

Recent Developments on the Transmission of Human Life

19 to 21 January 2023

Berlin, Germany

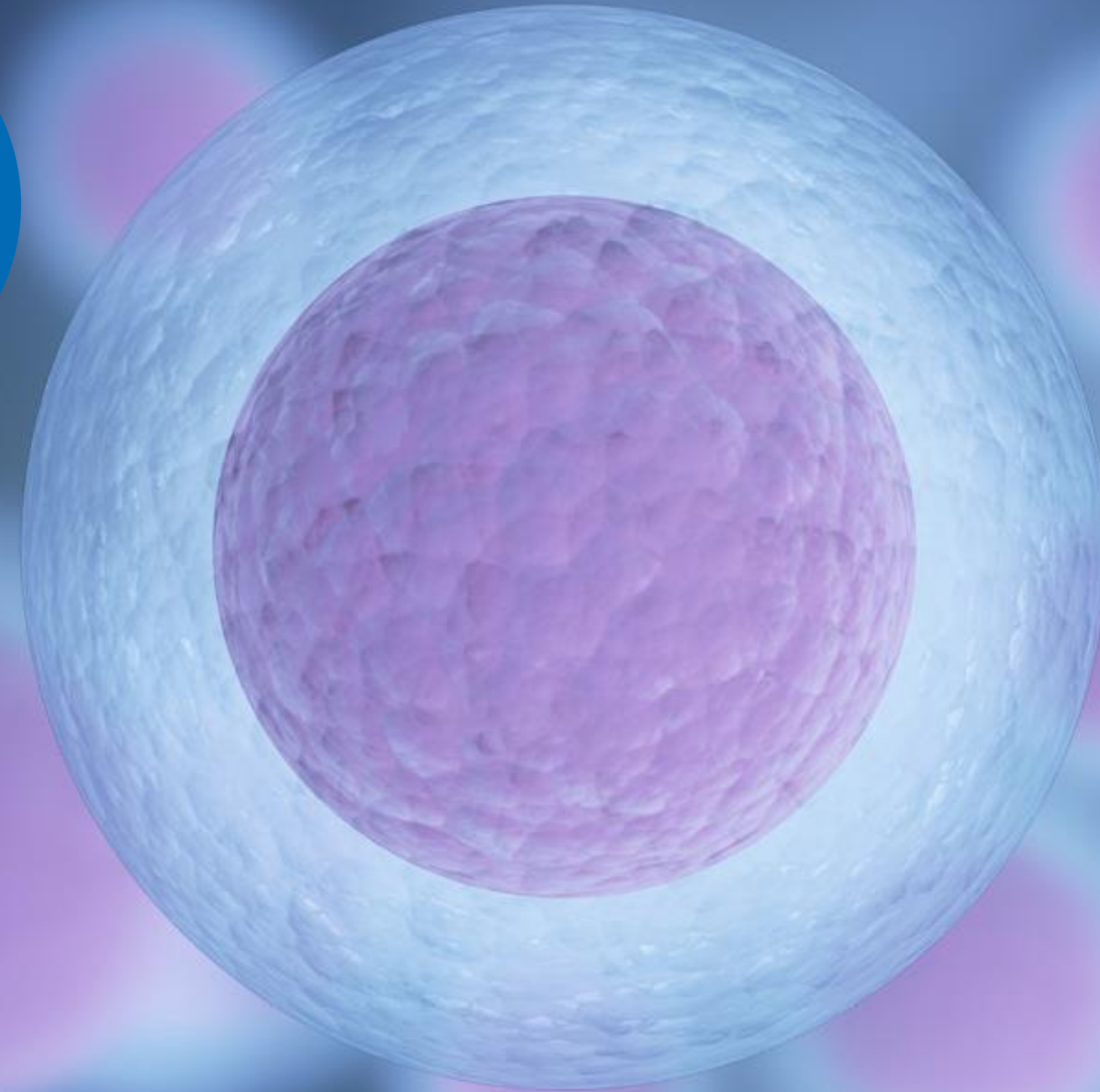
Welcome to all Participants



Recent Developments on the Transmission of Human Life

Endometritis and Embryos Implantation

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Italy



Faculty Disclosure

I have no potential conflict of interest to declare



MAIN POINTS

**Definition of chronic
endometritis (CE)**

Diagnosis of CE

**Impact on embryo
implantation: clinical studies**

Possible Therapy

MAIN POINTS

- Definition of chronic endometritis (CE)

What exactly is chronic endometritis?

DEFINITION

CE is a condition involving the breakdown of the peaceful co-existence between microorganisms and the host immune system in the endometrium.



DEFINITION

- Chronic endometritis is a chronic inflammation within the endometrial lining that depends on an **inbalance** **between the bacteria** present in uterine cavity.

DEFINITION

- Chronic endometritis is an inflammatory condition characterized by the **presence of plasma cells in the endometrium.**

Bayer-Garner and Korourian, 2001; Greenwood et al., 1981; Kasius et al., 2011; Kitaya et al., 2011; Smith et al., 2010

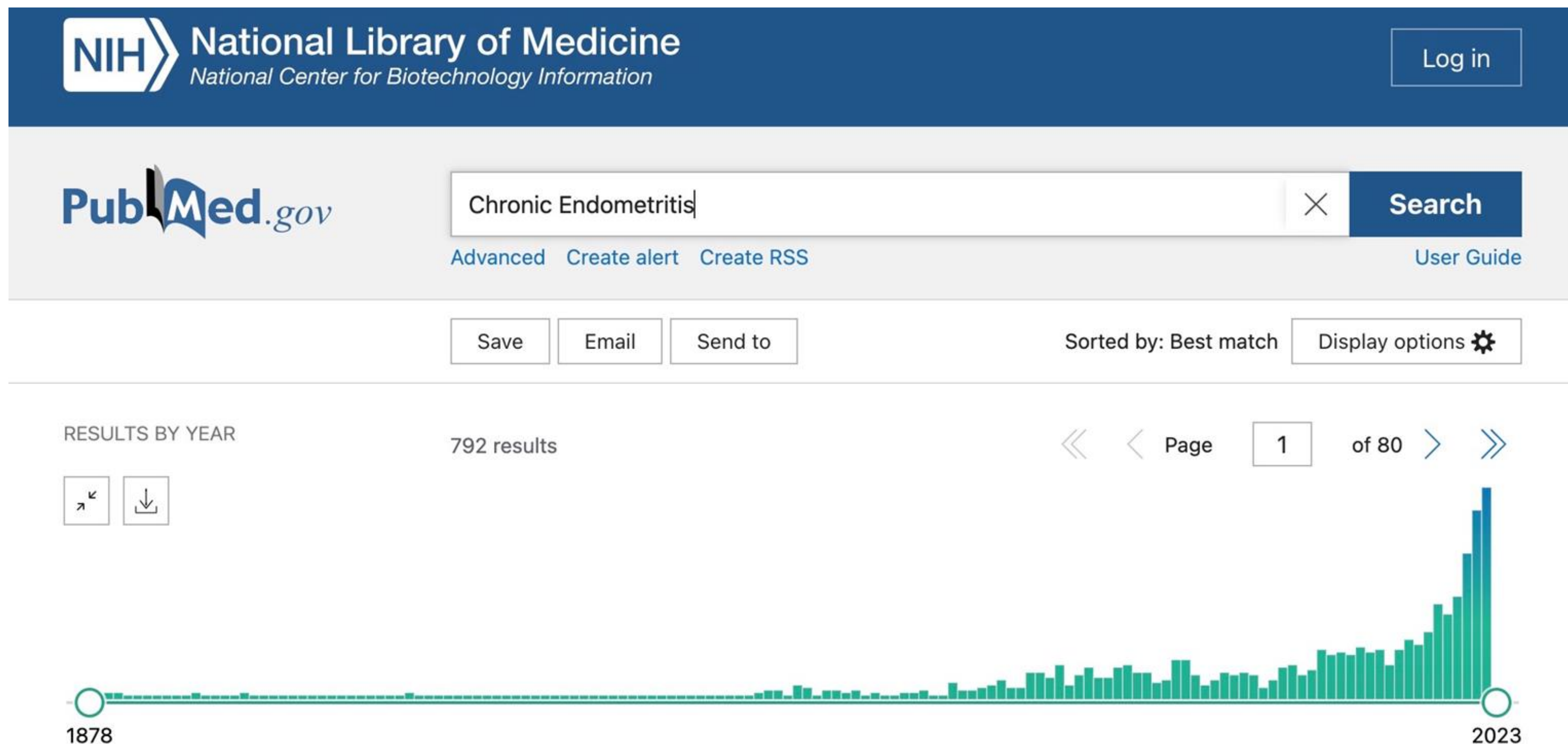
History of Chronic Endometritis

1878: Athil, Clinical lecture on the treatment of chronic endometritis

1907: Hirschman, “gold standard was presence of plasma cells”.

1911: Donald and Fletcher: “Whole subject is still in a state of confusion.

2023: today is still unclear how a mucous membrane that flakes and regenerates every month can be inflamed

