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Chapter 8

Microbiome, Infection and Inflammation in Infertility

Reza Peymani and Alan DeCherney

Additional information is available at the end of the chapter

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Abstract

The implantation mechanism and process are very complex and require a precise interaction between the embryo and endometrium. The failure to implant is thought to be due to implantation environment factors or embryonic factors.

A suitable condition of the uterine cavity is essential for successful reproduction. Inflammation can be a part of the normal physiologic process during implantation; however, there are also pathologic sources of inflammation that can adversely affect the uterine cavity and endometrial receptivity.

Chronic Endometritis is usually asymptomatic and is defined histologically by the presence of plasma cells in an endometrial biopsy. It is mostly associated with the gonorrheal or chlamydial also non-sexually transmitted infections including E-coli, streptococcus, staphylococcus, enterococcus faecalis, mycoplasma, urea plasma and yeast. However, often a causal organism can not be identified.

Available evidence suggests that chronic subclinical endometritis is relatively common in women with symptomatic lower genital tract infections, including cervicitis and recurrent bacterial vaginosis and may not be altogether rare even in asymptomatic infertile women.

Mucopurulent cervicitis is highly associated with chlamydial and mycoplasma infections and both organisms, in turn, are associated with chronic endometritis, which likely plays a role in the pathogenesis of tubal factor infertility.

There is also a growing interest in the Microbiome of the reproductive tract. The Vaginal and Uterine Microbiome have been partially characterized and shown to be related to obstetric outcomes. Given the large number of unexplained IVF failures, it is reasonable to consider the uterine Microbiome and its impact on female fertility.

Although routine serologic testing, cervical cultures and endometrial biopsies may be difficult to justify, further evaluation and treatment are appropriate and prudent in infertile women with clinical cervicitis, chronic or recurrent bacterial vaginosis or other symptoms that suggest pelvic infection as well as in women with unexplained IVF failures.

Compared to culture-dependent methods, culture-independent methods are estimated to have increased bacterial detection in the uterine cavity by about 50% and increased num-

ber of detected species by up to fivefold. The detection of bacteria in the intrauterine cavity by PCR, in the absence of signs of infection, confirms the proposition of a non-sterile uterus.

This chapter will focus on chronic endometritis, uterine microbiome and hydrosalpinges and will review the diagnosis, pathophysiology and the recommended treatments of these specific inflammatory processes that contribute to implantation failure.

Keywords: Uterine Microbiome, Chronic Endometritis, Hydrosalpinges, Implantation Failure, Infertility

1. Introduction

1.1. Inflammation and implantation

Implantation has a complex and multistep process and mechanism, resulting in the blastocyst being embedded in the uterine endometrium, which is often viewed as the rate-limiting step for the in vitro fertilization (IVF) success. Implantation failure is related to either maternal factors or embryonic causes. Maternal factors include uterine anatomic abnormalities, thrombophilia, non-receptive endometrium and immunological factors.

Although uterine abnormalities are considered to have a relevant impact on the chances to conceive through IVF, conventional infertility investigations, based on ultrasound and hysterosalpingography (HSG), may miss subtle intrauterine lesions. Fatemi et al. demonstrated that the prevalence of unsuspected intrauterine abnormalities in hysteroscopy before IVF ranges from 11 to 45% accordingly, recent reports suggest that hysteroscopy in the cycle preceding ovarian stimulation, could be useful for patients with recurrent implantation failure (RIF). It is well established that the success of embryo implantation depends on embryo quality and uterine integrity, including endometrial receptivity.

Although embryo quality is the most consistent factor for predicting implantation and pregnancy rates in IVF patients, this cannot be evaluated independently from uterine integrity or endometrial receptivity [1, 2].

The definition of RIF remains controversial, generally being defined as failure to conceive following two or three embryo transfer (ET) cycles, or cumulative transfer of >10 good-quality embryos [3].

Patients with RIF comprise a heterogeneous group that presents with diverse clinical problems, and need a thorough evaluation.

Evaluation of a couple with RIF includes assessment of maternal and paternal karyotypes, testing for antiphospholipid antibodies, and thorough assessment of the uterine cavity, including sampling of the endometrial lining.

Endometrial biopsy is one inexpensive and minimally invasive method to assess and study the physical and hormonal endometrial environment. If abnormalities are identified and corrected, implantation rates can be improved.

Chronic endometritis (CE) is a persistent inflammation of the endometrial lining. Histologically, the diagnosis of CE is generally based on finding plasma cells, which infiltrates in endometrial biopsies [4]. CE is thought to be related to infertility and spontaneous abortion, and is mostly asymptomatic and rarely suspected clinically [2, 5, 6].

Implantation is believed to be a process with physiologic inflammation, involved with inflammatory mediators, such as leukocytes, chemokines and other pro inflammatory mediators [7].

The great effect of inflammation on implantation was shown in mice by the localized implantation defect after the blockade of the pre-implantation cellular influx into one of the two uterine horns [8]. There is also notable histologic [9] and biochemical [10] supports on the role of physiologic inflammation in labor at term.

The decidua in human uterus has a great number of immune cells such as macrophages [11], natural killer (NK) cells [12, 13], T cells [14] and stromal cells [15] capable to produce soluble factors (e.g., prostaglandins, chemokines and cytokines) involved in regulating the immune response [16].

Wegmann et al. [17], through the classification of T cells into two subgroups by their cytokine profile (Th-1 producing INF, interleukin (IL)-2, and Th-2 producing IL-4, IL-5), suggested that pregnancy takes place in the content of a predominant Th-2 response, and that increase toward Th-1 levels would decrease the chance of successful pregnancy [11].

Although the discovery of newer cytokines and complexity of the inflammatory response at the feto-maternal interface has increased the understanding of the role of specific cellular subtypes (e.g., dendritic cells, natural killer cells, and regulatory T cells) [16] on implantation and pregnancy, the evidence that a Th-1 response in the human decidua may lead to spontaneous abortion remains substantial.

Regardless of how the micro organisms and inflammation effect the implantation, one thing is clear: the cause, prevalence and results of subclinical infection and inflammation in the endometrium and their effect on pregnancy failure (infertility, implantation failure, spontaneous abortion, preterm birth) deserve great attention as a mechanism of pathology [12].

The presence of high concentrations of endotoxins (components of gram negative Bacteria) induces a reaction of TH1 inflammatory cells. TH1 cells may predispose a hostile endometrial environment and thereby cause implantation failure, spontaneous abortion or even premature labor. Endometritis is also considered to be an inflammatory reaction. In contrast to acute endometritis, in CE, usually no causal pathogen can be identified.

2. Chronic endometritis

CE is the continuous inflammation of the uterine endometrium. The prevalence of CE ranges from 0.8 to 19% in the general population and 0.2 to 46% in patients with infertility, based on the population type and the diagnostic criteria of the studies [18]. The pregnancy rate following one cycle of IVF/ET can be as high as 60%. However, even in the very successful units, some couples fail repeatedly. Failure could be due to many different factors, such as inappropriate ovarian stimulation, suboptimal embryo culture conditions and faults in ET techniques. CE is one of the pathologies, which can not be evaluated and diagnosed by HSG and ultrasound, since it is a subtle abnormality, which is usually asymptomatic or with only mild symptoms.

Kasius et al. demonstrated the histological detection of plasma cells (Figure 1) in the endometrial stroma as the gold standard for the diagnosis of CE. It is good to note that even histology can miss the diagnosis due to the normal presence of leukocytes in the endometrium, mostly prior to menstruation.

Matteo et al. [18] showed that CE may reduce endometrial receptivity and may cause infertility because the endometrium is characterized by an abnormal pattern of lymphocyte subsets and, consequently, an aberrant endometrial microenvironment.

In a recent study by Cicinelli et al in 2014 [29], it was demonstrated that in women with repeated abortions, CE is a frequent finding and that women who received adequate antibiotic treatment had a significantly higher rate of successful pregnancies compared with women who were not treated or with persistent disease.

Moreover, in a research study by Quaas and Dokras [109], CE was identified in 30.3% of patients with repeated implantation failure at IVF, and women diagnosed with CE had lower implantation rates (11.5%) after an IVF cycle.

2.1. Chronic endometritis and IVF

Although CE has been related to infertility and recurrent abortion, it is usually asymptomatic, and the diagnosis is rarely clinically suspected [13]. It has also been demonstrated that a present or a recent inflammation of the upper genital tract can affect uterine receptivity and be implicated in worse results in IVF and ET (IVF-ET) [19].

The uterine cavity and its receptivity, despite the constant progress in the assisted reproductive technologies, seem somewhat left behind [20]. ET and implantation are the stages of IVF procedures that have the highest failure rate, and it seems that endometrial abnormality is an important causative factor in this failure. Consequently, any endometrial abnormality should be detected and treated before an IVF-ET cycle to improve pregnancy rates [21].

