

ECTOPIC PREGNANCIES AND ENDOMETRIAL MICROBIOTA

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Purpose of the review

In recent years there has been significant progress in the study of endometrial microbiota. This line of investigation has not been free of controversy and discussion. It is key for clinicians involved to remain updated with the most recent findings in microbiome and its clinical implications to be able to offer patients all possible treatments.

Recent findings

The existence of endometrial microbiota now seems undisputed. Current lines of work are centered on dysbiosis and its connection to other pathologies. It is in the field of assisted reproductive technology (ART) where this research plays an even more crucial role. In this case, we are focusing our attention towards the study of ectopic pregnancies (EP), searching for similarities in their etiopathogenesis and alterations in the endometrial microbiota.

Summary

Ectopic pregnancy has great repercussions for patients and for the healthcare system. We must continue researching to offer patients techniques and behaviors that can prevent it. The increase in its incidence makes ectopic pregnancy an entity that we must study.

Key words: Ectopic pregnancy, endometrial microbiota, tubal factor infertility, fallopian tube.

INTRODUCTION

We will try to connect the dots between alterations in endometrial microbiota and the pathogenesis of EP. The published literature on the matter does not provide support so far for this relationship, but we will try to point out similarities that lead to believe that both are actually related.

MICROBIOTA

We have known for over a decade that the upper genital tract (uterus, fallopian tubes, and ovaries) are not sterile structures and that tissues like the endometrium contains bacterial population that serves different purposes.

Due to this and taking into consideration the study techniques based on 16S rRNA gene sequencing for bacterial identification, we have been able to establish several

Assumptions that we will take on (1). It is well known that there is a direct implication of the microbiome in female reproductive health (1) and that a better study and understanding is necessary for clinical practice to be correct, thus improving our results as specialists.

ENDOMETRIAL MICROBIOTA

The endometrium has been the center of ambitious research and many papers conclude that there is a relationship between the existing endometrial microbiota and the processes needed to have healthy newborns heading home.

These investigations have led to a battlefield around the sampling on upper genital tract, a very controversial matter due to how limited access to these structures is. Drawing samples through the cervix is considered a questionable technique due to the possible contamination of the sample, but ultimately we can consider it a reliable method (2).

The bacterial profile found in the endometrium is specific and differs from the one found in the vagina. This was proven in the study carried out in a group of 25 women where their endometrial microbiota was similar to the one present in the cervix, yet significantly different to the one in the vagina (3).

Simultaneously, microbiota has started to be associated to different pathologies and results in female reproduction.

One of the first relevant breakthroughs by a research group in 2015 used sequencing techniques taking samples from the tip of an embryo transfer catheter. This provided evidence of the existence of endometrial microbiome, with two leading kinds of bacteria, *Flavobacterium* and *Lactobacillus* which represented the majority of bacteria isolated in the two study groups (women who had become pregnant and women who had not). They were also able to describe species that varied according to the result (4).

Later on, using aspirated endometrial liquid taken in the moment of the transference, the group of Moreno *et al.* managed to establish the impact of microbiota on reproductive results. The threshold was established at 90% presence of *Lactobacillus* (*Lactobacillus* dominated endometrium) and connected endometrium with <90% to obstetric poor results. This was the first time that there was a clear association between a specific microbiota profile and deficient in vitro fertility (IVF) results (5**).

This group also studied the variations of the microbiota throughout the hormonal cycle, proving the existence of a stable composition, despite the changes in endometrial receptivity. This idea has been highly discussed until this moment, due to the fact that the microbiota can be altered influenced by ovarian stimulation protocols and progesterone supplements, as some researchers have concluded (6*).

Endometrial microbiota, thus, began to be considered a key piece in the puzzle of understanding how the egg is implanted. This would lead to studying microbiota in pathologies and/or alterations that we know affect the process of implanting the egg.

A study analyzed the microbiota of 12 women diagnosed with chronic endometritis detected differences in the abundance of bacteria. The conclusions registered a lower proportion of *Lactobacillus* and a higher presence of pathogenic species like *Gardnerella* (7) in patients with endometritis compared to women in the control group.

Most of these studies have a small sample size. A recent study with a much bigger sample size, analyzed 392 women who had experienced problems to successfully implant the egg, were divided in two groups according to *Lactobacillus* dominance previously established by the group of Moreno *et al.* The results revealed a Non-*Lactobacillus* Dominated (NLD) endometrium in almost half of the patients and a higher abundance of this species in the luteal phase. In these patients with an NLD endometrium, other species, such as *Gardnerella*, were targeted. The purpose of this work was to evaluate the different treatment regimens and find the best improvement rate regarding the percentage of *Lactobacillus* after sequentially administering an oral antibiotic and a vaginal probiotic (8).