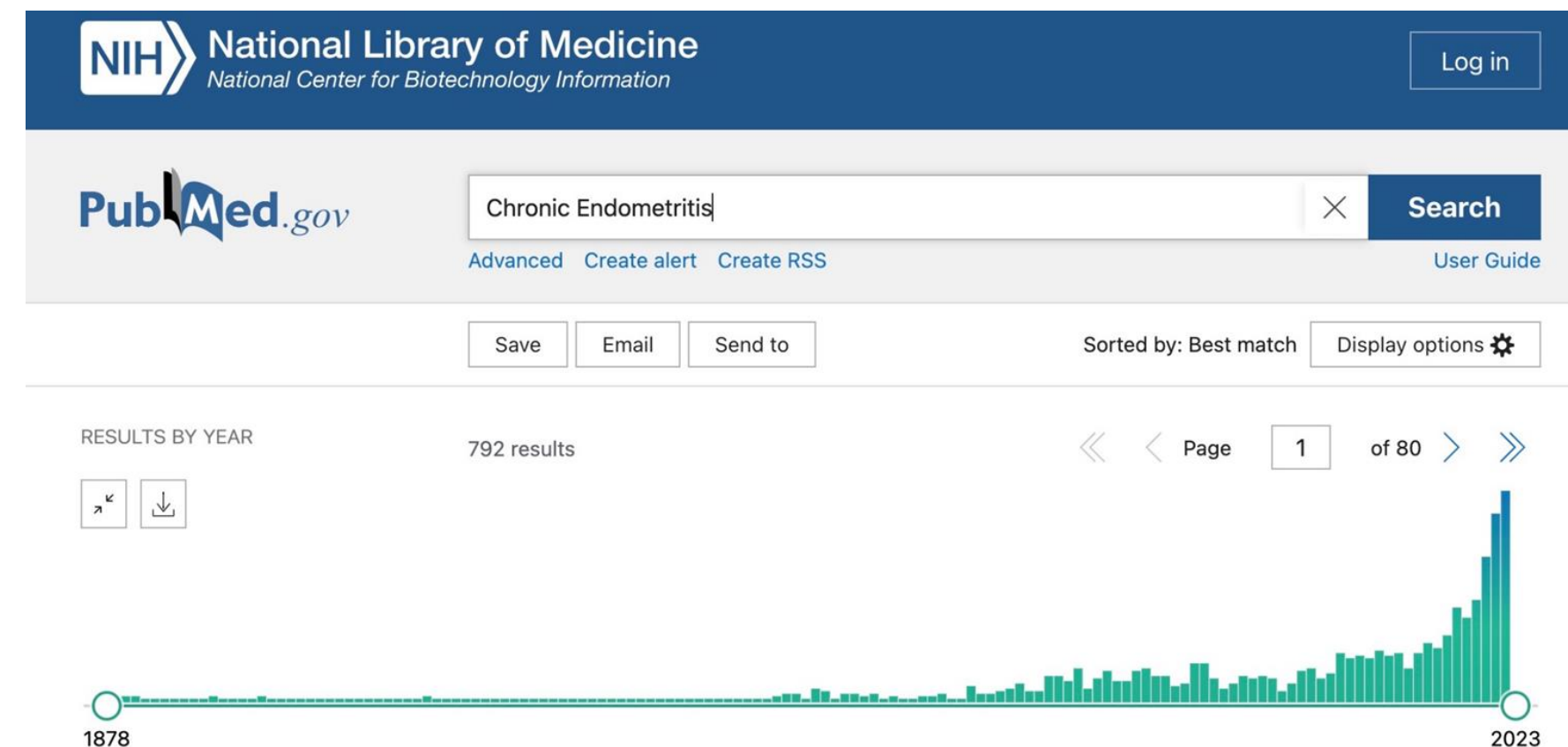


Why so many publications?

■ **Unexplained infertility** (Cicinelli et al., 2018; Liu et al., 2018)

■ **Recurrent miscarriage** (Kitaya, 2011; McQueen et al., 2005; Zolghadri et al., 2011)

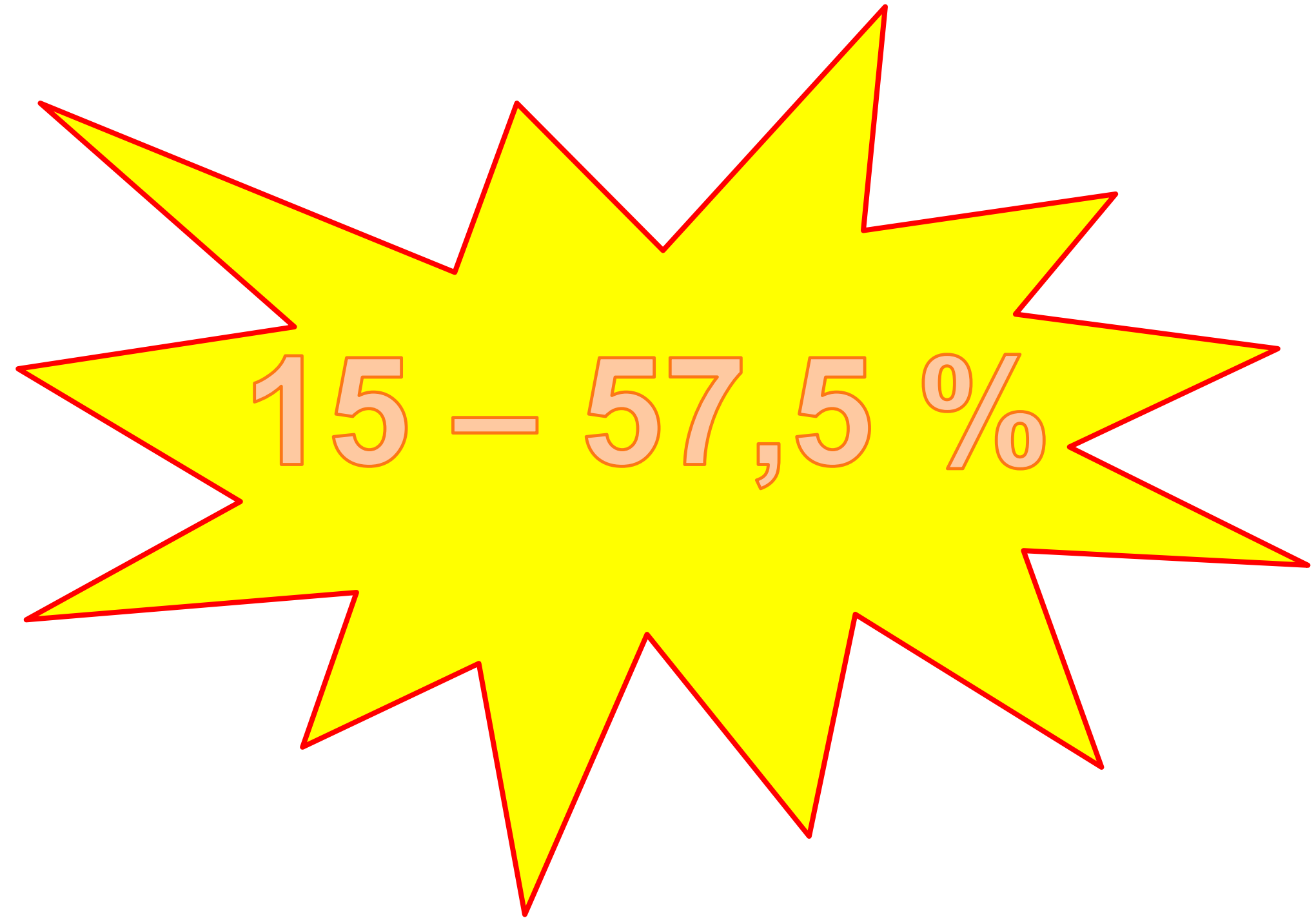
■ **Repeated implantation failure (RIF)** (Bouet et al., 2016; Cicinelli et al., 2005; Johnston MacAnanny et al., 2010; Song et al., 2018; Tersoglio et al., 2015; Yang et al., 2014).



INCIDENCE

In women suffering from:

- Infertility
- Implantation failure in IVF
- Unexplained recurrent miscarriage



Year- Publication	1°st Author	Recruited Patients (N°)	Diagnosis	CE (%)
2010 Fertil Steril	Johnston- MacAnanny E.B.	33	Recurrent Implantation Failure	10%
2011 Fertil Steril	Kitaya K.	54	Recurrent Pregnancy Loss	9,3%
2011 Eur J Ob/Gyn Repr/Biol	ZOLGHADRI J.	142	Unexplained Recurrent Miscarriage	42,9%
2011 Fertil Steril	Kasius J.C.	606	Infertility	2,8%
2018 Am J Reprod Immunol	Cicinelli E.	95	Unexplained Infertility	56,8%



Microorganisms often detected in endometrium with chronic endometritis include:

E. coli, Streptococcus, Enterococcus, Staphylococcus, Mycoplasma, . Ureaplasma urealyticum, Gardnerella vag, Proteus, Klebsiella pneum., Pseudomonas aeruginosa, Corynebacterium, and Mycobacterium tuberculosis.

The microbial infection would induce aberrant expression of pro-inflammatory molecules, triggering unusual immune responses in the endometrium.

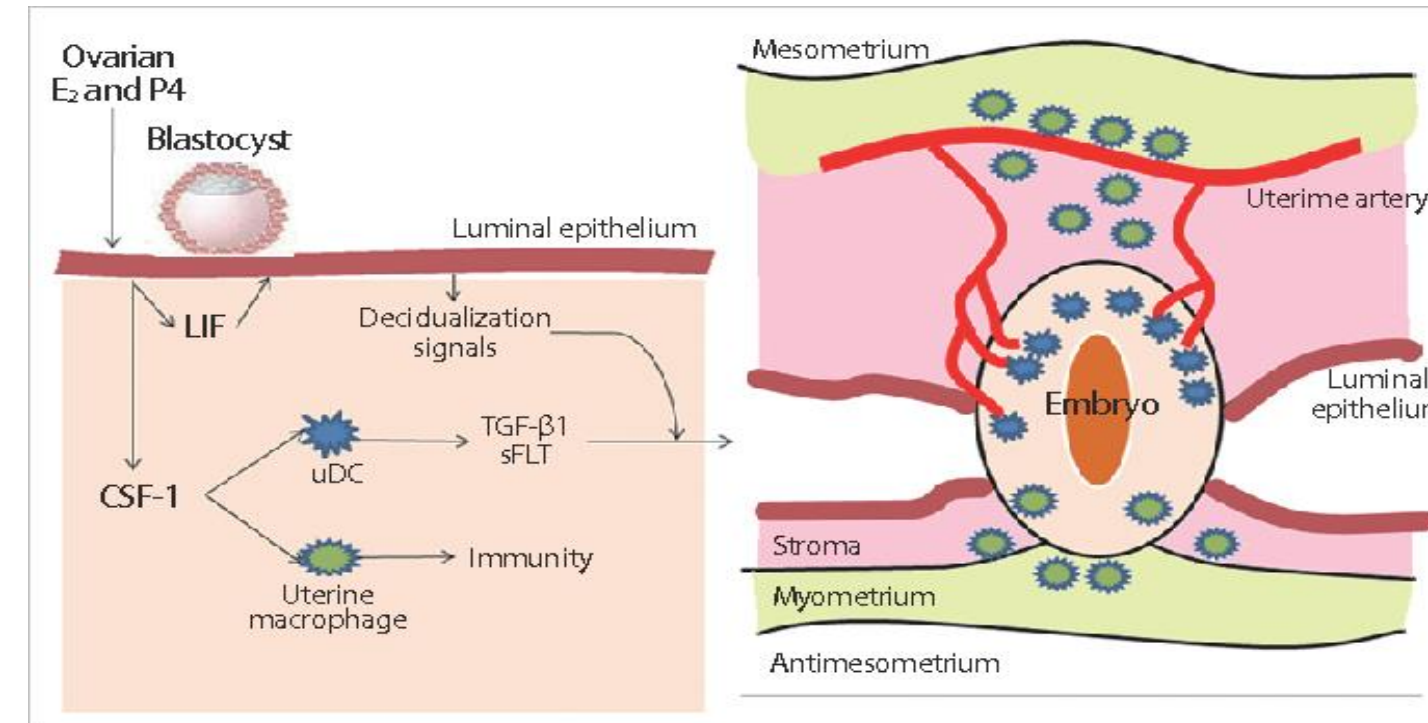
Kotaro Kitaya e Tadahiro Yasuo. Immunohistochemical and clinicopathological characterization of chronic endometritis. Am J Reprod Immunol. 2011 Nov;66(5):410-5.