Approximately, 8% of all couples seek for infertility treatment during their reproductive life. Although the frequency of infertility etiologically depends on the population studied, pelvic infections and their sequelae have been considered important causative factors. CE has been related to infertility and recurrent abortion, particularly because it could affect uterine receptivity and embryo implantation [22].

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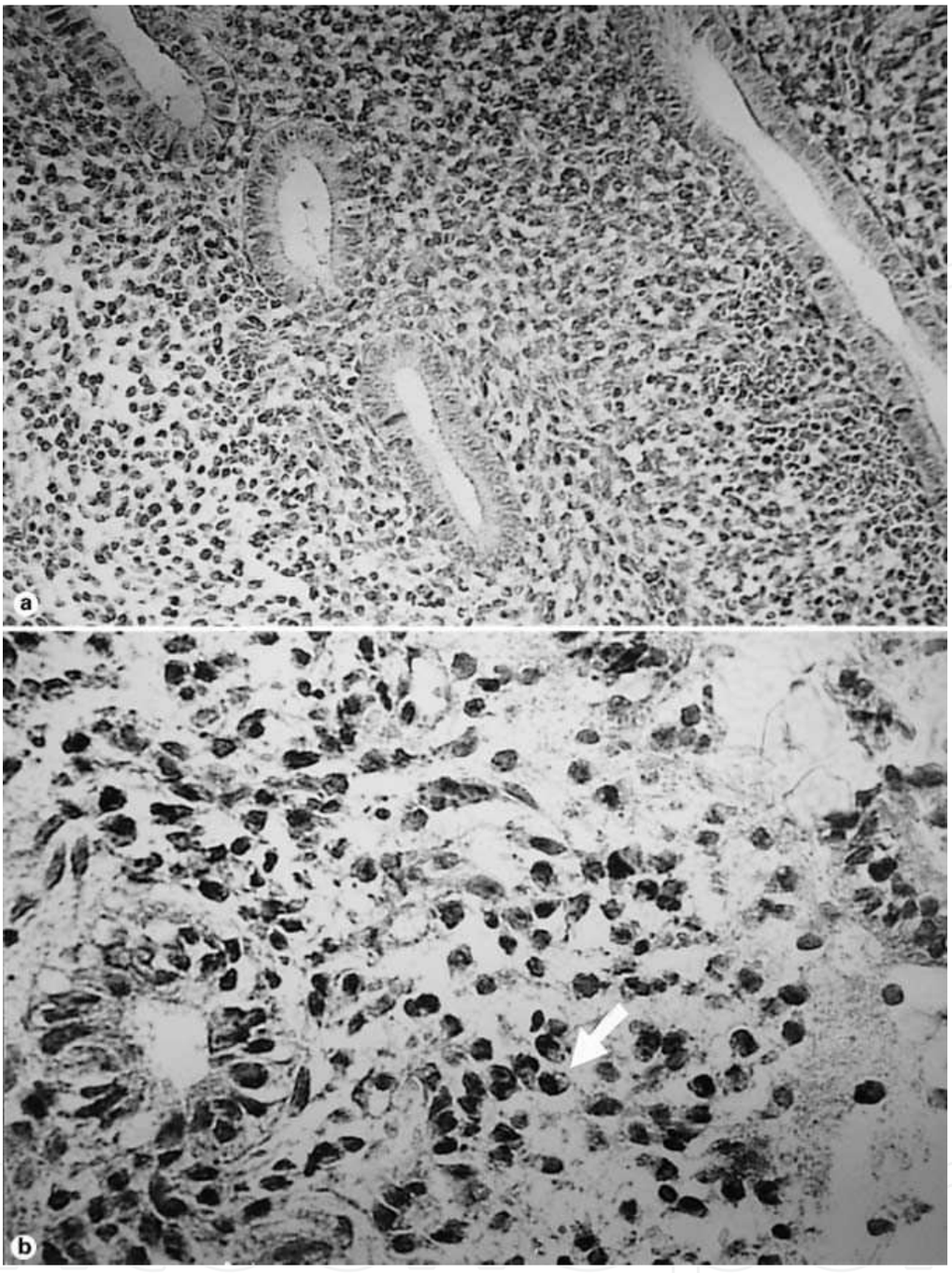

Reproduced with the permission from Polisseni et al. [5]. Copyright © 2003. *Detection of Chronic Endometritis by Diagnostic Hysteroscopy in Asymptomatic Infertile Patients Fernanda Polissenia Eduardo A. Bambirrab Aroldo F. Camargosa. Departments of Obstetrics and Gynecology, and Pathology and Legal Medicine, Faculty of Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil.*

Figure 1. A. Photomicrograph of an endometrial biopsy specimen showing a proliferative endometrium. B. Photomicrograph of an endometrial biopsy specimen showing plasma cell endometritis (arrow).

A specific therapy is required in these cases; so it is important to have a reliable diagnostic test to detect the pathology. Endometritis causes specific stromal, vascular, and glandular modifications that can be detected by hysteroscopy.

Very few studies have investigated the value of diagnostic hysteroscopy in the detection of CE in infertile patients, and they failed in analyzing the real value of this method in these cases.

In evaluating the role of diagnostic hysteroscopy (Figure 2) in the detection of asymptomatic endometritis in infertile patients, a high negative predictive value (89.1%) is identified. This means that when the hysteroscopy shows a negative result for endometritis, it is highly probable that the result is correct and that the patient does not really have an endometrial inflammation.

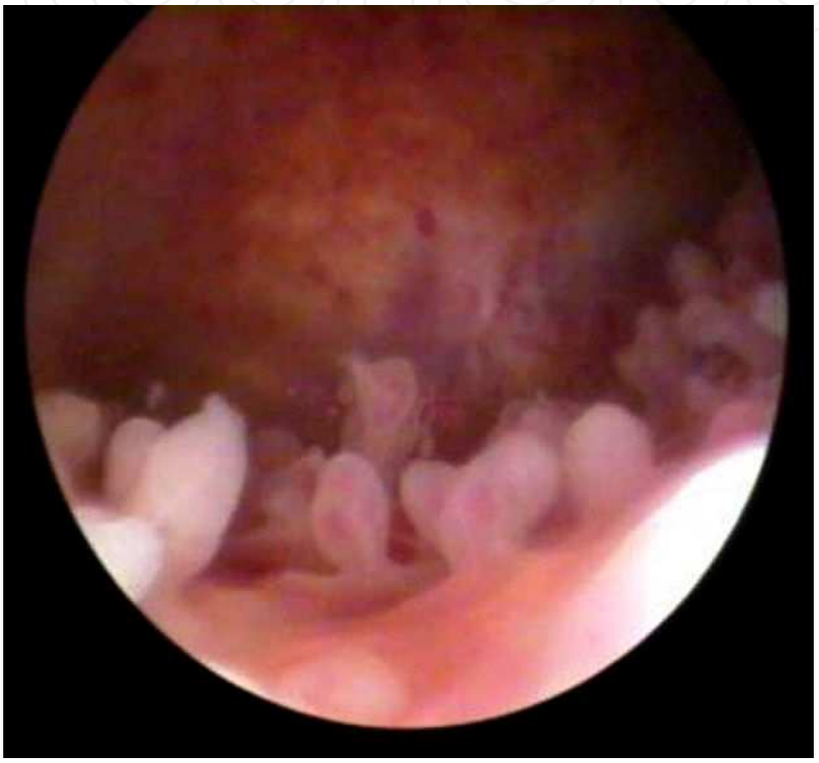

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Figure 2. Chronic endometritis at fluid hysteroscopy. Endometrial mucosa appears thick, edematous, hyperemic and covered by micropolyps (less than 1 mm size), which float into the uterine cavity.

If a diagnostic hysteroscopy shows the absence of endometritis, it is possible to assert, with a good margin of safety, that the patient does not exhibit an endometrial inflammation, which could hinder embryo implantation.

Hysteroscopy is also found to have a low positive predictive value in the detection of endometritis. This means that when the hysteroscopy shows a positive result for endometritis, it is better to consider that this could be an incorrect result and that the patient could have a normal endometrium.

Gump et al. [23] studied 204 infertile patients attending an infertility clinic, and all but 1 had negative cultures for *Chlamydia trachomatis* in cervical and endometrial specimens. These authors have found a significant correlation between prevalence of chlamydial antibodies and

prior pelvic inflammatory disease (PID), as documented by HSG and/or laparoscopy. From these data, Gump et al. [23] concluded that antecedent infection caused by *C. trachomatis*, as measured by antibody prevalence, is an important factor in infertility of tubal origin, but they could not find infections in the cervical or in the endometrial specimens.

In conclusion, data suggest that hysteroscopy is not useful in the screening for CE in asymptomatic infertile women.

2.2. Chronic endometritis and recurrent miscarriage

Recurrent miscarriage (RM) is defined as three or more miscarriages before 20 weeks of gestation and it affects 3% of couples.

