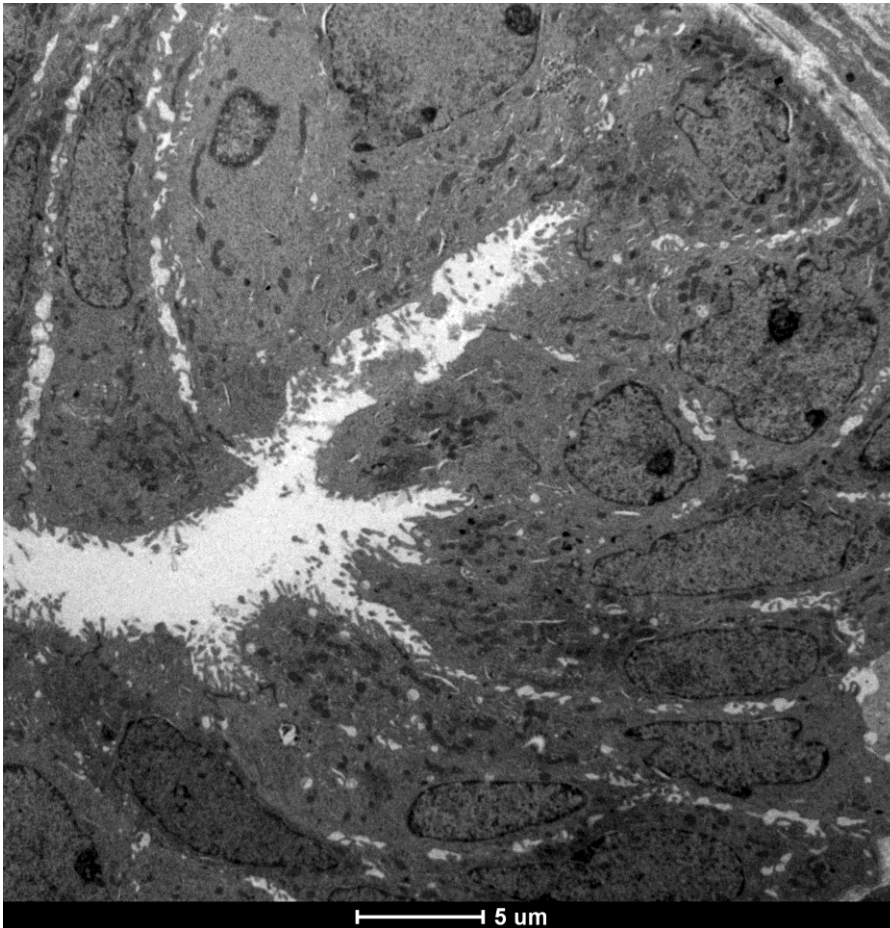
Figure 8. Transmission electron microscopy: Endometrium without chronic endometritis (Patient 2)

In **Figures 7-8**, two TEM pictures of healthy endometria are displayed. Pinopodes, namely protrusions of the uterine luminal epithelium present during the window of implantation, are clearly visible. Pinopodes display a regular suface, with a few micro-extensions (cilia, microvilli). Luminal cells are polarized in apico-basal fashion. Tight junctions between cells are well-marked, mitocondria and nucleoles are of small size. Inside the pinopodes, a small number of cytoplasmatic organelles are visible, including endosomes and glycogen aggregates.

Figure 9. Transmission electron microscopy: Endometrium with chronic endometritis (Patient 3)

Figure 10. Transmission electron microscopy: Endometrium with chronic endometritis (Patient 4)

Figures 9-10 show two TEM pictures of endometria in women with CE. Typical luminal protrusions of the WOI are less pronounced or absent, especially in **Figure 9**. Superficial

cellular extensions (in particular microvilli) are numerous in a similar fashion to the proliferative phase of the cycle. Luminal cells are non-polarized, with elongated mitochondria. In **Figure 10**, a considerable number of organelles are visible within the pinopode, with a dominance of endosomial vescicles and lisosomes as compared to healthy endometria (**Figures 7-8**).

Discussion

This PhD project includes different studies that are variously inter-twined, whose hearth is the aim to improve the available knowledge on CE. The multiplicity of objectives pursued in such a three-year project is the result of the active collaboration between many researchers, including some members of the "International Working Group for Standardization of Chronic Endometritis Diagnosis" (involving 23 researchers from eleven European, Asian, North American and South American countries) (39).

The decision to opt for a multifaceted PhD project arouse from the sincere need to evaluate CE all-round, including efforts for improving diagnosis and attempts for providing a deeper knowledge on the impact of CE on human reproduction and endometrial health.

The abovementioned working group, founded in 2017, had the original purpose of characterizing CE more precisely in order to establish comprehensible, standardized diagnostic criteria. Despite our efforts, this goal was not achieved as all the available diagnostic techniques for CE suffer from some limitations. Hysteroscopic examination, whose usefulness for CE diagnosis was demonstrated in a recent trial by our group (39), suffers from the fact that it is an operator-dependent method. As a matter of fact, the accuracy of hysteroscopy for CE diagnosis suffers from many additional problems, including the severity of CE, the quality of the equipment available, the day of the cycle in which the examination is performed, the presence of additional intrauterine pathologies (egs. polyps, myomas, hyperplasia, synechiae) and patient compliance during the examination. For these reasons, histopathological examination currently represents the diagnostic gold standard for CE. However, it should not be neglected that histopathological examination examines only a few fragments of the entire endometrium (unlike the hysteroscopic examination which allows the evaluation of the entire uterine cavity), and therefore is strictly dependent on the amount of tissue collected, as well as on the exact site of tissue sampling. For example, in the case of focal CE, a blind sampling can result in the inadvertent capture of healthy tissue, therefore leading to false negative diagnosis (40, 42).

Another limitation of the histopathological examination is inherent to the staining method used. Since the number of plasma cells in the entire section is often small, they can remain unnoticed or confused with other cellular elements. For this reason, the use of the immunomarker CD-138

has become widespread in recent years, as it makes the identification of plasma cells easier as compared to traditional staining methods (42, 43). Nonetheless, as discussed in the introduction, this marker is not specific for plasma cells and can sometimes lead to false positive diagnoses. For the latter reason, one of the objectives of this PhD project was to evaluate the accuracy and reproducibility of MUM-1 as a novel marker for the diagnosis of chronic endometritis.