Kotaro Kitaya et al. Endometritis: new time, new concepts. Fertil Steril 2018 Aug; 110(3):344-350.

WHAT WE FIND IN A NORMAL ENDOMETRIUM

A lot of immunocompetent cells:

- **natural killer (NK) cells,**
- **macrophages,**
- **neutrophils,**
- **dendritic cells and subsets of T cells.**



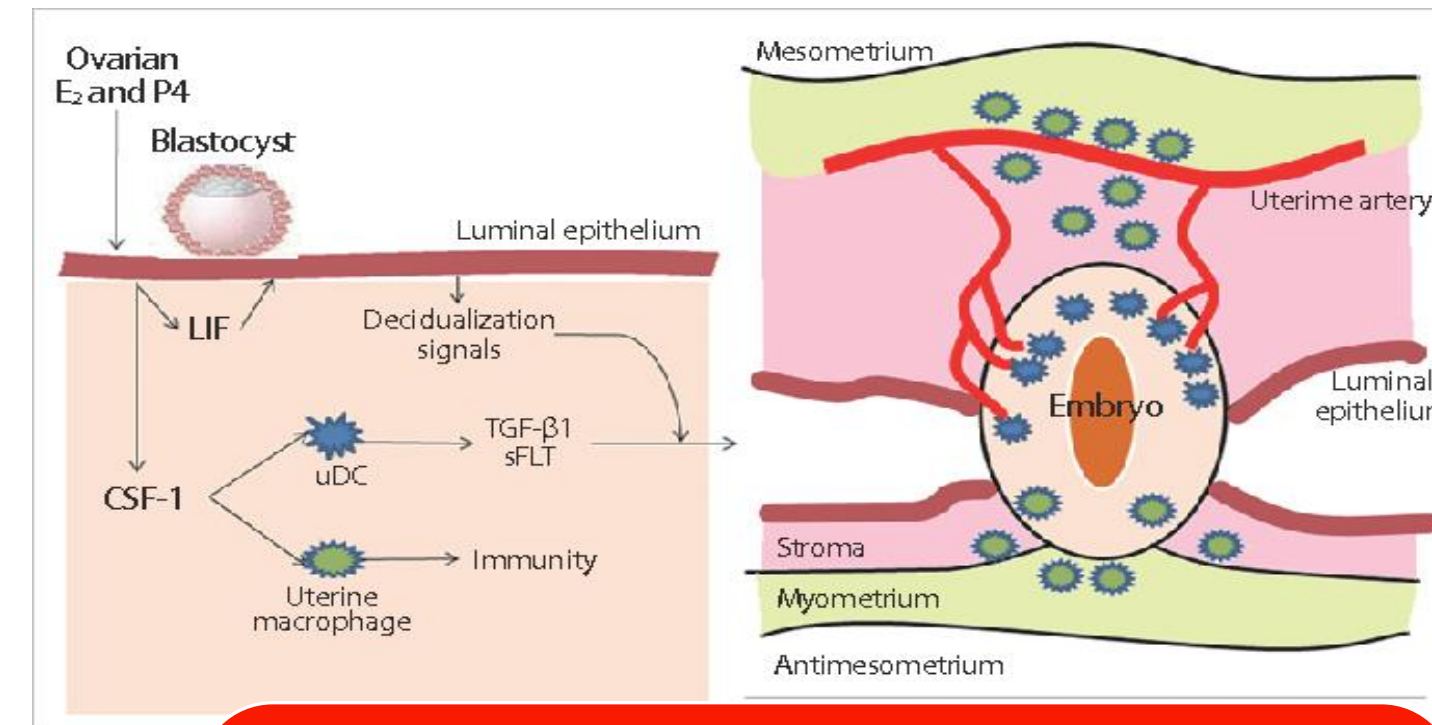
The composition and density of these endometrial leukocyte subpopulations also fluctuate periodically within a menstrual cycle.

The mucosal leukocyte subpopulations are considered to play a critical role in the establishment of embryo implantation and placentation.

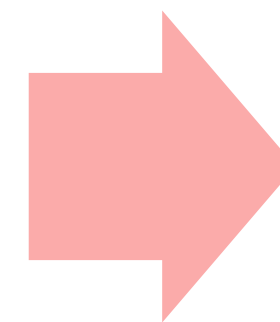
Kotaro Kitava et al. *Unusual inflammation in gynecologic pathology associated with defective endometrial receptivity*
Histol Histopathol 2014 Sep;29(9):1113-27

Lee J.Y et al. *Role of endometrial immune cells in implantation*
Clinical and Experimental Reproductive Medicine. 1 September 2011

WHAT WE FIND IN A NORMAL ENDOMETRIUM



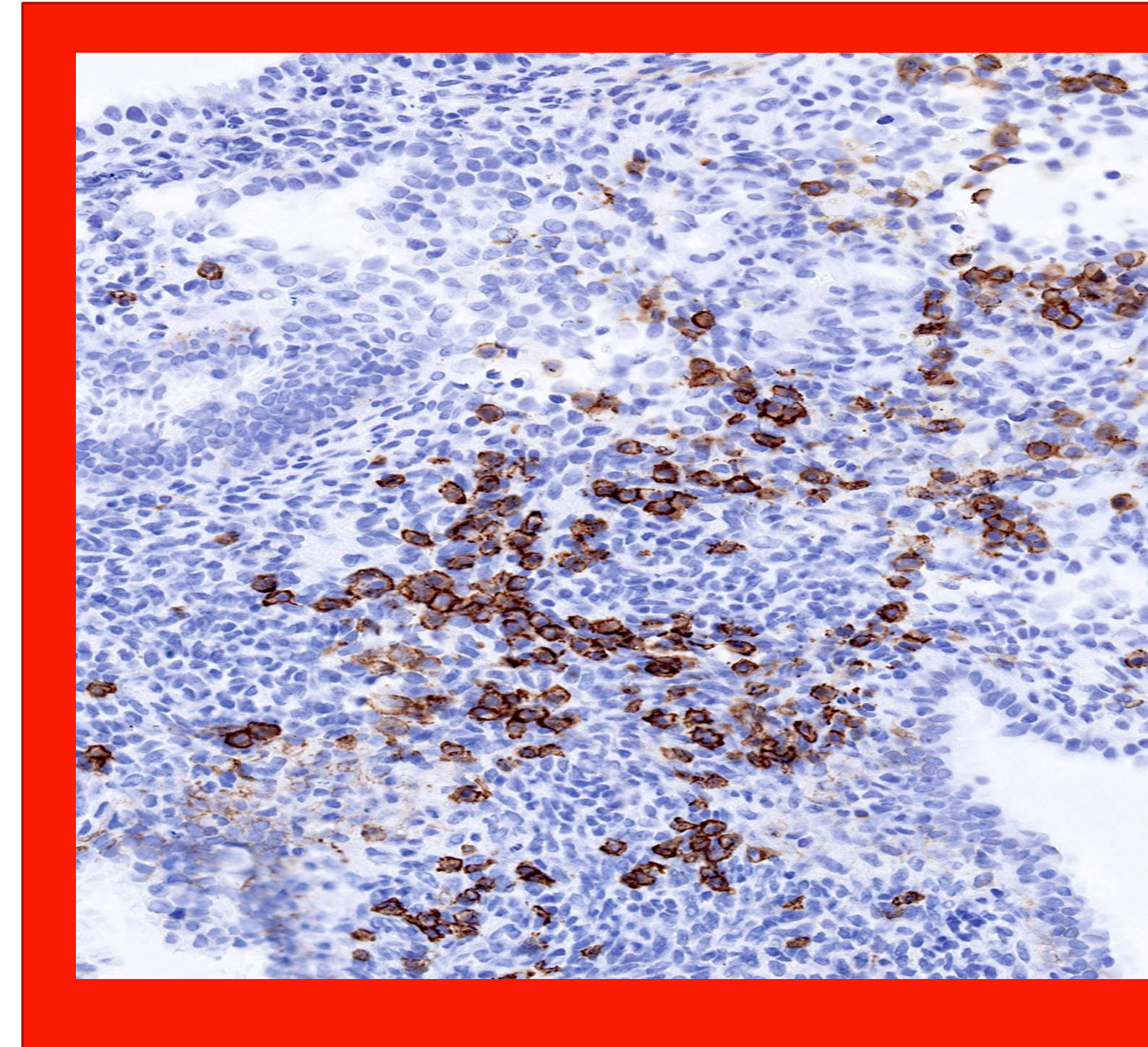
Lymphocytes of the B cell lineage are rare, representing approximately 3% of all endometrial lymphocytes and no more than 2% of all endometrial cells.



Rare Endometrial Stromal Plasmacytes (ESPCs) can occasionally be seen in the stromal compartments.

WHAT WE FIND IN A CHRONIC ENDOMETRITIS

- superficial mucosal edema
- increased endometrial stromal cell density
- unsynchronized differentiation between endometrial epithelial cells (EECs) and stromal cells
- unusual invasions by ESPCs



UTERINE MICROBIOTA COULD BE RELATED TO WOMEN'S REPRODUCTIVE DESEAS



[Front Cell Dev Biol.](#) 2021; 9: 693267.