Genetic abnormalities, anti-phospholipid syndrome, endocrine disorders, and uterine abnormalities have been diagnosed in 50% of these couples. The other 50% are diagnosed as with unexplained RM. The competence of the uterine environment is an issue of big interest these days. The regular infertility investigations, such as HSG and ultrasounds, are unable to detect small intrauterine pathologies, and the prevalence of unsuspected intrauterine abnormalities in hysteroscopy has been shown to range between 11% and 45%.

In recent years, the focus on CE, a slight and mostly asymptomatic inflammation of the endometrial lining, is growing [24, 25].

A retrospective pilot study [26] showed a relationship between RM and chronic endometrial inflammation, which could have several clinical implications.

First, they showed that CE is a common finding in women with RM. Second, this study showed that mycoplasmas and the bacteria are the most frequently involved micro organisms in CE.

Third, the study demonstrated hysteroscopy as a reliable diagnostic technique for CE and that the previously noted endometrial pathologies [26, 27] such as micropolyps, stromal edema, and diffuse or focal hyperemia are clearly related to the inflammatory state of the endometrium. Moreover, results indicated an improved reproductive outcome and a normal hysteroscopic exam after an appropriate antibiotic treatment in patients with CE.

Fourth, the results showed a restored fertility status in women with CE after appropriate antibiotic treatment, suggesting the appropriateness of performing a hysteroscopy in women with RM.

Fifth, the sustained signs of CE in hysteroscopy after treatment, even with negative endometrial cultures, were related to a worse reproductive outcome.

Their results are in concordance with those from the PID Evaluation and Clinical Health (PEACH) study, which showed non-gonococcal, and non-chlamydial infection in approximately 60% of women with PID [28].

Considering the high prevalence of bacterial vaginosis and the fact that ascending bacteria can colonize the uterine cavity, those data are not surprising [29]. In fact, bacterial vaginosis has been associated with PID and, in ART, may decrease implantation rates, increase early

miscarriages, and may also increase the risk of preterm labor [30]. This study suggested Mycoplasma and *Ureaplasma urealyticum* to be responsible for CE in about 24% of the cases.

This data do not disagree with the recent opinion that the uterine cavity is normally not sterile and that the presence of microorganisms does not mean inflammation [31–33]. Therefore, the main issue that causes the pathology is rather the interactions between the infectious agents and the endometrial environment than just the presence of infectious agents within the uterine cavity [30].

Fluid mini-hysteroscopy is a minimally invasive procedure that can be performed in an office without anesthesia [34], so the benefit in terms of evaluation, diagnosis and treatment sufficiently overcome the cost of hysteroscopy. The improvement of the reproductive outcome after both antibiogram-guided antibiotic therapy and the CDC guideline-based treatment supported the value of the hysteroscopic evaluation in women with RM.

Another important finding in the mentioned study data was that in the subjects with a negative endometrial culture, the ones with persistent CE diagnosed by hysteroscopy had a worse reproductive outcome compared to patients without CE in hysteroscopic evaluations after treatment. This shows that the hysteroscopic evaluation in patients with endometrial inflammation has a higher sensitivity than cultures and that a normal hysteroscopic evaluation could be more accurate in predicting of a successful pregnancy after treatment.

In conclusion, although some limitations are related to retrospective studies, in this study, Cicinelli et al, evaluating a large number of patients, demonstrated that CE is a condition commonly associated with RM. Mycoplasma and common bacteria were most prevalent microorganism for CE.

In women with RM, hysteroscopy reliably detects the existence of CE. The normalization of the hysteroscopic endometrial pattern seems to be associated with a significant improvement in the reproductive outcome [25].

In one study by Kitaya et al [36] using immuno-histochemical analysis of the stromal plasmacyte marker syndecan-1, CE was identified in approximate 10% of the women with RMs. In patients with RMs of unknown etiology, its prevalence was approximately 12-13%. It was lower compared with the reported prevalence (approximately 30%) in repeated embryo implantation failure after IVF-ET [35] and unexplained infertility [36]. All patients with CE had a history of RMs in the first trimester of pregnancy. Amassment of stromal B cells was observed in all the endometrium exhibiting CE. A flow cytometric study demonstrated that the proportion of B cells in endometrial mononuclear cells was threefold higher in the patients with RMs than in fertile women during the mid-secretory phase [37].

In addition, another morphometric study found that 2 of 22 patients with unexplained RMs had more endometrial B cells than any other patient or fertile woman in the mid-secretory phase [38].

2.3. Diagnosis

Most of the patients with CE are asymptomatic, which causes some diagnostic challenge.

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The gold standard for diagnosis of CE remains the histopathologic evaluation of the endometrial biopsy specimen, which is through the identification of plasma cells in the stroma of the endometrium by immunohistochemistry [4].

This method of diagnosis is limited by

1. Inadequate sampling of the endometrium
2. Inadequate staining of the specimen
3. Dependency on the existence of plasma cells, that can be hard to localize
4. Confounding factors like fibrotic stromal cells that can mimic plasma cells
5. Unknown clinical significance of the presence of few plasma cells

Considering these limitations, physicians have studied the role of hysteroscopic evaluation of the endometrium to diagnose CE with mixed results. The suggested hysteroscopic findings for the diagnosis for CE have been mucosal edema, areas of hyperemia, and micropolyps [39].

A study by Yang et al in patients with RIF showed a sensitivity and specificity of 35% and 68%, respectively, for diagnosis of CE by hysteroscopy. In addition, hysteroscopic and histological evaluations resulted in identification of 66% and 44% of CE cases, respectively [39].

Proponents of hysteroscopy as a diagnostic tool for CE argue that hysteroscopy allows a complete evaluation of the uterine cavity, whereas the endometrial biopsy is a random biopsy of the uterine cavity and therefore may miss focal points of inflammation.

The isolation of infectious agents (common bacteria, *Neisseria gonorrhea*, *Chlamydia trachomatis*, *Mycoplasma* species, *Ureaplasma urealyticum* and yeast) from the endometrial cavity is the last method of diagnosis for CE. A study completed by Cicinelli et al showed that among 483 women with hysteroscopic-diagnosed CE, 73% had at least a single microorganism isolated from endometrial cultures vs. 5% in the control group [18].

Syndecan-1 (Figure 3) is a plasma cell, cell membrane marker. It has been broadly used to detect plasma cells in flow cytometry and is a useful marker to detect both malignant and benign plasma cells in paraffin-embedded bone marrow biopsies [40]. Syndecan-1 is a cell surface proteoglycan that facilitates cell to cell adhesion, cell to extracellular matrix adhesion, cell proliferation and migration [41].

Syndecan-1 expression was evaluated in CE and dysfunctional uterine bleeding. In all subjects with CE, plasma cells were noted by light microscopic study of both hematoxylin and eosin (H&E) and syndecan-1 dyed slides. Immunohistochemical staining with syndecan-1 increased the plasma cell detection. Plasma cells have characteristic "clock-face chromatin in an eccentrically placed nucleus with a perinuclear halo" appearance. This characteristic can be easily visible by syndecan-1 staining. This staining also showed that some plasma cells lacking some of the classic features, were actually plasma cells.

Identifying plasma cells in the endometrial stroma of CE can be very challenging.

Some conditions can present with the histologic findings of CE or affect the search for plasma cells. These conditions are early proliferative endometrium or late menstrual, monocytic