Another unsolved issue for the diagnosis of CE is inherent to the fact that this condition has been traditionally associated with endometrial plasma cells. Theoretically, some CE cases may not display plasma cells infiltration in the endometrium. In particular, the duration of the inflammatory insult and different etiological agents may generate variable histological changes and local immune responses.

What we know today, the presence of CE has been associated with greater risk of reproductive failure (35), although not all the patients with CE are infertile (61). Thus, CE may exert variable damage on endometrial receptivity in different clinical situations. Starting from this principle, another goal of our project was to compare the TEM expression of endometrial organoids in the mid-secretory endometrium of women with CE versus women without CE. This study (still ongoing) opens the way for a further line of research on the microstructural abnormalities associated with CE.

A further objective of our project was inherent to previous studies published by our group on the correlation between CE and endometrial proliferative lesions (60). Specifically, we aimed to provide a summary of evidence on the causal relationship between CE and EPs. This study opens the way to future therapeutic strategies for EPs, potentially involving the combination of medical and surgical approaches. Moreover, this study raises the need for further research aimed to clarify the role of CE in carcinogenic processes of the endometrium.

Main findings and implications

-I: Accuracy and reliability of MUM-1 immunostaining for the identification of endometrial plasma cells in chronic endometritis: head to head comparison with CD-138. A multi-centre study.

Our study included a total number of 193 reproductive-aged patients for whom hysteroscopy plus endometrial biopsy was indicated due to infertility, recurrent miscarriages, abnormal uterine bleeding or the suspicion of polyps or myomas. For all patients, endometrial preparations with CD-138 and MUM-1 staining were realized besides traditional histology.

By using all the techniques (hysteroscopy, histology, CD-138 and MUM-1 immunohistochemistry), our study revealed high prevalence of CE in the entire sample of

patients, especially in those women suffering from reproductive disorders. These findings were in line with our previous data on women suffering from infertility, repeated embryo implantation failure and recurrent miscarriage (33, 38, 63). On the other hand, as compared to our recent study on women with intrauterine pathologies (60), this present study found lower prevalence of CE. However, the two studies were different in terms of research design and population characteristics.

Interestingly, in this study the accuracy for CE diagnosis of MUM-1 immunohistochemistry was similar to CD-138 staining (AUC=0.893 vs AUC= 0.844 by using a combination of hysteroscopy and histology as reference standard), and the closeness of agreement between the two techniques was high (ICC=0.831, 0.761-0.881, 95%CI). Notably, sensitivity and specificity of MUM-1 were slightly higher than CD-138 immunohistochemistry (93.48% and 85.03% vs 89.13% and 79.59%, respectively). These findings were further confirmed by individual matching of CE diagnoses achieved by the two immunohistochemical techniques with those obtained by hysteroscopy and histology (ICC=0.800 and ICC=0.592 vs ICC=0.696 and ICC=0.463 for MUM-1 and CD-138, respectively). These important results deserve some considerations concerning the peculiar characteristics of the techniques under comparison. As previously mentioned, CD-138 antigen is constitutively expressed not only by plasma cells, but also by the epithelial cells of endometrial glandular surface (41, 43). Therefore, epithelial cells can appear as marked elements in the endometrial staining with anti-CD-138 monoclonal antibodies, generating a focal or diffuse background reaction. In such cases, a correct diagnosis is based on cell morphology-based criteria. Nevertheless, we must stress that some endometrial sections with CD-138 staining may be misleading (egs. isolated or fragmented glandular epithelial cells in the sample) and potentially result in misidentification of epithelial cells as stromal plasma cells. Unlike CD-138, MUM-1 is not expressed by epithelial glandular cells but mainly by lymphoid cells, especially plasmacytes and activated B/T lymphocites (45, 46). Therefore, this latter staining technique does not display background reaction, but it should be noted that it marks also other lymphoid lineage cells that can be found in the endometrial stroma. Here, once again differential diagnosis is achieved through morphological criteria, where plasma cells display eccentric nuclei with clock-face chromatin and abundant cytoplasm. Thereby, the inner characteristics of the techniques employed in our study may potentially explain their imperfect agreement in terms of CE diagnoses. Additionally, the lack of background reaction with MUM-1 staining (but not with CD-138 staining) may justify our findings about higher inter-observer agreement between pathologists, as well as the higher mean number of plasma cells counted in paired samples by using MUM-1. In our opinion, these specific differences between CD-138 and MUM-1 immunohistochemistry emphasize the potential advantages of using both techniques contextually in CE. However, we need to stress that our results, although exciting, need further confirmation in other institutional settings. To

the best of our knowledge, this was the first study investigating the accuracy and reliability of MUM-1 immunohistochemistry for the diagnosis of CE. Originality, rigorous methodology, the multi-centre setting of the study and the relatively large sample size were the points of strength. The retrospective design was the main points of weakness of our study.

-II: Association between endometrial polyps and chronic endometritis: is it time for a paradigm shift in the pathophysiology of endometrial polyps in pre-menopausal women? Results of a systematic review and meta-analysis.

We found high prevalence of CE in pre-menopausal women with EPs (51.35%; 95% CI, 27.24%–75.13%), with high inconsistency (I^2 = 98.72%). Arguably, the high variability in CE prevalence across studies was ascribable to heterogeneity in the diagnostic criteria for CE and in populations' characteristics. High inconsistency however limits the overall accuracy of the prevalence estimates. Yet, we believe the data present a compelling argument for a renewed appraisal with a least one out of four pre-menopausal women with EPs will have concomitant CE (up to three women based on the 95% CI).

The results of the primary outcome were supported by secondary findings of our study. Indeed, exposure to EPs was associated with higher odds of being affected by CE compared to a non-polypoid endometrium (OR 3.07, 95% CI 1.59-5.95; p=0.0008), and exposure to multiple EPs increased the odds of CE compared to a single EP (OR 3.43, 95% CI 1.83-6.46, p=0.0001). Additionally, an extremely high prevalence of CD-138 immunoreactive plasma cells (ie. the typical markers of CE) within the EPs tissue was found (70.73%; 95% CI, 55.73%–83.68%).