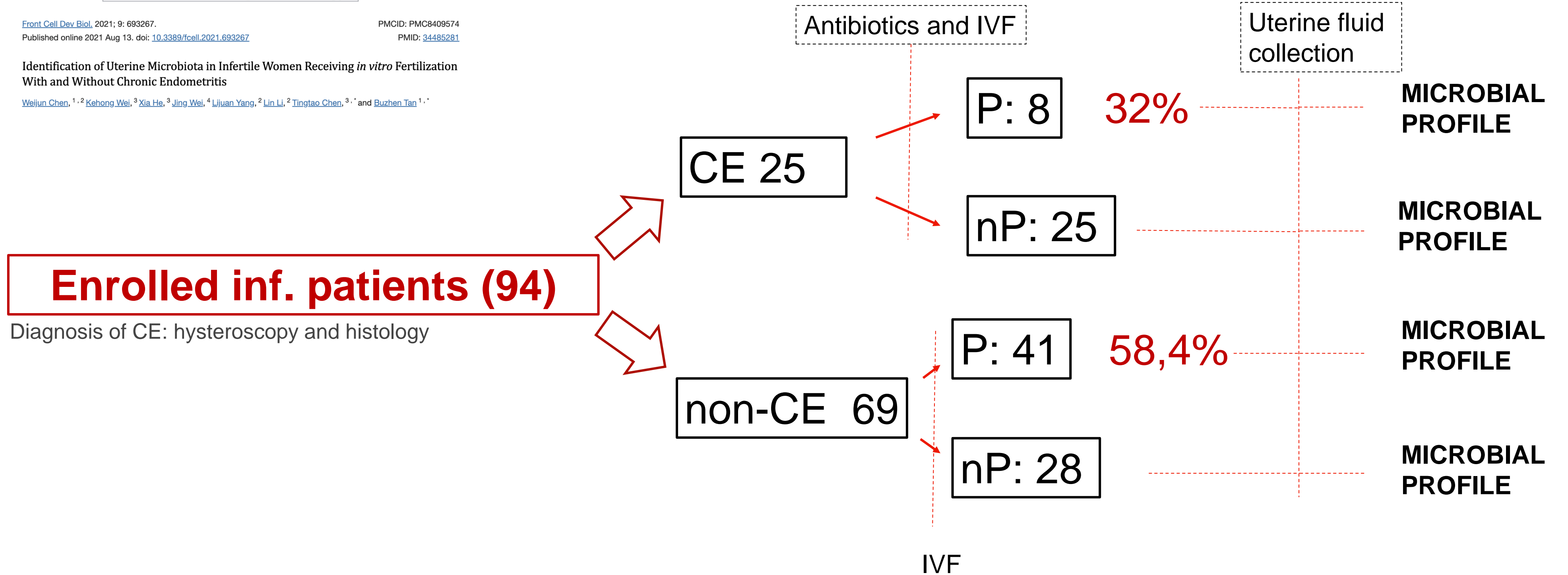
Published online 2021 Aug 13. doi: [10.3389/fcell.2021.693267](https://doi.org/10.3389/fcell.2021.693267)

PMCID: PMC8409574

PMID: [34485281](https://pubmed.ncbi.nlm.nih.gov/34485281/)

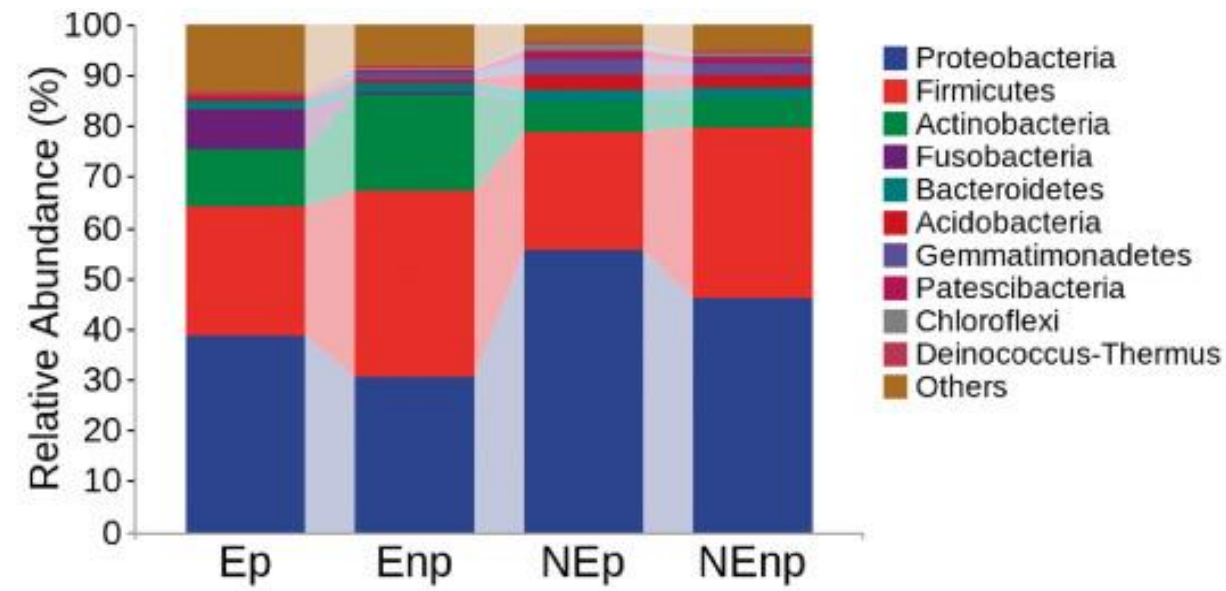
Identification of Uterine Microbiota in Infertile Women Receiving *in vitro* Fertilization
With and Without Chronic Endometritis

[Weijun Chen](#), ^{1,2} [Kehong Wei](#), ³ [Xia He](#), ³ [Jing Wei](#), ⁴ [Lijuan Yang](#), ² [Lin Li](#), ² [Tingtao Chen](#), ^{3,*} and [Buzhen Tan](#) ^{1,*}

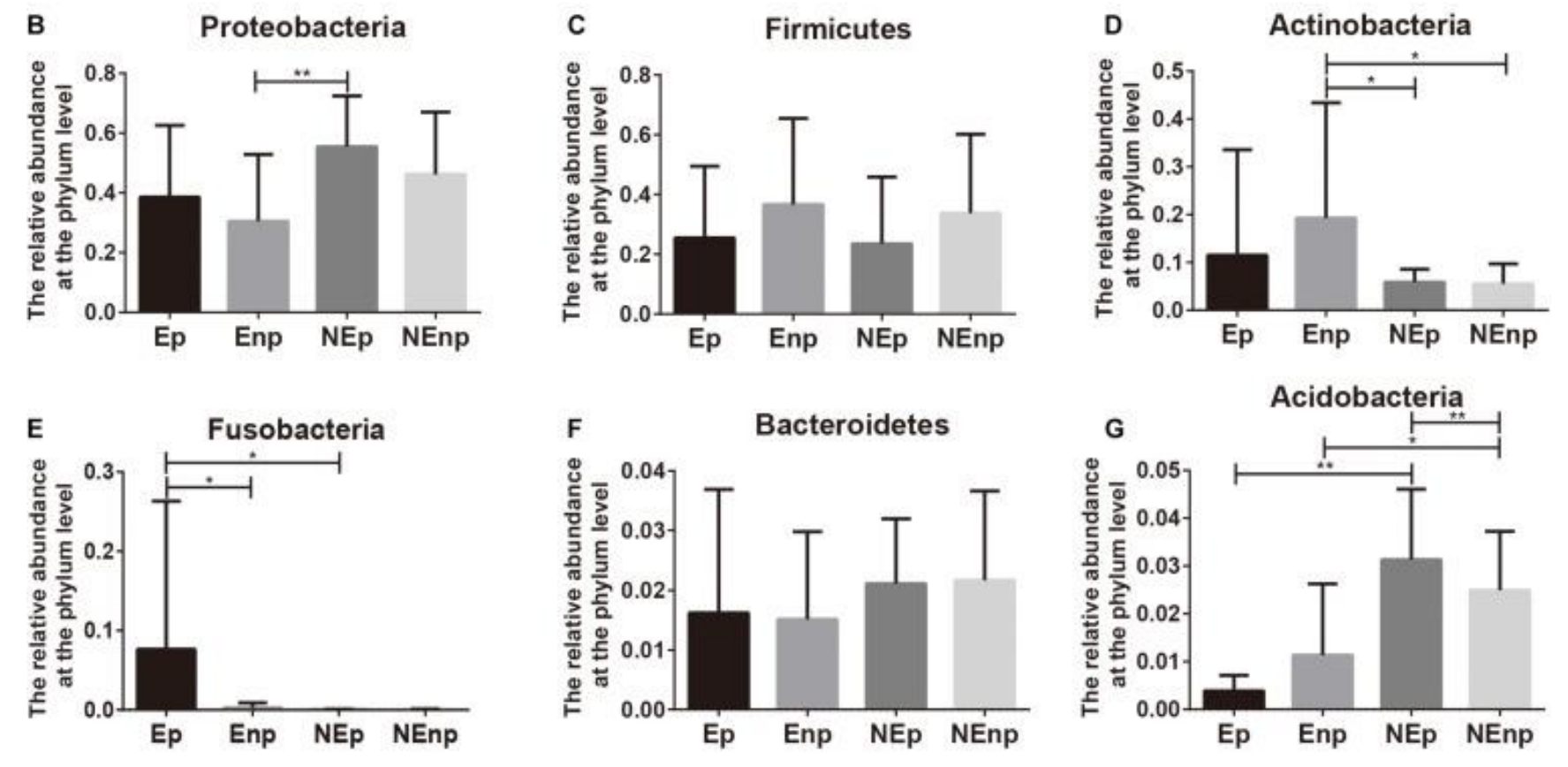




A



RESULTS



- PREGNANT: ↑ acidobacteria
- NON PREGNANT: ↑ proteobacteria ↑ gardnerella

CE group ↑ fusobacteria ↑ gardnerella
↑ actinobacteria ↑ B-Lymphocytes

non-CE group ↑ proteobacteria ↑ acidobacteria

- *Lactobacillus* was the most prevalent genera in uterine, but did not directly relate to the IVF outcomes.
- *Fusobacteria* was a risk factor for IVF failure

These results suggested that the dysbiosis is negative for embryo implantation and IVF outcome.

DIAGNOSIS

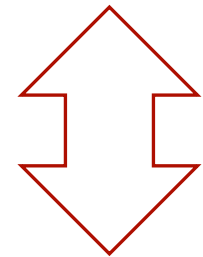
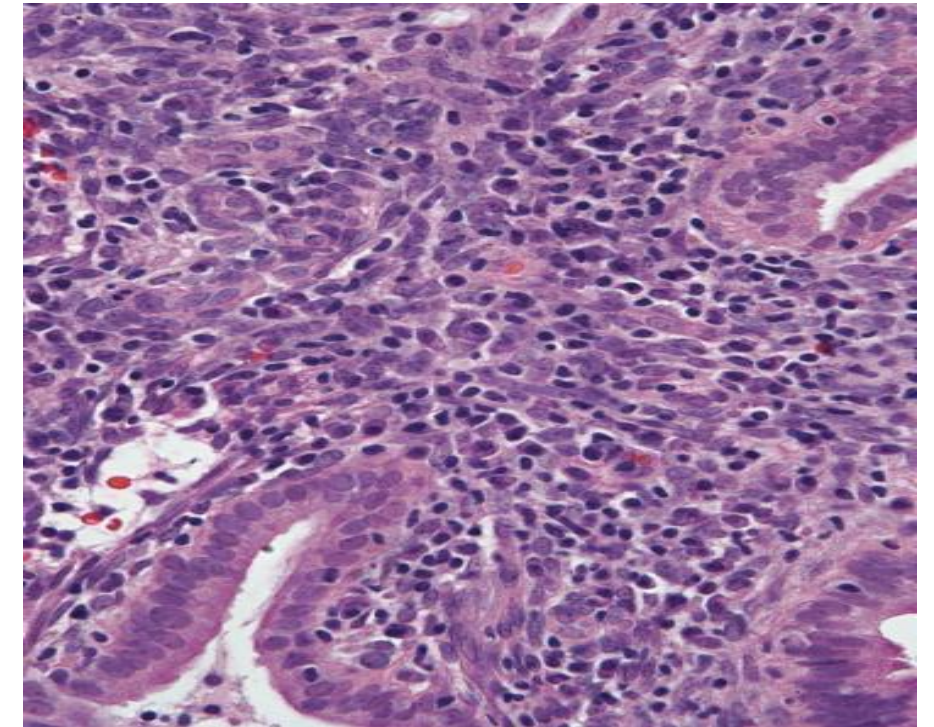
THERE ARE NO UNIVERSALLY ACCEPTED CRITERIA