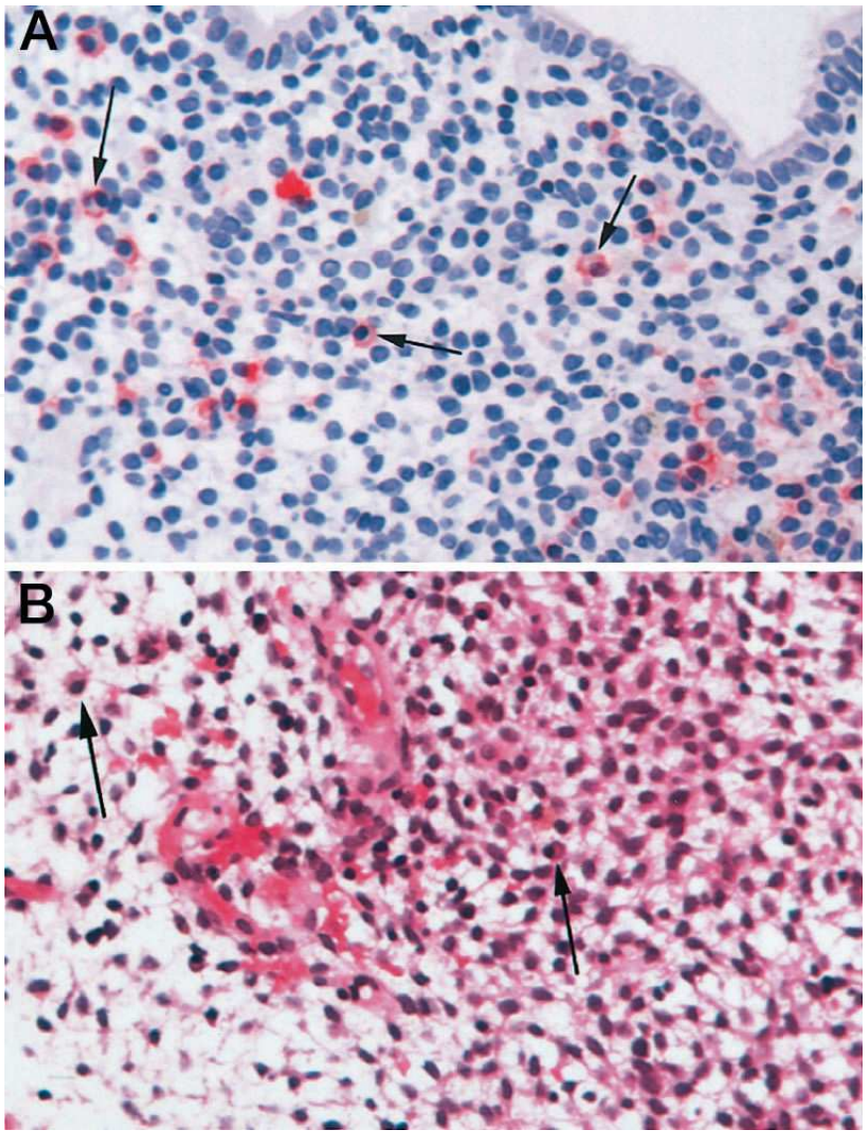

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Figure 3. A. Plasma cells membrane staining with syndecan-1 in endometrial tissue showing a prominent spindle cell stromal component in chronic endometritis. B. H&E staining of the same field showing plasma cells.

inflammatory infiltrates, ample stromal mitoses, the plasmacytoid appearance of stromal cells, stromal cell proliferation or a marked pre-decidual reaction in a late secretory endometrium [4, 42].

This is notable that plasmacytoid stromal cells and macrophages do not stain with syndecan-1 and plasma cells can be recognized by immunohistochemical staining with syndecan-1 even in conditions that may interfere with their identification on H&E.

The search to localize plasma cells, in order to establish CE, does not always involve a quick examination of the specimen. It is usually a time-consuming task. Many times there are suspicious for CE, when plasma cells cannot be detected in H&E slides, and syndecan-1

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immunohistochemistry staining helps in identifying the plasma cells and then the diagnosis of CE can be documented. Immunohistochemical syndecan-1 staining decreases the time consumption looking for plasma cells in patients suspicious for having CE, and will help the diagnosis in cases with other histological findings that can interfere with the detection of plasma cells.

It is of importance to mention that CE should not be the diagnosis when only mere number of plasma cells are detected, since endometrial stroma can contain limited number of plasma cells without an inflammatory process [43]. Only in the presence of a characteristic setting of CE, the diagnosis can be considered and the detection of plasma cells will be confirmatory.

In conclusion, syndecan-1 staining can be a great assistance and tool identifying the plasma cells and aid in the diagnosis of CE in patient with suspected CE when plasma cells can not be localized in H&E stained also when there are other histologic findings interfering the detection of plasma cells [44].

2.4. Pathophysiology

Studies characterizing the distribution of leukocytes in CE have noted an abnormal pattern of leukocytes. Not only the overall number of leukocytes rise, but also the accumulation of the leukocytes is superficial under the endometrial surface as well as around the superficial blood vessels and glands [4].

Plasma cells have been also identified in unusual locations, such as within the lumen of the glands or the intraepithelial [36]. It is believed that these plasma cells provide an abnormal local micro-environment, that decreases the endometrial receptivity.

Since plasma cells are in a minimal number in a healthy endometrial cavity, their presence in larger numbers in CE is considered pathologic. Immature B cells only normally express IgM immunoglobulins. Yet, when naïve B cell meets a particular antigen, a class-switch DNA recombination occurs, causing its differentiation into a plasma cell [45].

Based on the type of inflammatory response, plasmacells are able to produce a variety of immunoglobulins. The majority of the immunoglobulins in the endometrial cavity are IgM and IgA in the epithelial cells and IgG in the stromal cells.

The density of the Ig-bearing stromal cells, including IgM, IgA and IgG, is higher in patient with CE compared to people with normal healthy endometrium. Specially, IgG2 cells are noted to have an extremely higher density than other immunoglobulin subclasses in the endometrial stroma cells of the patients with CE [46]. This special Ig subclass expression is suggested to be a possible mechanism contributing to CE and implantation failure.

The role of matrix metalloproteinases (MMPs) is another interesting hypothesis contributing to inflammation, CE and implantation failure. Lately, a difference in the expression profile of several inflammatory cytokines has been demonstrated through endometrial gene expression studies in women with CE and infertility [47]. Endometrial stromal and epithelial cells by down-regulating the IL-11,could cause a dysregulation of trophoblast invasion. The noted

significant decline in CCL4 in CE, may also increase the employment of NK cells and macrophages into the endometrial environment [48].

2.5. Treatment

Mostly due to non-standardized treatment protocols and conflicting results about pregnancy outcomes, so far the management of CE to increase in the implantation rate has been controversial.

Some authors recommend treating CE first with of doxycycline for 2 weeks, then with persistent inflammation in a second biopsy, and ciprofloxacin and metronidazole for another 2 weeks [49]. Some others base the treatment on the identified infectious agent and the antibiotic sensitivity profile, giving ciprofloxacin for gram-negative bacteria, amoxicillin/clavulanate for gram-positive bacteria, and wide spectrum antibiotics (metronidazole ceftriaxone, doxycycline) for even negative cultures [18].

In one study, a treatment of antibiotics plus steroid was used to treat women with increased MMP activity in uterine fluid lavage [48].

The efficacy of treatment of CE to increase endometrial implantation and pregnancy rate is unclear. In a cohort study, clinical pregnancy per embryo transferred and live birth rates showed no change between the treated and non-treated patients with CE undergoing their first IVF/ICSI cycles. Both groups underwent endometrial biopsy and hysteroscopy as part of a larger RCT [18].

Another study on RIF compared treated women with a positive biopsy for CE (10) to women with a negative biopsy (23). Even after antibiotic therapy, the implantation rates stayed lower in women with CE (11.5 vs. 32.7%) [49]. Cicinelli et al, in a recently published retrospective study of women with RIF, showed an improvement in pregnancy rates and live birth rates with antibiotic treatment in patient with CE compared with the patient with persistent CE, 65 versus 33% and 60.8 versus 13.3%, respectively [18]. To date, this is the only study showing a significant higher live birth rates after antibiotic therapy of CE. Although, it is good to note before generalizing these results as subjects in this research had hysteroscopic evaluation as a first diagnostic module for diagnosis of CE, which can be subjective. Also, instead of blastocyst-stage embryos, cleavage-stage embryos, were transferred, which may not be applicable to all practices.

2.6. Summary

Physicians mostly look for the diagnosis of CE, in cases of RIF; however, because the lack of a universally accepted definition for RIF indirectly biases the pool of patients who are worked up for CE. The diagnosis of CE becomes even more complex and difficult by limitations in microbiology and immunohistopatholog, and the subjective nature of hysteroscopy. Management of CE is also non-standardized. Taken all together, the absence of definitive evidence to establish improved pregnancy outcomes after the treatment of CE precludes establishing a standard of care [48].

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3. Hydrosalpinges

Hydrosalpinx is defined as a chronic inflammatory condition in which a fallopian tube is filled with fluid as the result of distal obstruction.

PID caused by chlamydia or gonorrhea is considered as the primary cause of hydrosalpinx; however, other conditions that may cause tubal obstruction include adhesions from previous surgery, endometriosis, non-tubal infections (i.e., appendicitis, inflammatory bowel disease), salpingitis isthmica nodosa and pelvic tuberculosis.

The diagnosis rate of hydrosalpinges ranges from 10 to 13% by ultrasound, and up to 30% by laparoscopy or HSG [50]. Hydrosalpinges are documented in ultrasound as cystic elongated masses in the adnexa that can sometimes wrap around the ovaries [51].

There is some controversy for the definition of a "clinically significant hydrosalpinx," with some studies proposing that the diagnosis should be only made when hydrosalpinges are visible in ultrasound [52]. Findings of distal tubal occlusion on hysterosalpingogram would not qualify. Studies have shown low intra-observer reliability in detecting hydrosalpinges on HSG (kappa -0.28), when the intra-observer reliability for distal tubal obstruction was higher (kappa -0.71) [53]. This demonstrates the fact that hydrosalpinx and distal tubal occlusion are not synonymous conclusions.

A recent survey performed from members of the Society for Reproductive Surgeons and the Society for Reproductive Endocrinology and Infertility has highlighted the variation in clinical practice. The results showed that 60% of the members would make the diagnosis based on transvaginal ultrasound and 70% would make the diagnosis upon visualization of a dilated tube that is distally occluded on laparoscopy. However, commonly 80% of members would diagnose a hydrosalpinx based on a dilated tube that is distally occluded on hysterosalpingogram [54].