The findings from this present meta-analysis are in agreement with the results of our previous study showing an association between CE and EPs (60). The pooled percentage of CD-138 positive EPs were similar (i.e. 70.73% and 76.7% in this review and in our previous study (60), respectively). Notably, in our previous study (60), we also found that women with CD-138 positive EPs were more likely to suffer from CE compared to those with CD-138 negative EPs (64.1% vs 30.7%; p < .0001). The study by Kitaya et al (48) was in partial contrast with these data. In their prospective cross-sectional study on infertile patients undergoing hysteroscopy, the authors reported no plasma cells infiltration within specimens from EPs. Additionally, plasma cell density was significantly higher in micropolypoid tissue compared to EPs tissue. Nevertheless, as per authors' admission, the sample size of the study was small (n=23 women with EPs). Moreover, that study had not epidemiological purposes, but aimed exclusively to compare the mononuclear cell infiltration in macropolyps compared to micropolyps. Thus, women with EPs and concomitant signs of CE at hysteroscopy (egs. micropolyps) were plausibly excluded from the study.

If we depict a connecting line between the results here presented, an interdependent correlation between EPs and CE may be postulated. In particular, EPs and CE could rep-resent two consequent steps of a common pathological process. Such a theory was first proposed by Carvalho et al, in 2013 (52). The authors conducted a descriptive histological study on endometrial samples from 435 infertile women. Among those patients, the 24.6% were diagnosed CE and the 15.9% showed histological changes that were highly suspicious for CE. Interestingly, the authors observed specific endometrial vascular changes that were common in CE and EPs. In particular, 70% of vascular alterations in CE corresponded to vessel wall hyaline thickening, with similar morphology to the thick-walled vessels along the vascular axis of EPs. Moreover, the alteration was frequently associated with thrombi and/or fibrinoid degeneration of the vessel wall, potentially suggesting a vasculopathy caused by inflammation.

From a molecular point of view, chronic inflammation may promote EPs development by distorting the signaling pathways that control endometrial tissue proliferation. In this respect, we recently found an altered endometrial expression of genes involved in inflammatory, cell proliferation, and apoptosis processes in women with CE (including vascular endothelial growth factors [A, B, C], epidermal growth factor, tumor necrosis factor, interferon-γ, transforming growth factor β-1, cell division control protein variant, cyclin D3, cyclin B1, BCL-2 associated X protein, BCL-2 associated X protein transcript variant alpha and interleukin-12), with a dominance of proliferative and anti-apoptotic activity (37). These factors in CE may potentially justify the gradual development of endometrial proliferative lesions emerging from a scenario of chronic inflammation. Notably, similar pathogenic pathways have been previously highlighted with regard to the development of colorectal polyps in patients with inflammatory bowel diseases (64).

A pathogenetic link between CE and EPs is supported by indirect observations, too. In a retrospective study on 323 infertile women, Sun et al (65) found a significantly higher prevalence of EPs in women with fallopian tube obstruction compared to those with patent fallopian tubes (42.9% vs 20.1%, p<0.0001), suggesting a correlation between EPs and history of pelvic inflammatory disease (PID). Likewise, in women with hydrosalpinx, which is a frequent complication of PID, an increased risk of CE has been demonstrated (66). Common actors in the development of all these conditions may be some infectious agents. Notably, quantitative and qualitative alterations of endometrial microbiome were clearly demonstrated in CE, with a dominance of gram negative and intracellular bacteria (67-69). In women with EPs, significant alterations of endometrial microbiome were also found (ie. with increased percentages of Lactobacillus, Bifidobacterium, Gardnerella, Streptococcus, Alteromonas and decreased expression of Pseudomonas) (49). However, those changes in endometrial microbiome were not definitely attributed to causative bacteria of CE.

Last but not least, CE and EPs commonly coexist with endometriosis. Endometriosis is a chronic, estrogen-dependent disorder characterized by inflammatory reaction in the ectopic and eutopic endometrium (70-72). Endometriosis is a major cause of infertility and recurrent pregnancy loss after natural and medically assisted conception (73, 74). In a previous study, we found high prevalence of CE in severe endometriosis compared to controls (38.5% vs. 14.1%) (75). Our findings were in line with those from other studies on different ethnic groups (76, 77). Interestingly, infertile women with endometriosis show also higher prevalence of EPs compared to controls without endometriosis (68.35% vs. 20.51%; Shen et al study (77)). Theoretically, the correlation between endometriosis, CE and EPs may rely on common infectious and inflammatory factors (78). In women with endometriosis, Khan et al (79) found high levels of lipopolysaccharide (LPS) in peritoneal fluids and high contamination of Esherichia coli and LPS in the menstrual effluents. The "hypothesis of bacterial contamination in endometriosis" implies an initial bacterial stimulus, likely with Gram-bacteria (due to their high LPS content), followed by sustained inflammation which allows establishing a vicious circle involved in the development of endometriosis (78-80). In line with this theory, Chadchan et al (81) recently found that the administration of broad-spectrum antibiotics led to size reduction of endometriotic lesions in murine models. However, even if a common thread between endometriosis, CE and EPs is possible, we must recognize that current evidence is insufficient to draw firm conclu-sions.

 Our findings may have several future clinical and research implications. In pre-menopausal women with EPs undergoing hysteroscopy, physicians may be advised about the need of excluding CE diagnosis through a careful inspection of the uterine cavity and by undertaking targeted endometrial samples of the non-polypoid endometrium. This suggestion may be especially relevant in those patients with infertility or recurrent pregnancy loss, in whom CE persistence after surgery may be associated with poor reproductive prognosis. The investigation of CE may be relevant also for those women who are not seeking for pregnancy, as the persistence of CE after polypectomy may theoretically in-crease the risk of EPs recurrence in the long term. When CE is concomitant with EPs, the administration of antibiotics may be considered in addition to polypectomy. In this respect, a recent study by Kuroda et al (54) concluded discouraging empiric antibiotic therapy with doxicicline (200 mg daily for 14 days) in addition to polypectomy as it reduced the recovery rate from CE as well as the clinical pregnancy rate within six months in a group of infertile women undergoing IVF (54). However, these results are not conclusive for many reasons, including the observational study design, the short-term follow-up, the lack of data about live births. Additionally, a "scratching effect" due to hysteroscopy immediately prior to IVF (82-84) could not be excluded. Thus, the administration of antibiotic therapy in addition to polypectomy in infertile women with concomitant CE prior to infer-tility treatments needs future investigation. Similarly, the role of

antibiotic therapy against CE for the prevention of EPs recurrence after surgery is still undefined.