HISTOPATHOLOGICAL DIAGNOSIS

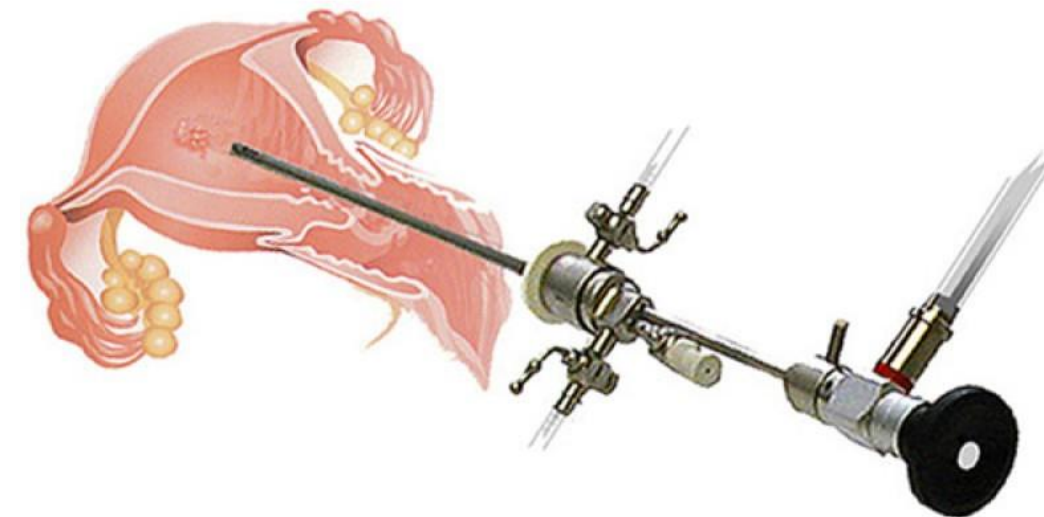
Hematoxylin – Eosin **staining method** has a low diagnostic rate (<10%)

Immunohistochemical (CD138) showed a dramatically higher sensitivity for diagnosing CE (56%).

Additional IHC staining for CD138 in H&E-stained tissue specimens increased the diagnostic rate.



DIAGNOSIS through FLUID HISTEROSCOPY

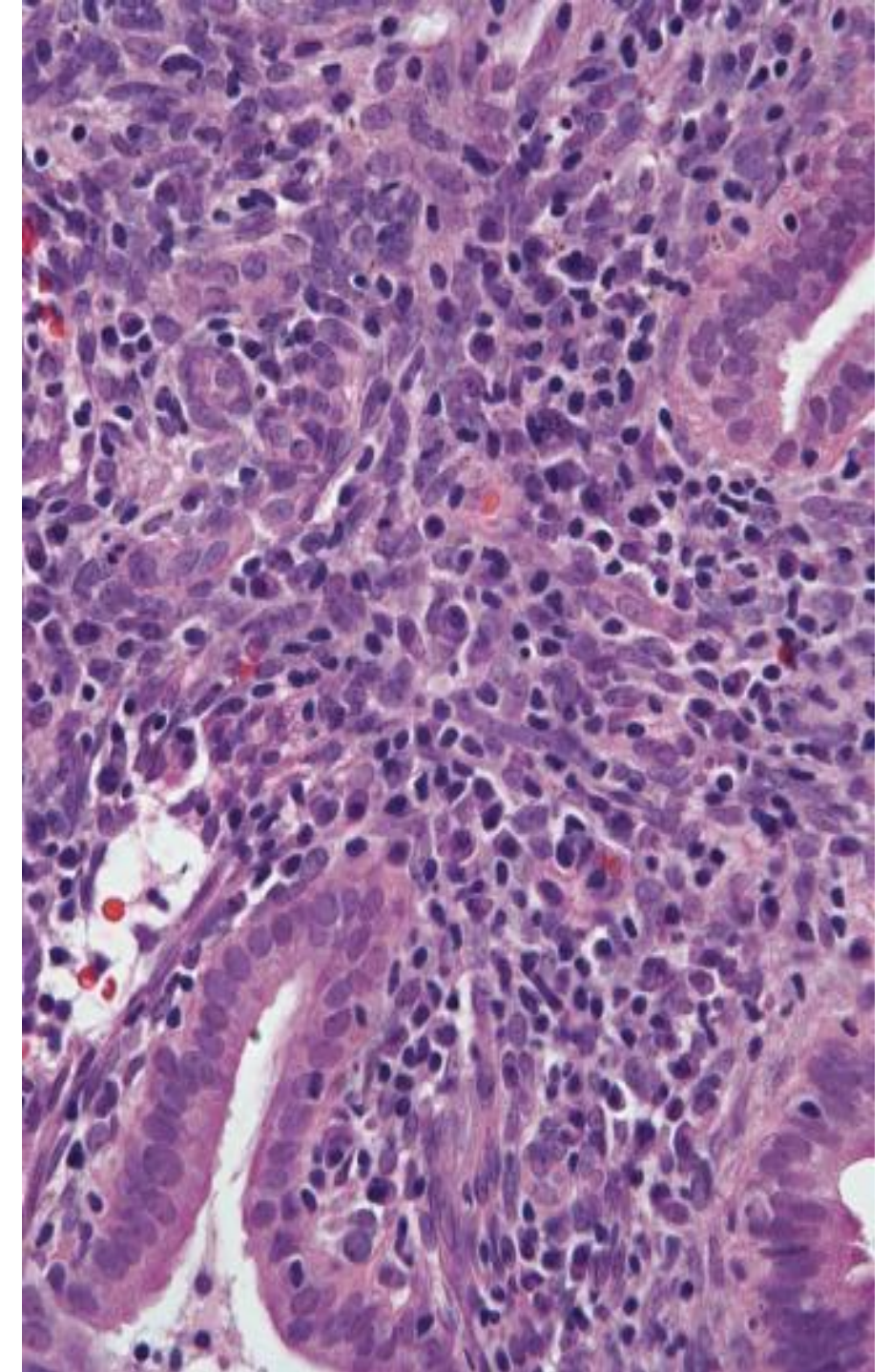


MICROBIAL CULTURE

Endometrial culture for the detection of Chlamydia, Neisseria gonorrhoeae, Ureaplasma, Mycoplasma, Trichomonas, Staphylococcus, Enterococcus, Streptococcus, Escherichia coli

HISTOPATHOLOGICAL DIAGNOSIS

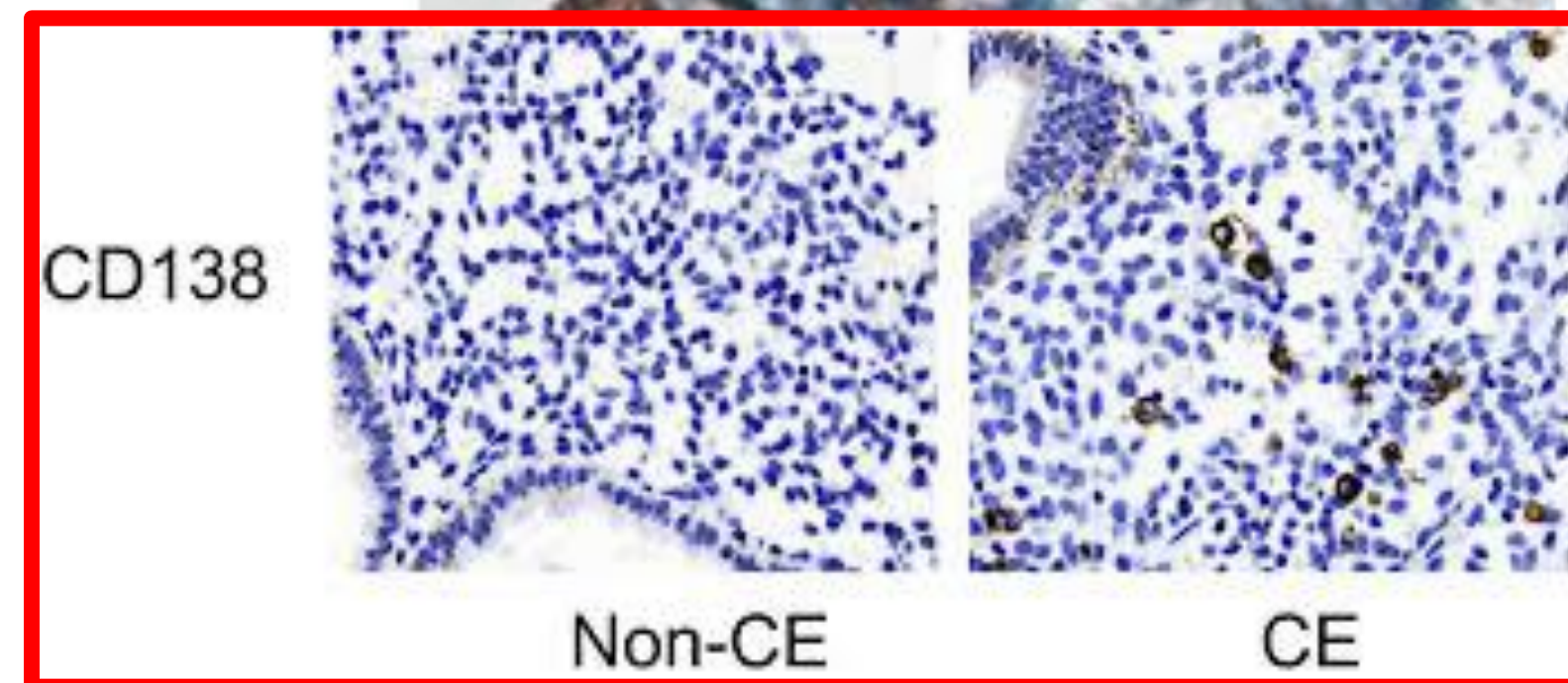
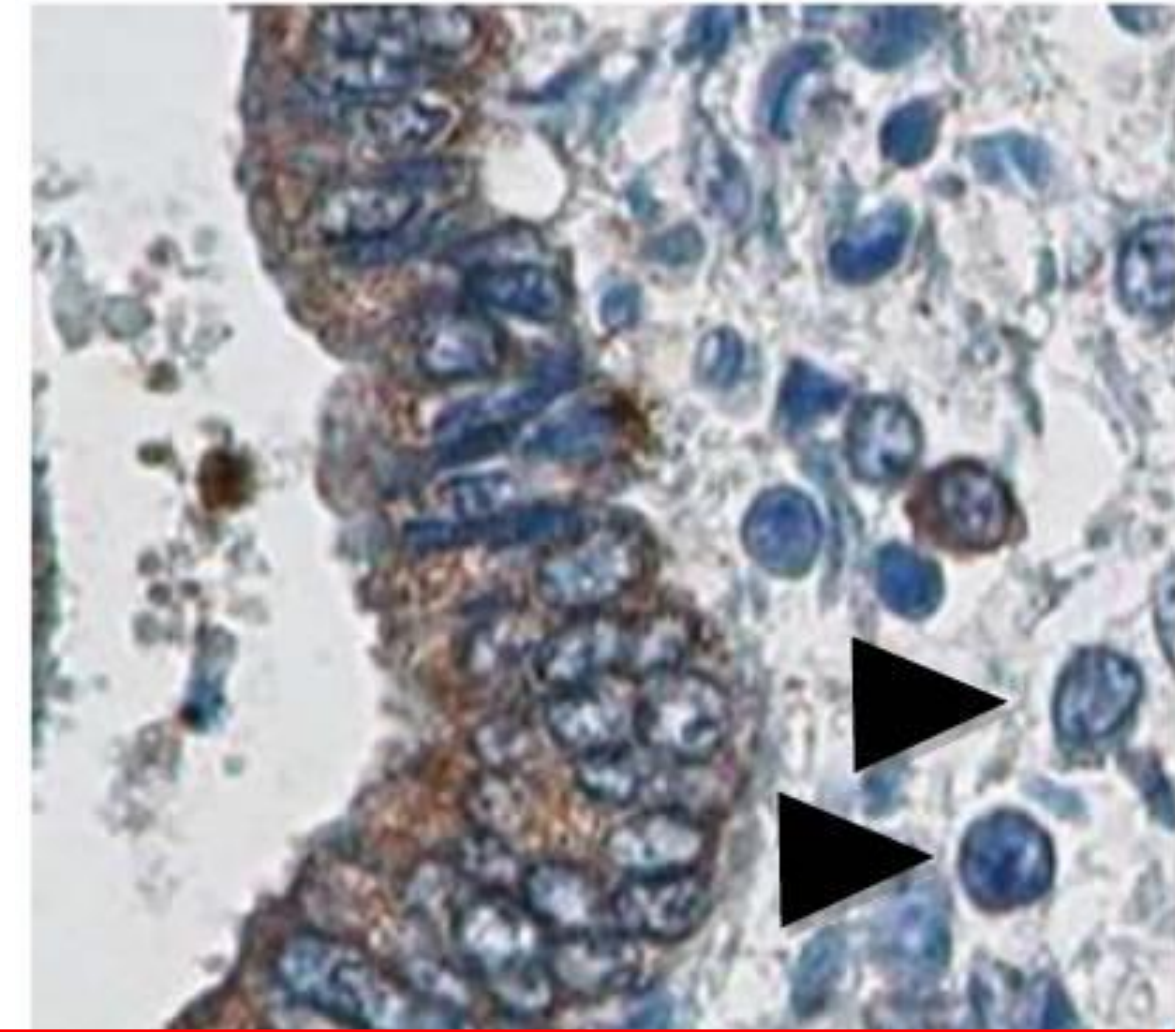
- Superficial endometrial stromal edema
- Increased stromal density
- Asynchronous maturation between stroma and epithelium
- Stromal inflammatory infiltrate dominated by lymphocytes
- **Endometrial Stromal Plasma cells infiltration (ESPS)**