The presence of unilateral or bilateral hydrosalpinges has been shown to adversely affect implantation or pregnancy rates for IVF in different clinical studies. There were two main meta-analyses performed. The first meta-analysis included over 6,700 treatment cycles from 11 studies, which identified that the implantation rate and the clinical pregnancy rate were 50% lower in patients with hydrosalpinges compared to patients without hydrosalpinges. Miscarriage in patients with hydrosalpinges had also a twofold higher rate [55].

3.1. Pathophysiology

There are multiple hypothesis as to how hydrosalpinges can affect and predispose to implantation failure. These include a direct embryotoxic effect, [56] decrease in subendometrial and endometrial blood flow [57] decrease in endometrial receptivity, [50] possible developmental abnormalities of the endometrium [58] and tubal fluid, which can compromise the contact between the embryo and the endometrial surface as mechanical effect [59].

In a retrospective case-control study, inflammation and inflammatory markers in hydrosalpinges were investigated. This study evaluated 21 cases of hydrosalpinges and 9 cases of

chronic salpingitis, and it was shown that the cases with hydrosalpinx were found to have an increase in inflammatory cells in the endometrium, including neutrophils and basophils. This correlation was statistically significant [60]. This case-control study showed that 65% of cases had high-intensity staining of IL-2, a marker for generalized inflammation, compared with only 7.4% in control group.

Inflammatory markers, especially Th-1 inflammatory cytokines, have been implicated in recurrent pregnancy loss, which is in favor of these markers playing a critical role in implantation failure [61]. In the presence of hydrosalpinx, similar to LIF, the expression of $\alpha v\beta 3$ integrins has been reported to be decreased [62] with an increase and return to normal levels following salpingectomy [63]. Integrins mainly play a role in cell-cell and cell-matrix adhesion and interaction in a variety of physiological processes, which include immune defense mechanisms and wound healing and should not be specifically considered inflammatory markers. Interestingly, $\beta 3$ integrins are cell-cycle-specific and are mainly expressed between cycle days 20-24, proposing the hypothesis that they might play a role during the window of implantation [64].

Poor outcomes have been seen with hydrosalpinx and the reasons are not clearly understood. The unfavorable effect seems to be directly related to the presence of a hydrosalpinx, rather than an embryo or oocyte factor.

Some investigators have researched the affect of the fluid from hydrosalpinx on the embryo and whether it has an embryotoxic effect or not and have determined that there might be an embryotoxic affect. On the contrary, recent studies have raised doubt about this toxin-induced effect. To further investigate this hypothesis, Strandell et al. [65] cultured human embryos in 50% hydrosalpinx fluid and realized that these embryos developed to blastocysts at the same degree as human embryos grew in a standard culture media.

The poor IVF outcomes seen in patients with a hydrosalpinx have been hypothesized to be related to tubal inflammation. A chronic inflammatory process can be present for multiple reasons. It could be observed with recurrent bacterial infections, including chlamydia, as a more common cause, or other bacteria. Acute inflammation can happen during an IVF procedure because of bacterial seeding of the hydrosalpinx during either ET or oocyte retrieval. As there is open communication between the tubes and the endometrium, the inflammatory process could directly spread from the fallopian tubes to the endometrium. The outcome of this process could then impact the development of the pre-implantation embryo and causes a change in the endometrial environment [66].

3.2. Treatment

The American Society of Reproductive Medicine has proposed that based on the clinical studies there is evidence that for every six women with hydrosalpinges, one more ongoing pregnancy will be successful if salpingectomy is performed prior to initiating IVF treatment [67].

A known approved alternative to salpingectomy for hydrosalpinges is laparoscopic proximal tubal occlusion.

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A randomized control trial was performed to evaluate the efficacy of laparoscopic salpingectomy and laparoscopic proximal tubal occlusion in 115 patients with bilateral or unilateral hydrosalpinges undergoing IVF treatment. These women were randomized to three groups: two treatment and one control. The outcome in group with laparoscopic proximal tubal occlusion was better than control (no surgery) group and compared to the laparoscopic salpingectomy group [68].

In a recent meta-analysis of eight RCTs evaluating the efficacy of salpingectomy versus proximal tubal occlusion in hydrosalpinges, same results as above was achieved in which the implantation and clinical pregnancy rates were not significantly different between the treatments, odds ratio (OR), 0.86 (95% CI: 0.53–1.4) and OR, 1.56 (95% CI: 0.81–3.0), respectively [69].

The use of hysteroscopic Essure microinsert placements to occlude hydrosalpinges has been studied, mainly in women who have contraindications to laparoscopic procedure. The Essure system is a spring device composed of nickel–titanium and Dacron fibers. The Food and drug Administration (FDA) has approved the Essure system as a sterilization method. The device is space occupying, and can induce an inflammatory response that can cause fibrosis in the tubal lumen. To this date, the studies and literature on the use of Essure for occluding hydrosalpinges is limited to small case series with unclear outcomes and mixed results.

The first live birth from IVF using Essure, following proximal occlusion of a hydrosalpinx, was reported by Rosenfield et al in 2005. This was performed in a 31-year-old nulligravid woman with a history of significant pelvic adhesions and a body mass index of 50 [70]. This unfavorable surgical candidate delivered a dichorionic-diamniotic twins at 34 weeks of gestation after the transfer of three cleavage-stage embryos.

In a case series of 15 patients who underwent Essure placement, 6 were reported to have successful pregnancies. On the other hand, there are also reports of complications using the Essure device, such as unsuccessful placement of the device, subsequent expansion of the hydrosalpinx and PID, which could potentially require an emergency laparotomy and bilateral adnexectomy. There also seems to be a hypothetical concern for having the Essure coils in the uterine cavity [48]. Larger scale studies and prospective multicenter clinical trials should be performed before recommendations regarding hysteroscopic tubal occlusion can become part of standard of care in patients with hydrosalpinx.

Retrospective studies have shown that the larger the hydrosalpinx, the worse the outcome after IVF, which raises the question of embryo toxic effect of the fluid.

Whatever the exact mechanism, an interruption of the communicating hydrosalpinx appears appropriate to improve the implantation in the endometrial environment [71].

In a study performed by Hurst et al, patients with an increased quantitative serum *Chlamydia trachomatis* IgG antibody titer were considered for treatment with doxycycline 100 mg twice daily for a total of 10 days before the first IVF cycle was initiated. This group also included 75% of the patients who had hydrosalpinx. The outcome of the study, which was implantation and pregnancy rates, was slightly lower in the group with hydrosalpinx than in the control

group; however, this difference was not statistically significant. In summary, patients treated with doxycycline for extended periods whom had hydrosalpinx did not have lower IVF success rates. On the contrary, the highest implantation rate was present in the group with hydrosalpinx [66].

An unfavorable effect of a hydrosalpinx could be caused by a chronic or acute tubal bacterial infection that could potentially affect the endometrium. This detrimental effect could be suppressed and become ineffective by using extended antibiotic therapy. In addition, healthcare cost can be prevented and minimized if initiating treatment with a 2-week course of an inexpensive antibiotic provides the same outcome comparable to surgical treatment of a hydrosalpinx prior to IVF treatment.

4. Salpingectomy

Studies have shown that patients with hydrosalpinx visible by ultrasound, are generally the ones that benefit from salpingectomy. As a result, this group of patients are the ones who are recommended to perform prophylactic salpingectomy prior to IVF treatment.

The psychological impact of this surgery and the removal of the tubes in an infertile patient are significantly important and should be emphasized. Even if a patient has a clear candidate for salpingectomy, it is essential that she is psychologically prepared for the procedure. At times, it has been necessary for the patient to undergo a few failed cycles before one can bring up the discussion of surgery and salpingectomy. Obviously, the final decision to perform salpingectomy should be based on appropriate evaluation of the tubal mucosa at laparoscopy and therefore avoid unnecessary surgery for the patient. It is crucial that surgeons identify carefully that whether a hydrosalpinx should be excised or is acceptable enough for a surgical repair [71].

4.1. Tubal ligation vs. salpingectomy

There are no results from randomized trials to answer this question. The data from two retrospective studies by Surrey/Schoolcraft [77], did not show any significant differences in pregnancy outcome, but the number of patients has been too low to allow for any conclusion. Today, there is no evidence that transvaginal aspiration is as effective as salpingectomy, but it is an option for patients who will not undergo salpingectomy, and for those who develop tubal fluid during stimulation.

Hydrosalpinges with destroyed mucosa are not suitable for reconstructive surgery. However, patients with these also have an impaired success rate after IVF, possibly due to the leakage of fluid into the uterus. Salpingectomy is the only method that has been properly evaluated as a surgical approach to overcome the negative influence of the hydrosalpingeal fluid.

From the Scandinavian study, a clear conclusion was drawn: patients with hydrosalpinges large enough to be visible on ultrasound examination can be recommended laparoscopic salpingectomy prior to IVF in order to enhance their chance of a full-term pregnancy. Patients

with large hydrosalpinges and without prospect of spontaneous conception should be recommended a salpingectomy, which truly increases their chances of a successful IVF treatment [71].