-III: Whether chronic endometritis modifies the endometrial interface for embryo implantation at transmission electron microscopy: a case-control study. (Ongoing study)

In humans, there is a finite time during which the endometrium becomes receptive for blastocyst attachment, known as the (WOI) (85). This period occurs during the mid-luteal phase of the menstrual cycle and lasts for a maximum of four days. During the WOI, a series of cellular modifications of the uterine luminal epithelium physiologically occur in response to sex steroids (86). Previous studies at TEM illustrated specific changes in microvilli and the formation of endometrial protrusions termed "pinopodes" or "uterodomes" (87, 88).

Pinopodes are 5-10 μm protrusions of the apical plasma membrane of uterine epithelial cells (89). Compared to other epithelial plasma membrane protrusions like microvilli and cilia (approximately from 0.2 μm to 90 nm in size) pinopodes are larger and appear only during WOI (90). The role of pinopodes in embryo implantation is still partly unclear (88, 89). Different authors demonstrated a role of these protrusions in the regulation of endoluminal fluid and in exocytosis of lipids, proteins, mRNAs, and miRNAs required for embryo-uterine crosstalk (91, 92). Other authors demonstrated that pinopodes are involved in the down-regulation of anti-adhesive glycoproteins (such as the anti-adhesive molecule mucin-16). Specifically, by unmasking the integrins of the luminal cells, pinopodes would promote the adhesion of the embryo on the endometrial surface (93, 94).

In women with CE, no study have still evaluated the expression of pinopodes and other organoids at the time of WOI. Preliminary data on four patients (n=2 with CE; n=2 without CE) from our ongoing study on TEM are intriguing. In women without CE, organoids were physiologically expressed (86). Pinopodes were well-pronounced, with a few cilia and microvilli. Conversely, in women with CE pinopodes were less pronounced or absent, with higher number of micro-extensions. Intriguingly, the endometrium in CE did not show the typical cell polarization of the WOI. Additionally, higher number of endosomial vescicles and lisosome were observed in women with CE.

The lack of pinopodes expression in CE deserves some reflections. As discussed above, the development of pinopodes physiologically occurs in response to stimulation of sex hormone receptors at the endometrial level (95-97). A previous study by Wu et al (98) found lower expression of endometrial decidualization markers such as prolactin and insulin-like growth factor binding protein-1 (IGFBP-1) in women with CE. Additionally, the authors identified increased expressions of estrogen receptors α and β, as well as progesterone receptors A and B

in the stromal cells of CE patients in comparison to controls. Whilst the effects of IGFBP-1 and PRL on pinopodes formation are still unknown, Martel et al and Singh et al (99, 100) demonstrated that estradiol rise causes pinopodes regression. Thus, we may speculate that the lack of expression of pinopodes in CE may be due to a hyperestrogenic background. Similarly, this scenario could be responsible of abnormal persistence of microvilli and cilia in women with CE.

Although preliminary findings are intriguing, we must stress that this study is still ongoing and full results are required to draw firm conclusions regarding any possible alteration of organoids in the mid-secretory endometrium of women with CE.

Conclusions

-I: Accuracy and reliability of MUM-1 immunostaining for the identification of endometrial plasma cells in chronic endometritis: head to head comparison with CD-138. A multi-centre study.

In our experience, immunohistochemistry for MUM-1 and CD-138 showed similar accuracy for detecting endometrial stromal plasma cells. Notably, MUM-1 showed higher reliability in the paired comparison of the individual samples than CD-138. Therefore, MUM-1 immunohistochemistry may represents a novel, promising add-on technique for improving the diagnostic process in women with CE.

-II: Association between endometrial polyps and chronic endometritis: is it time for a paradigm shift in the pathophysiology of endometrial polyps in pre-menopausal women? Results of a systematic review and meta-analysis.

Our systematic literature review found high prevalence of CE in pre-menopausal women suffering from EPs. Notably, the majority of EPs showed plasma cells infiltration at CD-138 immuno-histochemistry. The risk of CE was higher in women with EPs compared to women with a non-polypoid endometrium, as well as in those with three or more EPs compared to those with a single EP. Based on available evidence in pre-menopausal women, CE and EPs may have a dependent relationship and may represent two consequent steps of a common pathological process. However, a conclusive pathogenetic association between these two entities has not been demonstrated yet.

-III: Whether chronic endometritis modifies the endometrial interface for embryo implantation at transmission electron microscopy: a case-control study. (Ongoing study)

Mid-secretory endometrium of women with CE showed lower expression of pinopodes and higher expression of microvilli and cilia compared to controls. These endometrial features in CE

are potentially due to abnormal endometrial response to sex hormones during the WOI and may account for defective endometrial receptivity. As our study is still ongoing, full results will be required to draw firm conclusions regarding the expression of endometrial organoids in the mid-secretory endometrium of women with CE.

Financial disclosures: Nothing to declare.

Institutional disclosures: Member of the expert group of "Società Italiana della Riproduzione Umana" (SIRU) for the development of Italian Guidelines on the diagnosis and management of infertility from 2019 to 2021.

Two studies extracted from this PhD project were published in 2021 (101, 102).

References

1. Critchley HOD, Maybin JA, Armstrong GM, Williams ARW. Physiology of the Endometrium and Regulation of Menstruation. Physiol Rev. 2020 Jul 1;100(3):1149-1179.

2. Maybin JA, Critchley HO. Menstrual physiology: implications for endometrial pathology and beyond. Hum Reprod Update. 2015 Nov-Dec;21(6):748-61.

3. Hickey M, Fraser IS. The structure of endometrial microvessels. Hum Reprod. 2000 Aug;15 Suppl 3:57-66.

4. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1: 3–25, 1950.

5. Gargett CE, Chan RW, Schwab KE. Endometrial stem cells. Curr Opin Obstet Gynecol. 2007 Aug;19(4):377-83.

6. Figueira PG, Abrão MS, Krikun G, Taylor HS. Stem cells in endometrium and their role in the pathogenesis of endometriosis. Ann N Y Acad Sci. 2011 Mar;1221(1):10-7.

7. van der Horst PH, Wang Y, van der Zee M, Burger CW, Blok LJ. Interaction between sex hormones and WNT/β-catenin signal transduction in endometrial physiology and disease. Mol Cell Endocrinol. 2012 Jul 25;358(2):176-84.

8. Karaoğlan Ö, Kuyucu Y, Ürünsak İF, Gümürdülü D, Tap Ö. Morphological features of the secretory phase endometrium in women with unexplained infertility. Ultrastruct Pathol. 2021 May 4;45(3):243-256.