Gold standard is histology

IMMUNOHISTOCHEMISTRY: effectiveness of CD 138 for identification of ESPC

- Easy diagnosis
- More sensitivity (100% Vs 75%)
- More specificity (100% Vs 65%)
- Less interobserver and intraobserver variability in histopathologic diagnosis



CHRONIC ENDOMETRITIS. THE DIAGNOSIS

IMMUNOHISTOCHEMISTRY: CD 138

The questions:

- **Which sampling method?** It doesn't matter: endometrial biopsy or curettage
- **Where to perform the biopsy?** The sampling must not be too superficial and should reach the endometrial basal layer ⁽¹⁾
- **When to perform the biopsy?** the detection rate of ESPCs is higher in the proliferative phase than in the secretory phase ⁽²⁾⁽³⁾⁽⁴⁾
- **How many plasma cells?** There is no uniform consensus: $\geq 3 \approx \geq 5$ (in ≥ 2 high power fields)

1. Kitaya K., Yasuo T. Immunohistochemical and clinicopathological characterization of chronic endometritis. *Am. J. Reprod. Immunol.* 2011;66:410–415.

2. Song D. et al. Prevalence and confounders of chronic endometritis in premenopausal women with abnormal bleeding or reproductive failure. *Reprod. BioMed. Online.* 2018;36:78–83.

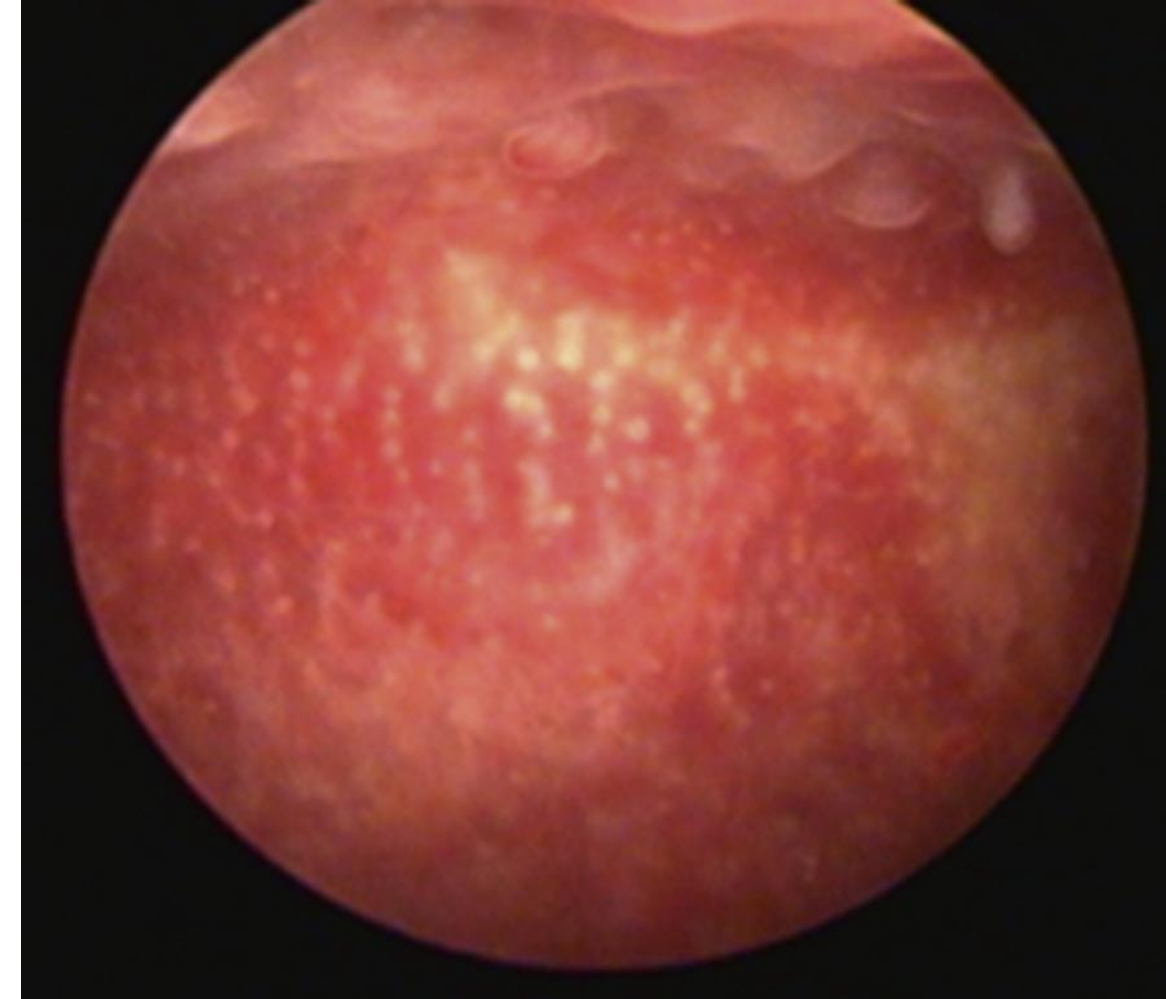
3. Eckert L.O. et al. Endometritis: The clinical-pathologic syndrome. *Am. J. Obstet. Gynecol.* 2002;186:690–695.

4. Punnonen R. et al. The relation between serum sex steroid levels and plasma cell infiltrates in endometritis. *Arch. Gynecol. Obstet.* 1989;244:185–191.