4.2. Surgical vs. medical management

Different hypothesis on medical management for hydrosalpinx have been considered. This is not just prophylactic antibiotics to patients after the puncture of hydrosalpinx. Sharara et al. in 1996 suggested giving prophylactic antibiotics to selected groups of patients with elevated serum Chlamydia trachomatis IgG antibody titers or considered as a routine before oocyte retrieval for all patients.

Nevertheless, this hypothesis of antibiotic treatment specifically in hydrosalpinx patients has not been studied or evaluated prospectively.

Hurst et al. in 2001 in a retrospective study worked on hydrosalpinx and antibiotic treatment. In this study, patients with hydrosalpinx who received prolonged doxycycline treatment during an IVF cycle were compared to those who did not receive antibiotics and had other conditions, such as (endometriosis/unexplained infertility or tubal occlusion without hydrosalpinx/adhesions). Implantation and pregnancy rates were similar in all groups. This concludes that antibiotic treatment could also minimize the detrimental effect of hydrosalpinx. Even though this method is simple and safe, its benefits should be evaluated in a prospective trial before it becomes a standard of care [71].

4.3. Salpingectomy vs. proximal tubal occlusion

One of the secondary benefits salpingectomy provides, is the removal of a mass that may possibly be infected and could also be a source of torsion. Hydrosalpinx left in situ, can potentially have a negative impact because it can cause an increase in the risk of infection and decrease in access to the ovary during oocyte aspiration.

On the contrary, there are potential disadvantages to the performance of salpingectomy. Salpingectomy is a procedure that would need expertise because it would be difficult to perform in patients with extensive pelvic adhesions, also considered an invasive procedure and could potentially increase the possibility of injury to the surrounding tissues and structures. In addition, transection of the tube too close to the cornua may also increase the risk of an interstitial pregnancy after ET, a devastating complication [72].

Salpingectomy could also hypothetically result in a decrease in ovarian perfusion, as part of the blood supply to the ovaries is provided by branches of the uterine artery and the mesosalpingeal vascular arcade [73]. Acute reduction in ovarian blood flow may have a direct impact on ovulatory function in the rat model [74]. In a study by Lass et al. [75], it was shown that fewer follicles were developed and fewer oocytes were retrieved from the ipsilateral ovary in women who had undergone unilateral salpingectomy.

On the contrary, Dar et al. [76] reported that ovarian response in assisted reproductive technology cycles that was performed before and after laparoscopic salpingectomy for ectopic pregnancy was not affected by surgery [76].

Of note, proximal tubal occlusion constitutes a significantly less invasive approach that includes minimal surgical intervention and less time allocated for the surgery, while at the same time eliminating retrograde flow of hydrosalpingeal fluid into the endometrial cavity. The current study demonstrated that laparoscopic proximal occlusion of the affected fallopian tube with bipolar cautery had similar ovarian response and IVF-ET cycle outcome as that of controls or as of those who have undergone laparoscopic salpingectomy [77].

In conclusion, prophylactic surgical management of hydrosalpinges by either proximal tubal occlusion or laparoscopic salpingectomy demonstrated statistically similar responses to controlled ovarian hyperstimulation and IVF-ET cycle outcomes. There is no evidence of any compromise in ovarian response induced by either surgical procedure [77].

4.4. Spontaneous or surgical drainage of the hydrosalpinx

Studies have shown that draining hydrosalpinges surgically or with the help of ultrasound guidance have improved pregnancy and implantation rates [78].

Even though this technique would potentially decrease the overall volume of hydrosalpingeal fluid, drainage cannot eliminate its source or its ability to flow into the endometrial cavity even in decreased amounts. Sowter et al. [79] have shown that surgical drainage of distended hydrosalpinges offered no benefits in enhancing implantation or pregnancy rates over untreated controls. In addition, Bloechle et al. [59] have demonstrated re-accumulation of hydrosalpingeal fluid within 3 days of aspiration performed at oocyte retrieval, which would precede the time of embryo implantation. As a result, this would hypothetically eliminate the benefit of drainage alone.

It appears that the drainage of a hydrosalpinx might not have much benefit to implantation. Generally, re-accumulation of fluid in the tubes can cause subsequent drainage into the uterus, resulting in the distention of the uterine cavity, which has been reported by Mansour et al. in 1991 [110] and many others.

Failure to benefit from drainage of hydrosalpinges might not necessarily be a result of incomplete drainage leaving some fluid to spill into the uterine cavity or from fluid re-accumulation. There could potentially be a functional destruction to the tube which would enhance retrograde passage of transferred embryos, both increasing the risk of ectopic pregnancy, and also contributing by wastage of embryos to the observed decrease of intrauterine implantation. If, indeed, this was a notable mechanism causing decreased intrauterine implantation, there would also be no advantage from undertaking distal salpingostomy simply to maintain tubal drainage.

The study results highly recommend that transvaginal drainage of hydrosalpinges provides no benefit at the time of oocyte retrieval for IVF treatment [80].

4.5. Summary

Hydrosalpinges is a chronic inflammatory condition that could unfavorably affect the chance of endometrial receptivity.

Several studies have shown changes in the inflammatory and immunological markers in the endometrium of patients exposed to hydrosalpingeal fluid. Markers identified were IL-2, NK-κB, and LIF.

Today, prior to IVF treatment, the routine approach is a surgical intervention performing laparoscopic salpingectomy or proximal tubal occlusion.

Some studies have demonstrated the most surgical benefit in the subset of patients with bilateral hydrosalpinges or ultrasound visible hydrosalpinges.

5. Microbiome of the reproductive tract

As more is learned about the human microbiome, it is becoming evident that it meaningfully affects the physiologic function of virtually every organ where bacteria are present.

The human body is colonized with an order of magnitude more bacteria than human cells in the body [81]. The majority of published medical literature focuses on the subset of the microbiome involved in pathogenesis, and only a subset focuses on the physiologic role that the microbiome plays.

The importance of this was recognized in 2001 at the time of the human genome published [82], when scientists called for a "second human genome project" that would investigate the normal microbiome colonies at various sites to understand the synergistic interactions between the microbiome and its host [83]. Several initiatives commenced worldwide, and in the United States the Human Microbiome Project led by the National Institutes of Health was launched in 2007, using high throughput sequencing technologies to characterize the human microbiome in 250 normal healthy volunteers at multiple body sites [81].

The female reproductive tract has long been known to have an active microbiome (Figure 4). Although the greatest focus has been on the vaginal milieu, data have been accumulating for decades demonstrating that the remainder of the female reproductive axis is not sterile. In fact, with more than 20 studies completed, virtually all of them have found that there is a small but active microbiome in the uterine cavity.

Importantly, many of these studies obtained their samples at the time of surgery with the use of transfundal collection techniques where there was no potential for contamination from transiting the vagina or endocervical canal. The majority of these studies were done with the use of traditional culture techniques to identify any bacteria that were present.

More recently, metagenomic techniques are confirming earlier findings and providing a more comprehensive definition of the endometrial microbiome.