In a published clinical case, persistence of *Gardnerella* was found (after 18 months of follow up and 6 samples) with unfavorable obstetric results (2 spontaneous abortions and 1 EP). There were favorable results after several cycles of antibiotics followed by probiotic treatment were completed (9).

However, in clinical practice, we must act with caution because we still do not have a clear picture of which antibiotic/probiotic treatments must be used and which combinations of both are more successful in the case of endometritis or a failure in the implanting of the egg. We must understand this relationship but we still need more studies to establish a common practice (1).

There is still much to unravel regarding endometrial microbiota but as we have indicated before it seems to be the clear protagonist. We have to be more skeptical, however, when it comes to the higher structures of the upper genital tract (fallopian tubes and ovaries). Some groups consider that the bacterial DNA found in the fallopian tubes could be transitory or simply remains, and that low levels of biomass should not be considered microbiota (10). There is insufficient research regarding the bacterial population in these structures. The data currently available shows a low presence, highly diverse, of microbiota in the fallopian tubes.

A group of investigators selected 16 women who had undergone a salpingectomy for benign causes. After studying several sections of the fallopian tubes bacterial population compatible with being microbiota was found both through analysis by electronic microscope as well as through 16S rRNA gene sequencing for bacterial identification(11).

There are groups that have studied the relationship between vaginal microbiota and pathologies in the fallopian tubes. Infertility due to tubaric factors has a high prevalence in patients with alterations in their vaginal microbiota. These patients are also prone to a higher risk of spontaneous in early stages (12). Additionally women with bacterial vaginosis seem less prone to have success at IVF treatments (13). This is why it is important to diagnose this condition and treat this pathology in women with infertility and/or those who are going to be submitted to ART (1).

We have reviewed the role that dysbiosis in the upper genital tract may have, not only in infertility problems but also in cases of early spontaneous abortion and the correct evolution of pregnancy.

There are insufficient studies that analyze endometrial microbiota during pregnancy, probably due to the potential consequences that collecting endometrial samples could have for the correct evolution of pregnancy. As a consequence we still have many things to learn in this field.

Several studies have proven that bacteria is able to colonize different materno-fetal tissue and amniotic liquid in healthy successful pregnancies. This bacteria seems to have a key role in pregnancies, though we still have to understand well which bacteria is needed and the role they play in the pregnancies. This matter has also been studied by the group of Moreno *et al.* (14*).

The study of endometrial microbiota in a group of 19 women that underwent a cesarian section revealed a low percentage (<1% in 15 patients) of *Lactobacillus*. This fact revealed the presence of different bacterial population in the decidua that is not dominated by *Lactobacillus* can also lead to successful pregnancies (16).

ECTOPIC PREGNANCY (EP)

As we have mentioned before, there are no studies where the microbiota is established as the factor to explain EP. However we do find in the related literature, similarities between the alterations that could lead to bacterial dysbiosis and risk factor and pathogenesis of EP.

EP is a well known complication, which is feared by all gynecologists because it is one the main threats to the fertility of women.

The main studies estimate an incidence of 2% in the case of spontaneous pregnancy, reaching up to 5% in the case of ART (17).

In 92% of the cases the fallopian tubes are the place where the egg is implanted. The region of the ampulla is the most frequent area where this takes place (18). Despite early diagnosis, it causes high morbidity and mortality rates, reaching 1.4% in certain series (19).

A study by Chouinard concluded that women that had suffered an EP had lower rates of later intrauterine pregnancies. These pregnancies were subject to a higher risk of obstetric complications, such as early delivery, underweight newborns, cesarean section births or placentation conditions (preclampsia, placenta previa), regardless of whether ART are used or not (20).

Taking into consideration this data it is important to continue studying EP in an attempt to prevent this condition, in the light of the close relationship it has with fertility and ART.

Risk factors are an important target to study and we believe the analysis should be focused on the impact on microbiota in the upper genital tract; from the more classic maternal age, consumption of tobacco, precedents in pelvic infections, previous EP, previous damage to fallopian tubes (such as obstruction), or abdominal surgery (17).

Risk factors in ART

EP are more common in patients who have undergone IVF. The reason for this remains unknown.