HYSTEROSCOPIC FEATURES



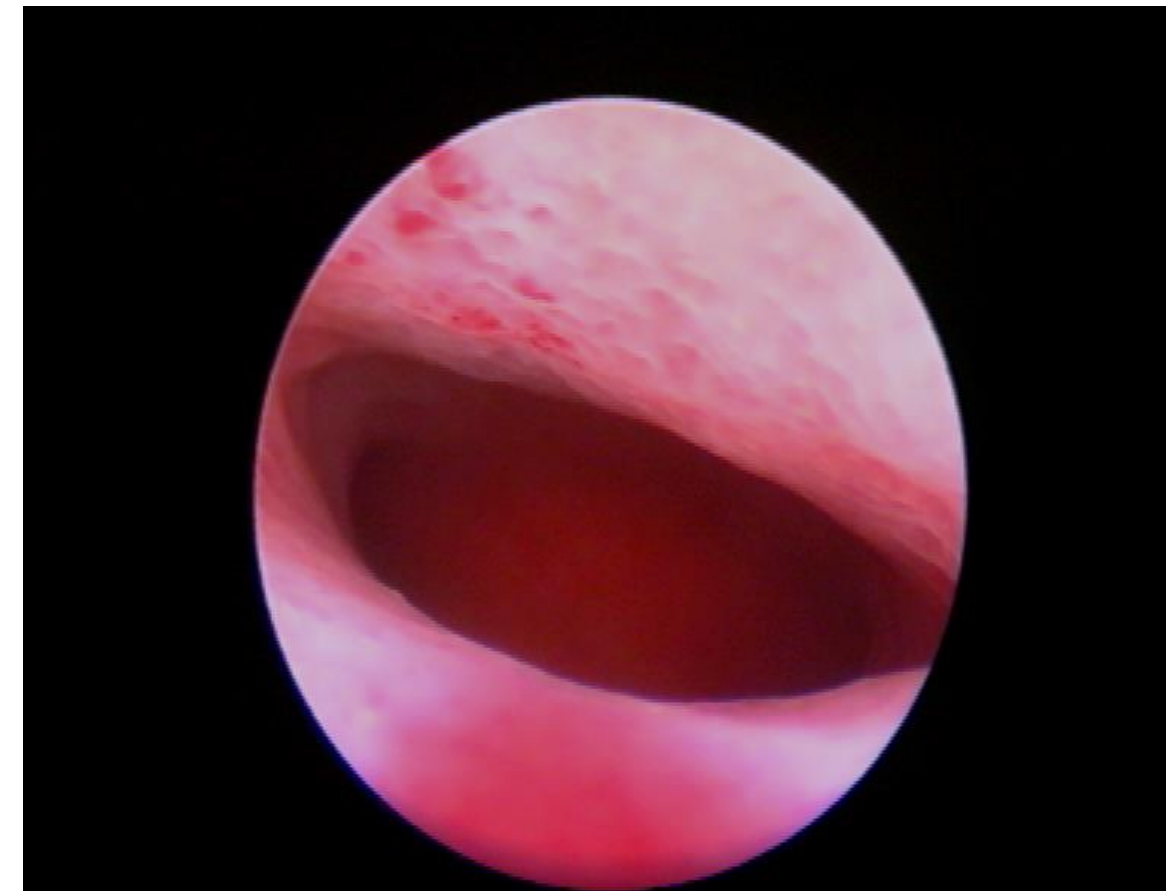
- endometrial hyperaemia



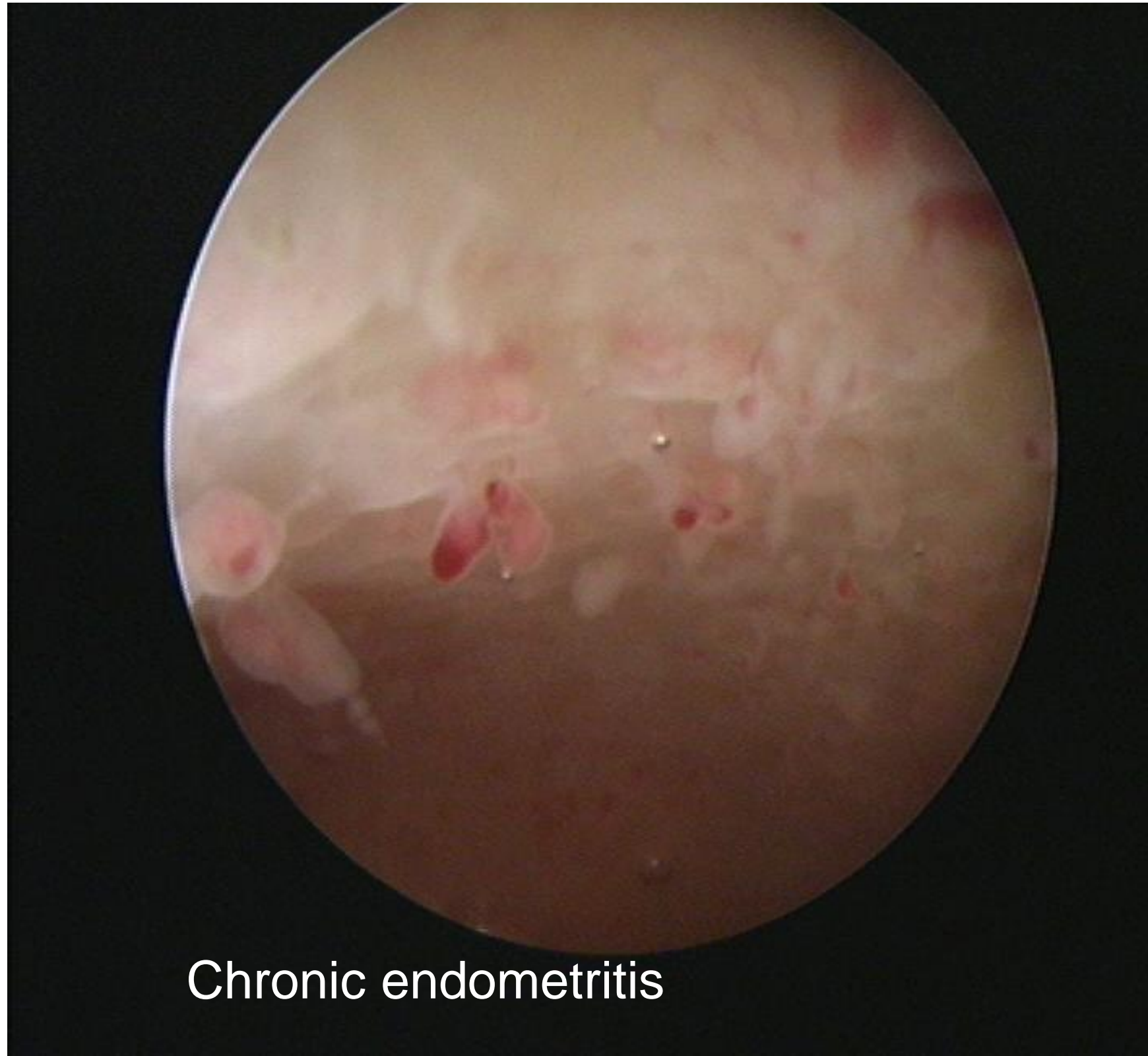
- micropolyps (<1 mm)



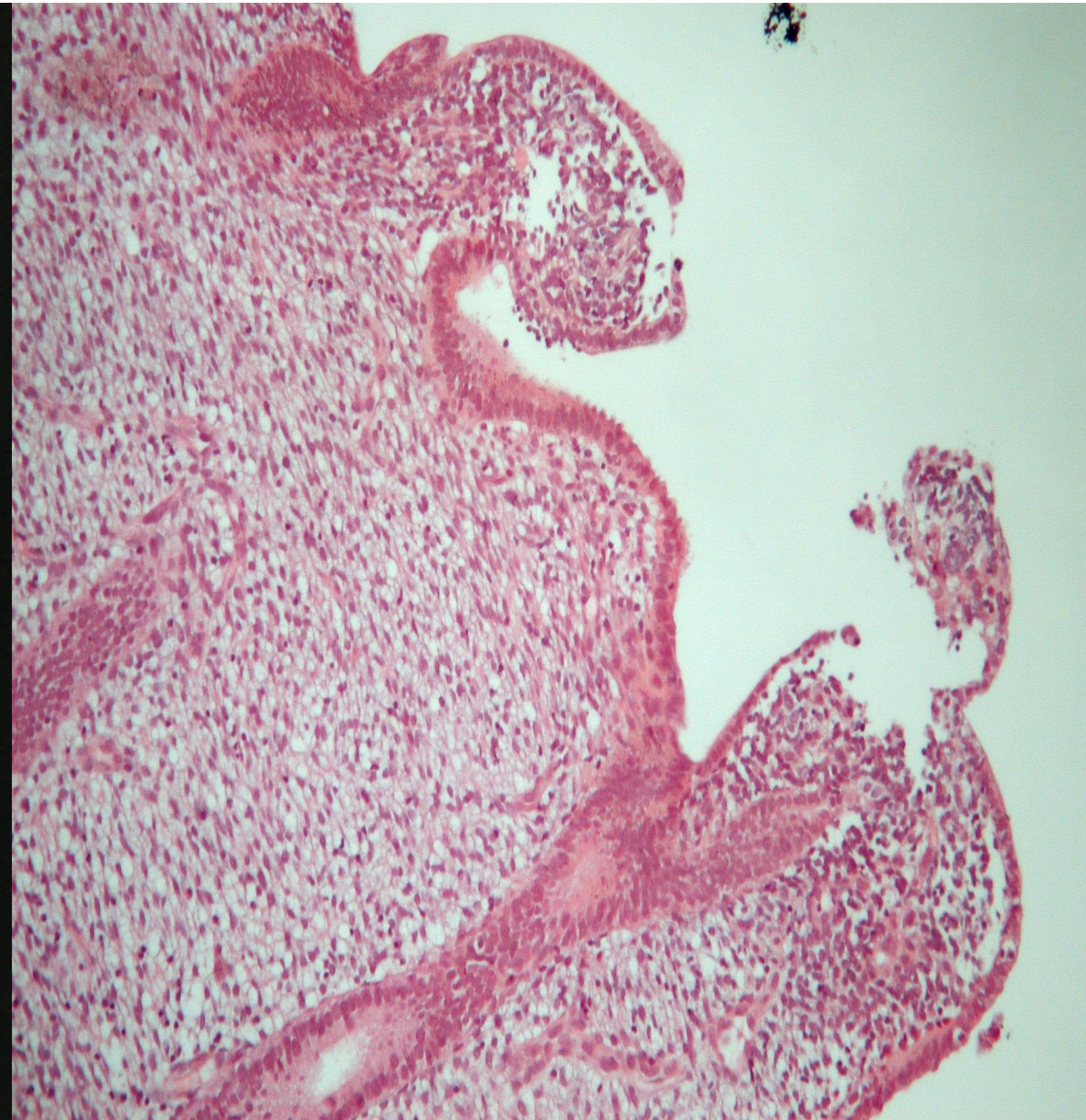
- mucosal oedema



Micropolyps



Chronic endometritis



HYSTEROSCOPIC FEATURES: MICROPOLYPS (1-2 mm)