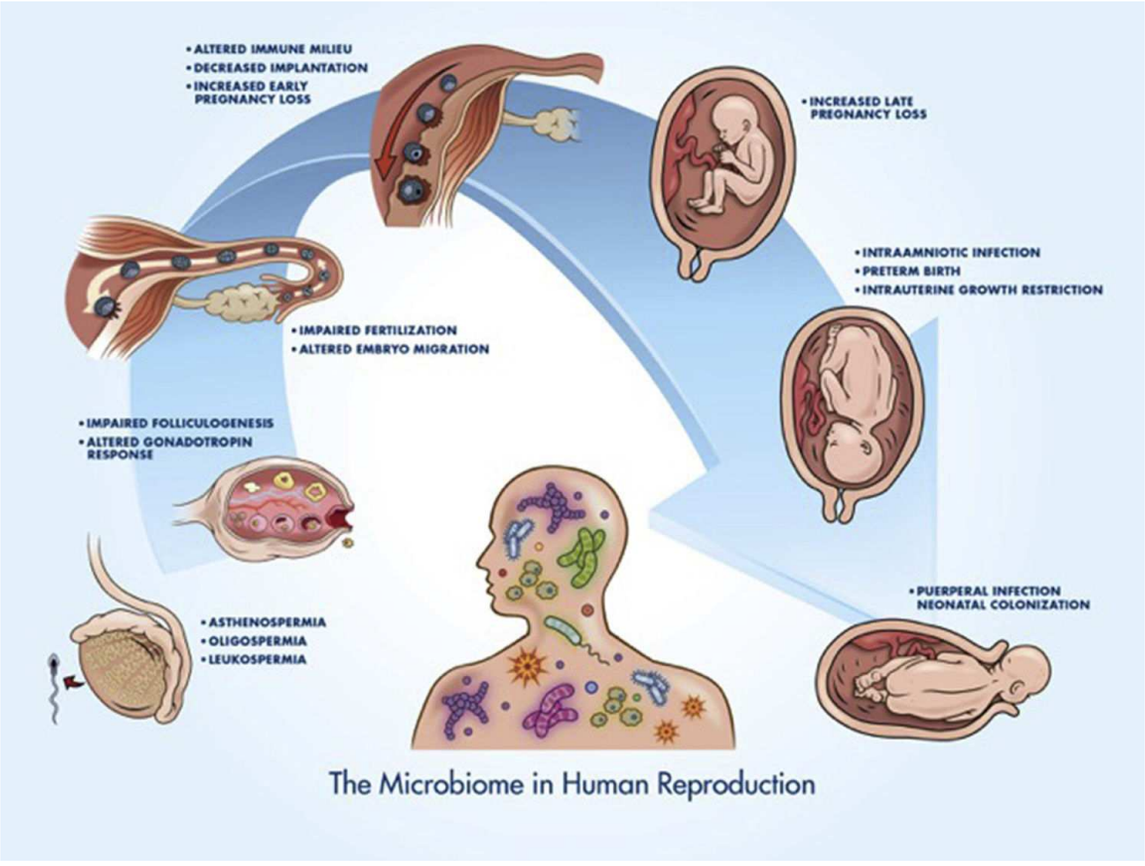

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Figure 4. The human microbiome affects all facets of reproduction from gametogenesis, to fertilization and embryo migration, to implantation with implications in early pregnancy failure, to involvement in late pregnancy loss, and poor obstetric outcomes during gestation and parturition in terms of intrauterine infection and preterm birth, among other things. A more complete characterization of this complex symbiosis is imperative as we understand its implications in human health and disease.

Interestingly, the microbiome extends above the endometrial cavity. Some studies have demonstrated bacteria in the fallopian tubes of women without obvious tubal pathology. Additional studies have demonstrated that the intra-follicular milieu may have an active microbiome in some patients.

Finally, there are now studies showing that the microbiome of the male reproductive axis is more complex than previously appreciated. The addition of metagenomic tools allowed descriptions of much broader and more complex microbiome, even in men without evidence of acute or chronic inflammation of their reproductive tract.

As the microbiome of the female and male reproductive axis has become more clearly defined, studies evaluating the clinical impact on ART treatment have followed. Given the influence which the microbiome has in virtually every organ systems, it is not surprising that subtle changes in the microbiome are associated with meaningful changes in gamete quality and ultimate clinical outcomes. In some cases, changes in the microbiome may provide insight into previously unexplained treatment failure [82].

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5.1. Diagnosis

It is important to briefly recognize that microbiome data are procured in one of two ways: culture-based or sequencing-based technology.

The various techniques available in metagenomics (fingerprinting, DNA microarrays, targeted sequencing, and whole genome sequencing) supply both strengths and weaknesses depending upon the primary purpose of the analysis.

Much of the early work describing the human microbiome comes from culture-based approaches using the 16S rRNA analysis of highly conserved genes as a way to characterize the diversity of the microbiome in a given environment [84]. However, data from the vaginal microbiome suggest that many organisms can not be identified with the use of culture-based techniques, which results in underestimating the diversity of the ecosystem as well as failing to identify potentially important organisms when describing their relationship to health and disease [85]. Thus, culture-based data, though still informative, must be interpreted within the limits of the technology.

Data presented more recently have relied on 16S rRNA gene sequencing, specifically the hypervariable regions within the gene, which serves as a molecular fingerprint down to the genus and species level [86]. Although to date, data that describe the microbiome of the reproductive tract have not widely used this technique, metagenomics is becoming an increasingly widespread approach to describing the microbiome [87]. Using this method, also termed community genomics, analysis of microorganisms occurs by means of direct extraction and cloning of DNA from a grouping of organisms. It allows analysis that extends beyond phylogenetic descriptions and attempts to study the physiology and ecology of the microbiome [82].

The study of the microbiome and its relationship to the efficiency of conception and early pregnancy maintenance is just beginning. Although there have been efforts to distinguish between normal or favorable microbiomes and those that impair or limit clinical outcomes, early investigations are also identifying alterations in several physiologic processes. These alterations provide insight into reproductive failure in some patients. They may also provide the foundational information to guide the development of new therapeutic interventions that could improve outcomes and previously recalcitrant clinical circumstances.

The association between clinically evident infection, inflammation, and altered reproductive function is well established. Much of this inflammation involves secretion of a number of pro-inflammatory cytokines and growth factors secreted by immune cells, which are activated in response to the presence of apparent pathogens. In the case of small shifts in the microbiome, the resulting subtle changes in the local milieu are typically not clinically evident but may remain clinically meaningful; however, the exact molecular mechanisms are not well characterized.

Accumulations of a particular interleukin or some other cytokine are described, but detailed mechanisms are still lacking. It is possible that the influence of some components of the microbiome is not via direct interaction with the local organ system.

The microbiome of the vagina is typically dominated by Lactobacilli [88]. In fact, a normal milieu is defined by the presence of specific subspecies of Lactobacilli that are capable of acting as probiotics and inhibiting the overgrowth of other bacterial species. For example, Lactobacilli species capable of producing high levels of H_2O_2 are generally considered to be most favorable. This demonstrates an important concept that some components of the microbiome's principal function may be to alter or limit some other component of the microbiome. A direct interaction with the actual tissue may occur but is not essential.

It is becoming increasingly evident that the aggregate microbiome is not a simple accumulation of free-floating bacteria on the surface of a human tissue. In many cases, complex three-dimensional lattices are formed, which may have one layer or may have an inner and an outer layer.

A protective outer coating composed of polysaccharide, nucleic acid, and protein may develop. At times, these biofilms may inhibit immune detection and reduce the effectiveness of antimicrobial treatment [89]. These three-dimensional structures spread across the surfaces of the tissues where they are located and are termed biofilms.

Biofilms are the subject of intensive investigation and may have important physiologic and pathophysiologic roles. Biofilms are routinely present in the vagina but commonly extend into the endometrial cavity [90] and even up into the fallopian tubes (Figure 5). Although no definitive conclusions regarding the role of biofilms of the reproductive axis have been established, it is important to understand that the relationship between the microbiome and the mullerian system may be more complex than the simple presence or absence of various species or bacteria or even their relative concentration. The interactions that lead to different biofilms and their subsequent impact on reproduction will provide important topics for future investigation.

The influence of the microbiome, most prevalent in the mullerian system, may extend to the remainder of the reproductive axis and may even affect gametogenesis. Indeed, ovarian follicles may have an active microbiome. Some investigators have found that some bacteria may adversely influence follicular development and may even inhibit gonadotropin responsiveness. Similarly, the male reproductive axis may be adversely affected, with subtle changes in the microbiome being associated with altered semen parameters [82].

Most studies characterizing the influence of the microbiome on ART and clinical outcomes are largely association studies. Detailed mechanistic studies that could lead to new therapeutic approaches are possible but remain to be done.

5.2. Vaginal microbiome

In contrast to the Human Microbiome Project, which investigated normal healthy volunteers, several investigators have looked at the link between the vaginal microbiome and infertility in patients undergoing various forms of ART [82].

One such study prospectively analyzed 152 patients undergoing IVF. Of the 152 patients, 133 (87.5%) tested positive for one or more microorganisms and 19 (12.5%) tested completely

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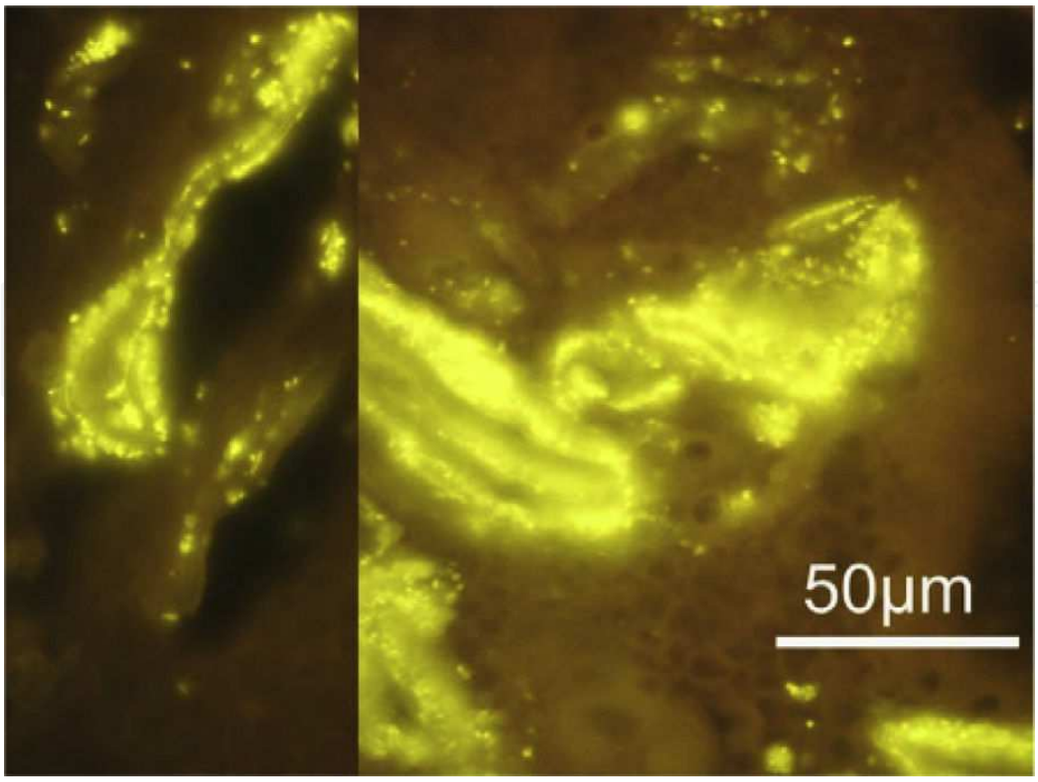