9. Pantos K, Nikas G, Makrakis E, Stavrou D, Karantzis P, Grammatis M. Clinical value of endometrial pinopodes detection in artificial donation cycles. Reprod Biomed Online. 2004 Jul;9(1):86-90.

10. Nikas G. Endometrial receptivity: changes in cell-surface morphology. Semin Reprod Med. 2000;18(3):229-35.

11. Horne AW, Lalani EN, Margara RA, Ryder TA, Mobberley MA, White JO. The expression pattern of MUC1 glycoforms and other biomarkers of endometrial receptivity in fertile and infertile women. Mol Reprod Dev. 2005 Oct;72(2):216-29.

12. Evans J, Hutchison J, Salamonsen LA, Greening DW. Proteomic Insights into Endometrial Receptivity and Embryo-Endometrial Epithelium Interaction for Implantation Reveal Critical Determinants of Fertility. Proteomics. 2020 Jan;20(1):e1900250.

13. Park DW, Yang KM. Hormonal regulation of uterine chemokines and immune cells. Clin Exp Reprod Med. 2011 Dec;38(4):179-85.

14. Salamonsen LA, Kovacs GT, Findlay JK. Current concepts of the mechanisms of menstruation. Baillieres Best Pract Res Clin Obstet Gynaecol. 1999 Jun;13(2):161-79.

15. Katzorke N, Vilella F, Ruiz M, Krüssel JS, Simón C. Diagnosis of Endometrial-Factor Infertility: Current Approaches and New Avenues for Research. *Geburtshilfe Frauenheilkd*. 2016;76(6):699-703.

16. Sanders B. Uterine factors and infertility. J Reprod Med. 2006 Mar;51(3):169-76.

17. Hatasaka H. Clinical management of the uterine factor in infertility. Clin Obstet Gynecol. 2011 Dec;54(4):696-709.

18. Pehlivan T, Rubio C, Rodrigo L, Romero J, Remohi J, Simón C, Pellicer A. Impact of preimplantation genetic diagnosis on IVF outcome in implantation failure patients. Reprod Biomed Online. 2003 Mar;6(2):232-7.

19. Segal TR, Kim K, Mumford SL, Goldfarb JM, Weinerman RS. How much does the uterus matter? Perinatal outcomes are improved when donor oocyte embryos are transferred to gestational carriers compared to intended parent recipients. Fertil Steril. 2018 Oct;110(5):888-895.

20. Taylor E, Gomel V. The uterus and fertility. Fertil Steril. 2008 Jan;89(1):1-16.

21. Carre G, Naud C. *Tissier: Recherches sur la flore intestinale des nourrissons (état normal et pathologique)*. Paris: Dissertation; (1900)

22. Benner M, Ferwerda G, Joosten I, van der Molen RG. How uterine microbiota might be responsible for a receptive, fertile endometrium. Hum Reprod Update. 2018 Jul 1;24(4):393-415.

23. Baker JM, Chase DM, Herbst-Kralovetz MM. Uterine Microbiota: Residents, Tourists, or Invaders?. *Front Immunol*. 2018;9:208. Published 2018 Mar 2. doi:10.3389/fimmu.2018.00208

24. Moller BR, Kristiansen FV, Thorsen P, Frost L, Mogensen SC. Sterility of the uterine cavity. *Acta Obstet Gynecol Scand* (1995) 74(3):216–9.10.3109/00016349509008942.

25. 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) 93(1):102–8.10.1111/aogs.12296.

26. Zervomanolakis I, Ott HW, Hadziomerovic D, Mattle V, Seeber BE, Virgolini I, et al. Physiology of upward transport in the human female genital tract. *Ann N Y Acad Sci* (2007) 1101:1–20.10.1196/annals.1389.032.

27. Moreno I, Franasiak JM. Endometrial microbiota-new player in town. Fertil Steril. 2017 Jul;108(1):32-39.

28. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Bieda J, Chaemsaithong P, Miranda J, Chaiworapongsa T, Ravel J. The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term. Microbiome. 2014 May 27;2:18.

29. Ralph SG, Rutherford AJ, Wilson JD. Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. BMJ. 1999 Jul 24;319(7204):220-3.

30. Liu Y, Ko EY, Wong KK, Chen X, Cheung WC, Law TS, Chung JP, Tsui SK, Li TC, Chim SS. Endometrial microbiota in infertile women with and without chronic endometritis as diagnosed using a quantitative and reference range-based method. Fertil Steril. 2019 Oct;112(4):707-717.e1.

31. Kitaya K, Takeuchi T, Mizuta S, Matsubayashi H, Ishikawa T. Endometritis: new time, new concepts. Fertil Steril. 2018;110:344-350.

32. Park HJ, Kim YS, Yoon TK, Lee WS. Chronic endometritis and infertility. Clin Exp Reprod Med. 2016;43:185-192.

33. Kimura F, Takebayashi A, Ishida M, Nakamura A, Kitazawa J, Morimune A, Hirata K, Takahashi A, Tsuji S, Takashima A, Amano T, Tsuji S, Ono T, Kaku S, Kasahara K, Moritani S, Kushima R, Murakami T. Review: Chronic endometritis and its effect on reproduction. J Obstet Gynaecol Res. 2019 May;45(5):951-960.

34. Kushnir VA, Solouki S, Sarig-Meth T, Vega MG, Albertini DF, Darmon SK, Deligdisch L, Barad DH, Gleicher N. Systemic Inflammation and Autoimmunity in Women with Chronic Endometritis. Am J Reprod Immunol. 2016 Jun;75(6):672-7.

35. Vitagliano A, Saccardi C, Noventa M, Di Spiezio Sardo A, Saccone G, Cicinelli E, Pizzi S, Andrisani A, Litta PS. Effects of chronic endometritis therapy on in vitro fertilization outcome in women with repeated implantation failure: a systematic review and meta-analysis. Fertil Steril. 2018 Jul 1;110(1):103-112.e1.

36. Buzzaccarini G, Vitagliano A, Andrisani A, Santarsiero CM, Cicinelli R, Nardelli C, Ambrosini G, Cicinelli E. Chronic endometritis and altered embryo implantation: a unified pathophysiological theory from a literature systematic review. J Assist Reprod Genet. 2020 Dec;37(12):2897-2911.

37. Cicinelli E, Vitagliano A, Loizzi V, De Ziegler D, Fanelli M, Bettocchi S, Nardelli C, Trojano G, Cicinelli R, Minervini CF, Leronni D, Viggiano L. Altered Gene Expression Encoding Cytochines, Grow Factors and Cell Cycle Regulators in the Endometrium of Women with Chronic Endometritis. Diagnostics (Basel). 2021 Mar 8;11(3):471.