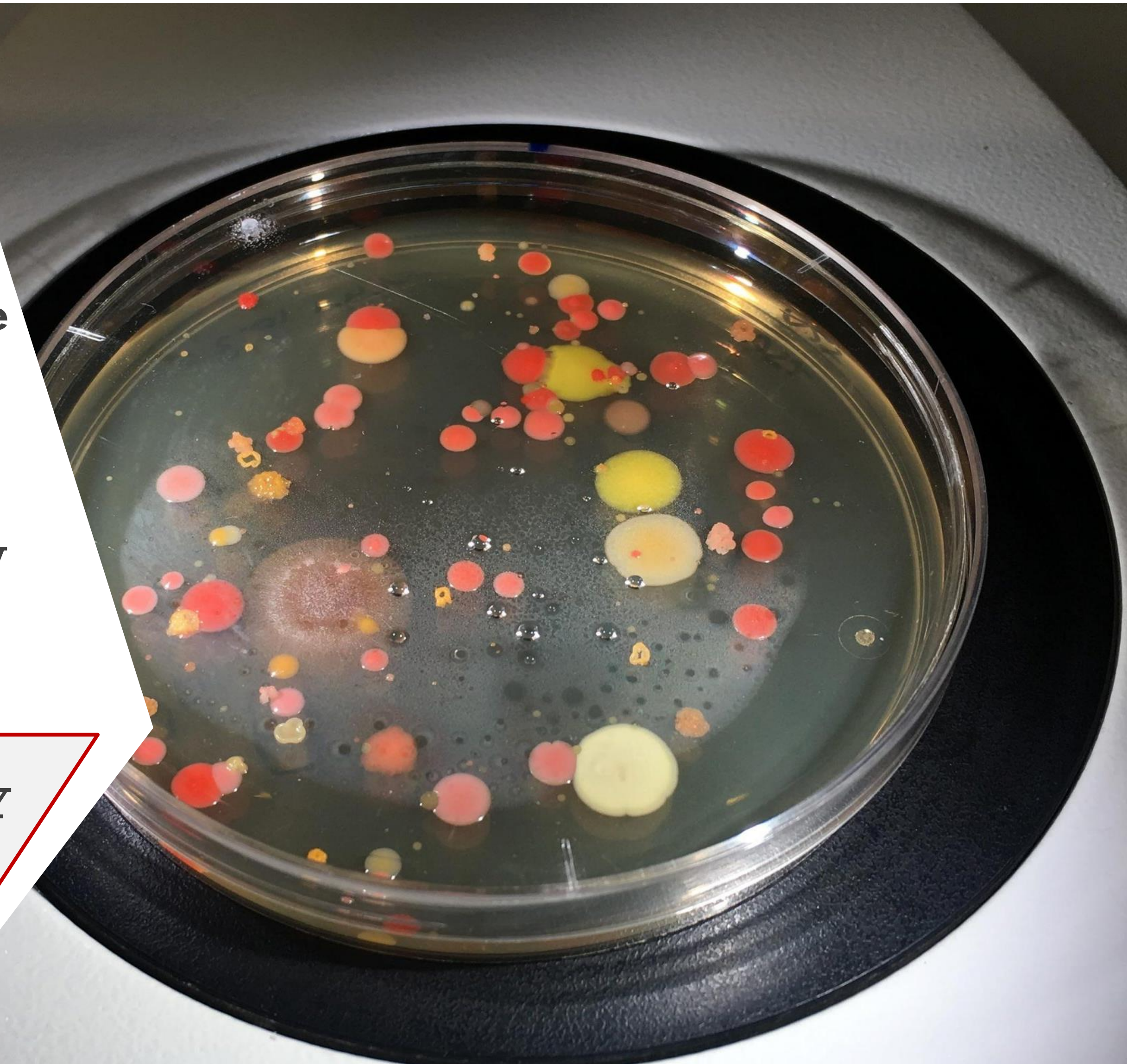


Protrusions or polyps arising from the endometrium with associated endometrial stromal thickening and oedema, **found normally in 11% patients on routine hysteroscopy and present in 50 to 67% of women with infertility** having either recurrent pregnancy loss (RPL) or repeated implantation failure (RIF) **with tissue diagnosis of chronic endometritis.**

The correlation between micropolyposis of the endometrium and chronic endometritis is still unclear.

MICROBIAL CULTURE

- ▶ **This technique allows the identification of pathogens and the prescription of targeted therapy.**
 - ▶ **Limitations:** contamination with vaginal bacteria, limited culturability of some organisms
- ▶ **The MOLECULAR MICROBIOLOGY (PCR) (NGS) provides additional detection of nonculturable microorganism**





DIAGNOSIS: is possible only by symptoms?

NO!

- **No symptoms**
- **Mild symptoms**
- **Chronic pelvic pain or pelvic discomfort**
- **Abnormal uterine bleeding**
- **Leukorrhea**



Are women with a history of recurrent early pregnancy loss (REPL) and/or fetal demise (FD) affected by CE?

ORIGINAL ARTICLE: EARLY PREGNANCY

Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise

Dana B. McQueen, M.D.,^a Lia A. Bernardi, M.D.,^{a,b} and Mary D. Stephenson, M.D., M.Sc.^{a,b}
^a University of Chicago Recurrent Pregnancy Loss Program, Department of Obstetrics and Gynecology, University of Chicago; and ^b Department of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, Illinois

Fertil Steril® 2014;101:1026-30



PATIENTS: 395 ≥ 2 REPL ≤ 10 wks or FD ≥ 10 wks

DESIGN: observational study using prospectively collected data.

INTERVENTIONS: All women had an endometrial biopsy. CE was treated with antibiotics, and a second endometrial biopsy was recommended as a "test of cure."

MAIN OUTCOME MEASURES: Subsequent live-birth rate (LBR).



RESULTS: The overall prevalence of chronic endometritis was 9% (35/395) in this cohort; 7% (21/285) in the REPL group, 14% (8/57) in the FD group, and 11% (6/53) in the combined REPL/FD group. The cure rate was 100% after a course(s) of antibiotics. The subsequent cumulative LBR was 88% (21/24) for the treated chronic endometritis group versus 74% (180/244) for the group without chronic endometritis.

The per-pregnancy LBR for the treated chronic endometritis group was 7% (7/98) before treatment versus 56% (28/50) after treatment.

CONCLUSIONS: there was a high prevalence of CE in this cohort. The test of cure was normal with antibiotics. Subsequent LBRs after treatment were encouraging.

Hypothesis of pathophysiology link between Endometritis and RIF

- microbial infection
- abnormal microenvironment
- aberrant expression of pro-inflammatory molecules,
- unusual immune responses.
- recruitment of circulating B cells into the stromal glandular areas.
- fraction of endometrial B cells differentiate into plasma cells
- increase in the presence of antibodies in the mucosa which has a negative impact on the embryo implantation process

CHRONIC ENDOMETRITIS: The TREATMENTS

ORAL ANTIBIOTICS

There is no standard antibiotic regimen

Different antibiotics and dosages have been prescribed

Endometrial receptivity tends to improve after antibiotic therapy

The first line regimen is Tab **Doxycycline 100mg** BD for 14 days.

For Gram neg. Bact.: **Ciprofloxacin 500 mg** twice a day for 10 days

Second-line therapy includes **Ciprofloxacin and Metronidazole 500 mg** OD for 2 wks or

Ofloxacin 400 mg OD for two weeks and **Metronidazole 500 mg** OD for two weeks.



CHRONIC ENDOMETRITIS: The TREATMENTS

Effect of antibiotic therapy on chronic endometritis cure rates and reproductive outcomes														
Cicinelli et al. (2015)	T1. Doxycycline 200 mg/	T1. Doxycycline 200 mg/day for 14 days)												
	T2. Ciprofloxacin and me	T2. Ciprofloxacin and metronidazole (500 mg of each for 14 days)												
Kitaya et al. (2017)	T1. Doxycycline (200 mg/day for 14 days)													
	T2. Ciprofloxacin and metronidazole (500 mg of each for 14 days)													
Johnston- MacAnanny et	T1. Doxycycline (200 mg/day for 14 days)													
	T2. Ciprofloxacin and metronidazole (500 mg of each for 14 days)													
Tersoglio et al. (2015)	Doxycycline 200 mg/day for 14 days, with metronidazole 1 g/day and ciprofloxacin 1 g/day for 14 days													
	If no remission, scheme is repeated + linezolid 600 mg/day orally for 10 days + meprednisone orally 4 to 8 daily mg;													
McQueen et al. (2014)	T1. Ofloxacin (800 mg) and metronidazole (100 mg) for 2 weeks													
	T2. Doxycycline alone, doxycycline and metronidazole, or ciprofloxacin and metronidazole													

UNEXPLAINED INFERTILITY

SHORT COMMUNICATION

Chronic endometritis in patients with unexplained infertility: Prevalence and effects of antibiotic treatment on spontaneous conception

Ettore Cicinelli, Maria Matteo, Giuseppe Trojano, Paola C. Mitola, Raffaele Tinelli, Amerigo Vitagliano ✉, Francesco M. Crupano, Achirpita Lepera, Giuseppe Miragliotta, Leonardo Resta

First published: 14 November 2017 | <https://doi.org/10.1111/aji.12782> | Citations: 108



Does the therapy work?

Hysteroscopy and Biopsy ➤

AIM:
 CE Incidence
 Effects of Antibiotic Therapy

95 ♀ Unexplained Infertility

CE 56,8% (n.53)

no CE 43,2% (n.42)

Targeted Antibiotic

2nd Biopsy

CE Resolution n.38

Still CE n.15

PR: 76,3% (n.29)
LBR: 65,8% (n.25)

PR: 20% (n.3)
LBR: 6,6% (n.1)

PR: 9,5 % (n.4)
LBR: 4,,8% (n.2)

**CE is prevalent in pts with unexpl. infert
 Treatment improve spont. PR and LBR**

CHRONIC ENDOMETRITIS: The TREATMENTS

BUT ...

no randomized studies that analyse the possible benefit of treatment for chronic endometritis have been published



(Kitaya et al., 2018).

CHRONIC ENDOMETRITIS: The TREATMENTS

- ✓ **Antibiotics seem to improve implantation rate and pregnancy rates in women with CE**
- ✓ **IVF outcomes after successful treatment are comparable to those without CE**
- ✓ **Adequate treatment can reverse histological damage, and leads to a doubling of the implantation rate and the live birth rate in oocyte donation programs**

- Cicinelli et al Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy *Hum. Reprod.*, 30 (2015), pp. 323-330
- Kitaya, et al Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure *Am. J. Reprod. Immunol.*, 78 (2017), p. E12719
- Tersoglio et al Repeated implantation failure in oocyte donation. What to do to improve the endometrial receptivity? *JBRA Assist. Reprod.*, 19 (2015), pp. 44-52
- Vitagliano et al Effects of chronic endometritis therapy on invitro fertilization outcome in women with repeated implantation failure: a systematic review and meta-analysis *Fertil. Steril.*, 110 (2018), p. 103

CONCLUSIONS

- Chronic endometritis is a subtle pathology associated with reproductive insuccesses.
- CE is not due to STDs pathogens but to altered microbiome
- Histology with CD138 IHC is at the moment the gold standard
- Endometrial Microbioma composition before ET could be a useful biomarker to predict reproductive outcome, offering an opportunity for new treatment strategies.
- Adequate antibiotic treatment improves reproductive outcomes

CONCLUSIONS

CE is often associated with reproductive failure but **there is still no guideline** today nor it is included among the pathologies to be excluded before starting an IVF program

We need randomized studies assessing the impact of antibiotic treatment as a possible therapeutic option

Actually, pending new evidence, it would be advisable not to include CE in the in initial baseline study before assisted reproduction in order not to delay other ART treatments.
It would be advisable in cases of recurrent implantation failure

Take-home message

CE is implicated in infertility and recurrent pregnancy loss

Endometrial stromal plasma cells is the most sensitive and specific finding for the diagnosis of CE

The cure for histopathologic CE improves the reproductive outcomes

We need a definitive diagnostic criteria or universal guidelines for CE

THANK YOU



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