Patients subject to IVF with the highest rate of EP are those who had previously undergone abdominal surgery and those diagnosed with infertility due to the tubaric factors (21). Both groups are more at risk of suffering heterotopic pregnancies.

If instead of taking into consideration the type of patients we consider the factors to be considered in the case of ART, as well as treatments or techniques, we come to the conclusion that the incidence of EP does not differ depending on the protocols of ovarian stimulation used (22).

We do not find differences regarding the number of embryos, the day the transference is carried out (on day 3 or day 5), or whether the embryos are fresh

or frozen (21). Therefore, none of these can be considered risk factors. Another group reached similar results, but did find slow development blastocyst transfers to be a risk factor (23).

We also find factors that protect against EP in ART such as having an endometrium with the adequate thickness (24). This proves that endometrial health is fundamental to ensure a correct implant of the egg.

Risk factors in spontaneous pregnancies

Pelvic inflammatory disease (PID) is a condition that plays a significant role in EP and infertility and is the main cause of sexually transmitted infections (STI).

PID seem significant risk factors in themselves and could seem independent from the circumstances that lead to an EP (25*). Gonorrhoea and chlamydia are conditions traditionally related to PID. But, nowadays we know that microorganisms that appear related to PID and infertility are independent from gonorrhoea and chlamydia. If several of these conditions are present simultaneously, the risk spikes (26).

According to a meta-analysis (27), infection by *Chlamydia trachomatis* (CT) the risk of PID, EP and tubaric originated infertility increases (28). Despite having an asymptomatic and low incidence presence, the risk posed is higher than more visible conditions such as gonorrhoea (29), even after targeted antibiotic treatment has been completed (30).

The information we currently have regarding early detection and treatment diminishes the risk of pelvic inflammatory disease (and its consequences) is still unclear. We still do not have good diagnose techniques, screening strategies, nor prevention despite the rapid growth of STI (31).

What we do know is that an altered microbiota could stop being the necessary barrier to avoid a fatal outcome in these infections. If they do occur they can lead to transformation (dysbiosis among others) responsible of the consequences (tubaric infertility, chronic endometritis...) (14).

CONCLUSION

Relation between the microbiota and ectopic pregnancies.

The mechanisms involved in an EP, such as abnormal tubaric transport and/or an inflammatory environment can be behind the faulty implantation in the ampular region of the fallopian tube. These theories do not manage to pinpoint the precise pathogenesis of EP which still remains unknown.

There is not a consensus on whether an altered microbioma is the cause or the effect of an illness (14). Several authors speculate with the relevant interaction of endometrial microbiome with the epithelium and its population of immune cells, which might give way to an altered receptivity, and a faulty implantation of the egg

(32). There is probably a synchronized task carried out by the microbiota and the immune system creating a balanced medium for the egg to be successfully implanted. Any alteration of this balance could lead to a faulty implantation (33).

We know that there are several cytokines involved both in the endometrial receptivity and in the development of the embryo. They are influenced by inflammatory and infectious mechanisms, and therefore are affected, as well, by the microbiota (34**).

There seems to be a significant increase in the appearance of pro-inflammatory genes (TNF- α , IL-6, and IL-8) and the levels of PROKR (prokineticin receptors that regulate the contractility of the smooth muscle) in the fallopian tubes of patients with tubaric EP. PROKR2 and IL-8 are risk factor that can lead to EP and are involved in its pathogenesis (35).

Other molecules have a relevant role as an anti-inflammatory agent in the mucus of the female genital tract. Elafin (elastase-specific inhibitor) is significantly augmented in the epithelium and connective tissue in the point where the egg is implanted in tubaric EP (36). Adrenomedullin (ADM) is a peptide hormone with a potent anti-inflammatory activity that is highly present in the fallopian tubes and it is present in lower levels in patients diagnosed with salpingitis or a tubaric EP. This is due to an increase in macrophage response that triggers an excess of pro-inflammatory and pro-implantation cytokines IL-6 and IL-8 (37).

It seems obvious that the immunitary deregulation is closely tied to different pathogenic ways in the female reproductive system. One of these ways could be endometrial dysbiosis.

Our work group has begun to study endometrial microbiota in patients diagnosed with EP. The purpose of the study is to establish whether there is dysbiosis in the samples collected, which could be a factor that led to some women being predisposed to suffer EP. We take into consideration the endometrial microbiota of intrauterine pregnancies in comparison to EP in order to prove that endometrial dysbiosis can be a determining factor in EP.

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Conflicts of interest

None.

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