38. Cicinelli E, Matteo M, Trojano G, Mitola PC, Tinelli R, Vitagliano A, Crupano FM, Lepera A, Miragliotta G, Resta L. Chronic endometritis in patients with unexplained infertility: Prevalence and effects of antibiotic treatment on spontaneous conception. Am J Reprod Immunol. 2018 Jan;79(1).

39. Cicinelli E, Vitagliano A, Kumar A, Lasmar RB, Bettocchi S, Haimovich S; International Working Group for Standardization of Chronic Endometritis Diagnosis. Unified diagnostic criteria for chronic endometritis at fluid hysteroscopy: proposal and reliability evaluation through an international randomized-controlled observer study. Fertil Steril. 2019 Jul;112(1):162-173.e2.

40. Li Y, Xu S, Yu S, Huang C, Lin S, Chen W, Mo M, Lian R, Diao L, Ding L, Zeng Y. Diagnosis of chronic endometritis: How many CD138^{+} cells/HPF in endometrial stroma affect pregnancy outcome of infertile women? Am J Reprod Immunol. 2020 Nov 5:e13369.

41. Parks RN, Kim CJ, Al-Safi ZA, Armstrong AA, Zore T, Moatamed NA. Multiple Myeloma 1 Transcription Factor Is Superior to CD138 as a Marker of Plasma Cells in Endometrium. Int J Surg Pathol. 2019 Jun;27(4):372-379.

42. Kannar V., Lingaiah HK., Sunita V,.Evaluation of Endometrium for Chronic Endometritis by Using Syndecan-1 in Abnormal Uterine Bleeding. J Lab Physicians. 15 2012;4,69–73.

43. Kitaya K, Yasuo T. Inter-observer and intra-observer variability in immunohistochemical detection of endometrial stromal plasmacytes in chronic endometritis. Exp Ther Med. 2013 Feb;5(2):485-488.

44. Kitaya K, Yasuo T. Immunohistochemistrical and clinicopathological characterization of chronic endometritis. Am J Reprod Immunol.2011;66:410–5.

45. Falini B, Fizzotti M, Pucciarini A, Bigerna B, Marafioti T, Gambacorta M, Pacini R, Alunni C, Natali-Tanci L, Ugolini B, Sebastiani C, Cattoretti G, Pileri S, Dalla-Favera R, Stein H. A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. Blood. 2000 Mar 15;95(6):2084-92. Erratum in: Blood. 2013 Oct 3;122(14):2523. PMID: 10706878.

46. Wasco MJ, Fullen D, Su L, Ma L. The expression of MUM1 in cutaneous T-cell lymphoproliferative disorders. Hum Pathol. 2008 Apr;39(4):557-63.

47. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. **2009** Jul 21;6(7):e1000097.

48. Kitaya, K.; Tada, Y.; Taguchi, S.; Funabiki, M.; Hayashi, T.; Nakamura, Y. Local mononuclear cell infiltrates in infertile patients with endometrial macropolyps versus micropolyps. *Hum. Reprod.* **2012**, *27*, 3474–80.

49. Fang RL, Chen LX, Shu WS, Yao SZ, Wang SW, Chen YQ. Barcoded sequencing reveals diverse intrauterine microbiomes in patients suffering with endometrial polyps. Am J Transl Res. 2016;8(3):1581-1592.

50. Kosei N, Zakharenko N, Herman D. ENDOMETRIAL POLYPS IN WOMEN OF REPRODUCTIVE AGE: CLINICAL AND PATHOGENE-TIC VARIATIONS. Georgian Med News. 2017 Dec;(273):16-22.

51. Mollo A, Stile A, Alviggi C, Granata M, De Placido G, Perrella A, d'Antonio A, Cicinelli E. Endometrial polyps in infertile patients: do high concentrations of interferon-gamma play a role? Fertil Steril. 2011 Nov;96(5):1209-12.

52. 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. Eur J Obstet Gynecol Reprod Biol. 2013 Sep;170(1):152-6.

53. Tolani A, Ryan EE, Folkins AK, Lathi RB. DOES THE TIMING OF ENDOMETRIAL BIOPSY IMPACT THE DETECTION OF ENDOMETRIAL PLASMA CELLS? Fertility and Sterility, Volume 114, Issue 3, e192; 2020.

54. Kuroda K, Takamizawa S, Motoyama H, Tsutsumi R, Sugiyama R, Nakagawa K, Sugiyama R, Kuribayashi Y. Analysis of the therapeutic effects of hysteroscopic polypectomy with and without doxycycline treatment on chronic endometritis with endometrial polyps. Am J Reprod Immunol. 2021 Jun;85(6):e13392.

55. Sklyarova V, Kyshakevych I, Volosovsky P, Sklyarov P, Kupchak I. ; EPIDEMIOLOGICAL FEATURES OF CHRONIC ENDOMETRITIS IN REPRODUCTIVE AGE WOMEN WITH DISORDERS OF REPRODUCTIVE HEALTH. *Georgian Med News*. **2020** Jul-Aug;(304-305):27-32.

56. Inaba K, Wada-Hiraike O, Harada M, Hirota Y, Koga K, Fujii T, Osuga Y. Dienogest suppresses cellular proliferation status of endometrial polyps and acts differently depending on the morphological type. Womens Health (Lond). 2020 Jan-Dec;16:1745506520952003.

57. Song D, Feng X, Zhang Q, Xia E, Xiao Y, Xie W, Li TC. Prevalence and confounders of chronic endometritis in premenopausal women with abnormal bleeding or reproductive failure. Reprod Biomed Online. 2018 Jan;36(1):78-83.

58. Nomiyama M, Yamasaki F, Tokunaga M, et al. Endometrial polyps with increased plasma cells are associated with chronic endometritis in infertility patients: Hysteroscopic findings and post-polypectomy pregnancy rates. Reprod Med Biol. 2021;20(4):494-504.

59. Guo L, Gu F, Tan J, Luo L, Gao J, Zhou C. Multiple endometrial polyps is associated with higher risk of chronic endometritis in reproductive-aged women. J Obstet Gynaecol Res. 2021 Jan;47(1):389-396.