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Figure 5. Polymicrobial biofilm dominated by Gardnerella attached to the endometrium. The left panel shows follicular and the right panel shows luteal endometrium.

negative for bacterial contamination. The most common microorganisms identified were *Lactobacillus* species, *Staphylococcus* species, and Enterobacteriaceae, including *Escherichia coli*, Klebsiella, and Proteus. Outcomes data showed that implantation rates were 12.4% in those with one or more bacteria present versus 14% in those completely negative ($P<0.001$).

Additionally, patients testing positive for Enterobacteriaceae and *Staphylococcus* had lower pregnancy rates than the negative culture group. Although this study provided some insight into the microbiome during IVF treatment, it highlighted the limitations associated with culture-based technology for evaluation of the microbiome. The fact that 12.5% of patients were completely negative for bacterial contamination suggests that the culture-based technique significantly underrepresents both the presence and the diversity of the microbiome at the time of ET.

A subsequent study with the use of 16S sequencing technology took a more robust look at the vaginal microbiome in the infertile patient undergoing IVF [90]. The investigators approached the study design with the hypothesis that, given that the vaginal microbiome has changes during the normal menstrual cycle with varied estrogen levels in the physiologic range [91], controlled ovarian hyperstimulation required to achieve success in IVF would also affect the vaginal microbiome [82].

5.3. Uterine microbiome

Just as alterations in the microbial environment affect vaginal health, these fluctuations may also predispose to upper genital tract infection, such as PID.

Direct culture or sequencing of samples from upper genital tract structures has been performed less frequently than the evaluation of vaginal samples, but available data confirm that BV-associated bacteria can be isolated from the upper genital tract [92].

In a study of 45 women with laparoscopically confirmed acute salpingitis (cases) and 44 women seeking bilateral tubal ligation (controls), 16S rDNA PCR detected bacteria in the fallopian tubes of 24% of cases and none of the controls [93]. Several of the specimens contained bacteria associated with BV, such as *Atopobium vaginae*, as well as *Leptotrichia* species and *N. gonorrhoea*.

The identification of causal microorganisms in upper genital infection is important for understanding disease pathogenesis and provides insight into why some cases are resistant to conventional treatment. The presence of certain organisms in the vagina is physiologic, and bacteria may also exist in the upper cervix and uterus during states of health.

In a study, by performing quantitative PCR on endometrial and upper cervical swabs from 58 women undergoing hysterectomy for benign conditions, at least one bacterial species was found in the upper genital tract of 95% of the subjects. The most frequently detected species were *Lactobacillus iners* (45%), *Prevotella* species (33%), and *Lactobacillus crispatus* (33%) [94].

An important consideration is that the upper cervix and uterus were grouped together as the ''upper genital tract'';however, these sites may contain different bacterial species or proportions of bacteria. For instance, there was a statistically significant difference in the proportion of upper genital tract bacteria based on race. African American and Hispanic women were more likely to harbor an upper genital tract microbiome dominated by a non-*Lactobacilli* species (83% and 75%, respectively) compared with Caucasian women (54%). These results mirror those of vaginal microbiome in that non-*Lactobacilli* species were more common in African American and Hispanic women, but the clinical implications of these findings are unclear.

Furthermore, there was no evidence of significant inflammation in the endometrial samples that contained bacteria typically found in the vaginal tract. Possible explanations for the lack of inflammation include vaginal contamination of the uterine samples or that molecular methods detected RNA of non-living organisms that did not affect clinical status.

Alternatively, it remains a possibility that certain bacteria in the upper cervix and uterus may serve important roles in maintaining homeostasis and may not necessarily represent pathology [92].

5.4. Ovarian follicle microbiome

Human follicular fluids have been extensively cultured and found to have an active microbiome in many patients.

Although some specimens were collected from follicular aspirate attained at the time of transvaginal oocyte retrieval, others were collected laparoscopically [95]. It is not clearly established whether the bacteria that were cultured represent true colonization or merely contamination of the ovarian follicular fluid at the time of puncture for transvaginal oocyte aspiration [96].

Studies simultaneously evaluating the vagina, endocervix, endometrium, fallopian tube, follicular fluid, and peritoneal cavity are lacking.

Current studies looking at the microbiome of the follicle have used culture techniques. Early culture studies have suggested that an active follicular microbiome does affect ART outcomes. Interestingly, the impact of the microbiome is influenced by the clinical diagnosis of the female partner.

Diminished fertilization and development rates as well as reduced transfer and implantation rates have been noted in women with endometriosis, but not in women with ovulatory dysfunction or male-factor infertility [97, 98]. This may suggest that a more complex mechanism with an altered immune response present in women with endometriosis may produce a different reaction to the presence of an active microbiome and then also influence the developing oocyte.

It may also be important to note that an active microbiome is not always a negative finding. Pelzer et al. [99] noted that outcomes improved when Lactobacilli were present. This is in sharp contrast to the presence of other species, such as *Propionibacterium* and *Actinomyces*, among others, where impaired clinical outcomes were documented. They also observed differences in the microbiome between the left and right ovaries, which were attributed to differences in hematogenous spread. The clinical relevance of this finding remains to be fully characterized [82].

At the present time, data are still accumulating regarding the significance of the follicular microbiome and the need for screening. Additional studies, particularly those using metagenomic approaches, are needed.

5.5. ART and microbiome

Data have been gathered on the microbiome at every stage and level of human reproduction from the ovary, follicle and oocyte to testes and semen/spermatozoa and to the fallopian tube, uterus, cervix, and vagina. Both the male and female reproductive tracts exhibit complexity and diversity only realized within the last decade.

Furthermore, it is not enough to simply qualitatively or even quantitatively explore the reproductive tract microbiome using metagenomics. Understanding that these bacteria are not simply free-floating on the surface of tissue, but form their own three-dimensional biofilms with inner and outer layers, adds an additional complexity and could be of great importance if they were further explored. The fact these biofilms exist from the vagina to the fallopian tubes, allows complex and dynamic interactions between the gametes and embryo, as well as the maternal tissue interface.

To date, the assisted reproductive technology literature describing attempts to alter the microbiome in the reproductive tract in order to impact outcomes has been operating on a rudimentary understanding of this complex environment at best. However, this approach, although perhaps to blunt a tool at present, may indeed be an important key to altering both the microbiome and subsequently the immune system as we further explore enhancement of reproductive competence in assisted reproductive technology.

5.6. Conclusion

Knowledge regarding the interactions between the microbiome and the human reproductive axis is growing rapidly. A deeper understanding of normal physiology, identification of different dysbioses, and characterizing the microbiome's impact on reproductive outcomes promise meaningful enhancements in clinical care. While much has been learned since the early contributions of Semmelweis, the most insightful and powerful findings may lie just ahead [100].

6. Antimicrobials and ART

Although the reproductive tract microbiome remains relatively poorly understood in terms of its relationship to reproductive outcomes, there is a long history of attempting to influence it with the use of prophylactic antibiotics at the time of procedures during ART. This has been a practice ingrained since 1978, when it was suggested that contamination during ART procedures could negatively affect outcomes [101]. Because antiseptics, such as povidone iodine, can have a negative impact on embryos, antibiotics were turned to as a way of manipulating the microbiome [102].

A common time for antimicrobial prophylaxis is at the time of ET. Given the concern for colonization of the transfer catheter tip with microbiota from the upper genital tract, antibiotics have been proposed as a way to decrease inoculation of the uterine cavity and thereby increase pregnancy rates. Despite this widespread practice, relatively little data exist to support or refute antibiotic use.

A recent Cochrane review analyzed randomized controlled trials in the literature that investigated antibiotics at ET [103]. Only four potential studies were identified, of which three were excluded. The remaining study reported on clinical pregnancy rates as the primary outcome. Although administration of antibiotics reduced microbial contamination as defined by culture of ET catheter tips, the clinical pregnancy rate was 36% in those receiving antibiotics and 35.5% in those not receiving antibiotics (odds ratio 1.02, 95% confidence interval 0.66–1.58) [104]. The reviewers concluded that more evidence is needed with live birth as the primary outcome.

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