60. Cicinelli E, Bettocchi S, de Ziegler D, Loizzi V, Cormio G, Marinaccio M, Trojano G, Crupano FM, Francescato R, Vitagliano A, Resta L. Chronic Endometritis, a Common Disease Hidden Behind Endometrial Polyps in Premenopausal Women: First Evidence From a Case-Control Study. J Minim Invasive Gynecol. 2019 Nov-Dec;26(7):1346-1350.

61. Volodarsky-Perel A, Badeghiesh A, Shrem G, Steiner N, Tulandi T. Chronic Endometritis in Fertile and Infertile Women Who Underwent Hysteroscopic Polypectomy. J Minim Invasive Gynecol. 2020 Jul-Aug;27(5):1112-1118.

62. Li Y, Xu S, Yu S, Huang C, Lin S, Chen W, Mo M, Lian R, Diao L, Ding L, Zeng Y. Diagnosis of chronic endometritis: How many CD138^{+} cells/HPF in endometrial stroma affect pregnancy outcome of infertile women? Am J Reprod Immunol. 2021 May;85(5):e13369.

63. Cicinelli E, Matteo M, Tinelli R, Pinto V, Marinaccio M, Indraccolo U, De Ziegler D, Resta L. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. Reprod Sci. 2014 May;21(5):640-7.

64. Terzić J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology. 2010 Jun;138(6):2101-2114.e5.

65. Sun Y, Zhang J, Bai W. Higher Prevalence of Endometrial Polyps in Patients with Fallopian Tube Obstruction: A Case-control Study. *J Minim Invasive Gynecol*. **2019** Jul-Aug;26(5):935-940. doi: 10.1016/j.jmig.2018.07.024.

66. Holzer I, Ott J, Kurz C, Hofstetter G, Hager M, Kuessel L, Parry JP. Is Chronic Endometritis Associated with Tubal Infertility? A Prospective Cohort Study. J Minim Invasive Gynecol. 2021 Apr 21:S1553-4650(21)00188-6. Chen P,

67. Chen P, Guo Y, Fang C, Li T. Interaction Between Chronic Endometritis Caused Endometrial Microbiota Disorder and Endometrial Immune Environment Change in Recurrent Implantation Failure. Front Immunol. 2021 Oct 4;12:748447.

68. Chen W, Wei K, He X, Wei J, Yang L, Li L, Chen T, Tan B. Identification of Uterine Microbiota in Infertile Women Receiving in vitro Fertilization With and Without Chronic Endometritis. Front Cell Dev Biol. 2021 Aug 13;9:693267.

69. Moreno I, Cicinelli E, Garcia-Grau I, Gonzalez-Monfort M, Bau D, Vilella F, De Ziegler D, Resta L, Valbuena D, Simon C. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. Am J Obstet Gynecol. 2018 Jun;218(6):602.e1-602.e16.

70. Pirtea P, Cicinelli E, De Nola R, de Ziegler D, Ayoubi JM. Endometrial causes of recurrent pregnancy losses: endometriosis, adenomyosis, and chronic endometritis. Fertil Steril. 2021 Mar;115(3):546-560.

71. Vitale SG, Capriglione S, Peterlunger I, La Rosa VL, Vitagliano A, Noventa M, Valenti G, Sapia F, Angioli R, Lopez S, Sarpietro G, Rossetti D, Zito G. The Role of Oxidative Stress and Membrane Transport Systems during Endometriosis: A Fresh Look at a Busy Corner. Oxid Med Cell Longev. 2018 Mar 21;2018:7924021.

72. Freitag N, Pour SJ, Fehm TN, Toth B, Markert UR, Weber M, Togawa R, Kruessel JS, Baston-Buest DM, Bielfeld AP. Are uterine natural killer and plasma cells in infertility patients associated with endometriosis, repeated implantation failure, or recurrent pregnancy loss? Arch Gynecol Obstet. 2020 Dec;302(6):1487-1494.

73. Noventa M, Scioscia M, Schincariol M, Cavallin F, Pontrelli G, Virgilio B, Vitale SG, Laganà AS, Dessole F, Cosmi E, D'Antona D, Andrisani A, Saccardi C, Vitagliano A, Ambrosini G. Imaging Modalities for Diagnosis of Deep Pelvic Endometriosis: Comparison between Trans-Vaginal Sonography, Rectal Endoscopy Sonography and Magnetic Resonance Imaging. A Head-to-Head Meta-Analysis. Diagnostics (Basel). 2019 Dec 17;9(4):225.

74. Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, Takahashi K, Suginami H, Murakami T. The association between endometriosis and chronic endometritis. PLoS One. 2014 Feb 18;9(2):e88354.

75. Cicinelli E, Trojano G, Mastromauro M, Vimercati A, Marinaccio M, Mitola PC, Resta L, de Ziegler D. Higher prevalence of chronic endometritis in women with endometriosis: a possible etiopathogenetic link. Fertil Steril. 2017 Aug;108(2):289-295.e1.

76. Kim MR, Kim YA, Jo MY, Hwang KJ, Ryu HS. High frequency of endometrial polyps in endometriosis. J Am Assoc Gynecol Laparosc. 2003 Feb;10(1):46-8.

77. Shen L, Wang Q, Huang W, Wang Q, Yuan Q, Huang Y, Lei H. High prevalence of endometrial polyps in endometriosis-associated infertility. Fertil Steril. 2011 Jun 30;95(8):2722-4.e1.

78. García-Peñarrubia P, Ruiz-Alcaraz AJ, Martínez-Esparza M, Marín P, Machado-Linde F. Hypothetical roadmap towards endometriosis: prenatal endocrine-disrupting chemical pollutant exposure, anogenital distance, gut-genital microbiota and subclinical infections. Hum Reprod Update. 2020 Feb 28;26(2):214-246.

79. Khan KN, Kitajima M, Yamaguchi N, Fujishita A, Nakashima M, Ishimaru T, Masuzaki H. Role of prostaglandin E2 in bacterial growth in women with endometriosis. Hum Reprod 2012;27:3417–3424.

80. Kobayashi H, Higashiura Y, Shigetomi H, Kajihara H. Pathogenesis of endometriosis: the role of initial infection and subsequent sterile inflammation (review). Mol Med Rep 2014;9:9–15.

81. Chadchan SB, Cheng M, Parnell LA, Yin Y, Schriefer A, Mysorekar IU, Kommagani R. Antibiotic therapy with metronidazole reduces endometriosis disease progression in mice: a potential role for gut microbiota. Hum Reprod 2019;34:1106–1116.

82. Vitagliano A, Di Spiezio Sardo A, Saccone G, Valenti G, Sapia F, Kamath MS, Blaganje M, Andrisani A, Ambrosini G. Endometrial scratch injury for women with one or more previous failed embryo transfers: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril. 2018 Sep;110(4):687-702.e2.

83. Seval MM, Şükür YE, Özmen B, Kan Ö, Sönmezer M, Berker B, Atabekoğlu C. Does adding endometrial scratching to diagnostic hysteroscopy improve pregnancy rates in women with recurrent in-vitro fertilization failure? Gynecol Endocrinol. 2016 Dec;32(12):957-960.

84. Vitagliano A, Andrisani A, Alviggi C, Vitale SG, Valenti G, Sapia F, Favilli A, Martins WP, Raine-Ferring N, Polanski L, Ambrosini G. Endometrial scratching for infertile women undergoing a first embryo transfer: a systematic review and meta-analysis of published and unpublished data from randomized controlled trials. Fertil Steril. 2019 Apr;111(4):734-746.e2.

85. Harper MJ. The implantation window. Baillieres Clin Obstet Gynaecol. 1992 Jun;6(2):351-71.

86. Lessey BA. Endometrial receptivity and the window of implantation. Baillieres Best Pract Res Clin Obstet Gynaecol. 2000 Oct;14(5):775-88.

87. Luddi, A.; Pavone, V.; Semplici, B.; Governini, L.; Criscuoli, M.; Paccagnini, E.; Gentile, M.; Morgante, G.; De Leo, V.; Belmonte, G.; Zarovni, N.; Piomboni, P. Organoids of Human Endometrium: A Powerful In Vitro Model for the Endometrium-Embryo Cross-Talk at the Implantation Site. *Cells* **2020**, *9*, 1121.

88. Quinn KE, Matson BC, Wetendorf M, Caron KM. Pinopodes: Recent advancements, current perspectives, and future directions. Mol Cell Endocrinol. 2020 Feb 5;501:110644.

89. C.E. Quinn, R.F. Casper, Pinopodes: a questionable role in endometrial receptivity, *Human Reproduction Update*, Volume 15, Issue 2, March-April 2009, Pages 229–236.

90. Nikas G. Endometrial receptivity: changes in cell-surface morphology. Semin Reprod Med. 2000;18(3):229-35.

91. Tung HN, Parr EL, Parr MB. Endocytosis in the uterine luminal and glandular epithelial cells of mice during early pregnancy. Am J Anat. 1988 Jun;182(2):120-9.

92. Bentin-Ley U, Sjögren A, Nilsson L, Hamberger L, Larsen JF, Horn T. Presence of uterine pinopodes at the embryo-endometrial interface during human implantation in vitro. Hum Reprod. 1999 Feb;14(2):515-20.

93. Gipson IK, Blalock T, Tisdale A, Spurr-Michaud S, Allcorn S, Stavreus-Evers A, Gemzell K. MUC16 is lost from the uterodome (pinopode) surface of the receptive human endometrium: in vitro evidence that MUC16 is a barrier to trophoblast adherence. Biol Reprod. 2008 Jan;78(1):134-42. doi: 10.1095/biolreprod.106.058347. Epub 2007 Oct 17. Erratum in: Biol Reprod. 2013 Dec;89(6):149.

94. Li L, Xu BF, Chen QJ, Sun XX. Effects of hydrosalpinx on pinopodes, leukaemia inhibitory factor, integrin beta3 and MUC1 expression in the peri-implantation endometrium. Eur J Obstet Gynecol Reprod Biol. 2010 Aug;151(2):171-5.

95. Nikas G, Drakakis P, Loutradis D, Mara-Skoufari C, Koumantakis E, Michalas S, Psychoyos A. Uterine pinopodes as markers of the 'nidation window' in cycling women receiving exogenous oestradiol and progesterone. Hum Reprod. 1995 May;10(5):1208-13.

96. Sarantis L, Roche D, Psychoyos A. Displacement of receptivity for nidation in the rat by the progesterone antagonist RU 486: a scanning electron microscopy study. Hum Reprod. 1988 Feb;3(2):251-5.

97. Stavreus-Evers A, Mandelin E, Koistinen R, Aghajanova L, Hovatta O, Seppälä M. Glycodelin is present in pinopodes of receptive-phase human endometrium and is associated with down-regulation of progesterone receptor B. Fertil Steril. 2006 Jun;85(6):1803-11.

98. Wu D, Kimura F, Zheng L, Ishida M, Niwa Y, Hirata K, Takebayashi A, Takashima A, Takahashi K, Kushima R, Zhang G, Murakami T. Chronic endometritis modifies decidualization in human endometrial stromal cells. Reprod Biol Endocrinol. 2017 Mar 4;15(1):16.

99. Martel D, Monier MN, Roche D, Psychoyos A. Hormonal dependence of pinopode formation at the uterine luminal surface. Hum Reprod. 1991 Apr;6(4):597-603.

100. Singh MM, Trivedi RN, Chauhan SC, Srivastava VM, Makker A, Chowdhury SR, Kamboj VP. Uterine estradiol and progesterone receptor concentration, activities of certain antioxidant enzymes and dehydrogenases and histoarchitecture in relation to time of secretion of nidatory estrogen and high endometrial sensitivity in rat. J Steroid Biochem Mol Biol. 1996 Oct;59(2):215-24.

101. Cicinelli E, Haimovich S, De Ziegler D, Raz N, Ben-Tzur D, Andrisani A, Ambrosini G, Picardi N, Cataldo V, Balzani M, Cicinelli R, Noventa M, Marin L, Greco P, Resta L, Saccardi C, Buzzaccarini G, Vitagliano A; International Working Group for Standardization of Chronic Endometritis Diagnosis. MUM-1 immunohistochemistry has high accuracy and reliability in the diagnosis of chronic endometritis: a multi-centre comparative study with CD-138 immunostaining. J Assist Reprod Genet. 2021 Nov 17.

102. Vitagliano A, Cialdella M, Cicinelli R, Santarsiero CM, Greco P, Buzzaccarini G, Noventa M, Cicinelli E. Association between Endometrial Polyps and Chronic Endometritis: Is It Time for a Paradigm Shift in the Pathophysiology of Endometrial Polyps in Pre-Menopausal Women? Results of a Systematic Review and Meta-Analysis. Diagnostics (Basel). 2021 Nov 24;11(12):2182.