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Endometrial microbiota — do they mean more than we have expected?

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ABSTRACT

Low biomass microbiome has an increasing importance in today's fertility studies. There are more and more indications for incorporating upper gynecological tract microbiome content in patients diagnostic and in vitro fertilization process, as doing so may help to evaluate chances for a positive outcome. An abnormal endometrial microbiota has been associated with implantation failure, pregnancy loss, and other gynecological and obstetrical conditions. Furthermore it has been shown, that using molecular methods in addition to routine diagnostics may help diagnose chronic endometritis or even indicate cancerogenic changes. Understanding the significance of microbiome in endometrium may completely change therapeutic approach in treatment of this part of reproductive tract. Next generation sequencing (NGS) has allowed to isolate culturable and unculturable bacteria from female reproductive tract and is a cheaper and quicker alternative for other widely known and used methods.

Key words: endometrium; microbiota; reproductive health; next generation sequencing

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INTRODUCTION

For almost a century gynecologists and scientists were convinced that a healthy uterus is sterile. Henry Tissier, who in early 1930's has isolated bacteria from the stool of healthy breastfed infants, believed that an infant develops in a sterile womb and its first contact with the bacteria occurs during entering the birth canal [1, 2]. Further studies on this subject has shown that meconium is not sterile, and bacteria were also detected in amniotic fluid, umbilical cord and fetal membranes of healthy term babies [3–6]. These findings prompted further research as more proof of nonsterile fetus cast doubt on the assumption of has no commensal microflora in the upper genital tract. The importance of microbiome in the entire fetal life is currently studied by many researchers [7].

UTERINE MICROBIOME

Until very recent, the cervix had been seen as a perfect barrier between the vagina, uterus and the fallopian tubes, which were believed to be sterile. However, some studies have proven, that the changes in relative concentration of mucins present in the cervix, are leading to changes in their aggregation. Such changes dependent on pH variations during menstrual cycle and may allow bacteria passage under certain conditions [8].

In 1995 Moller et al. [9] published a study describing bacterial culture isolated from the cervix and the uterus of 99 patients undergoing a hysterectomy, where main indications for the procedure were persistent vaginal bleeding (n = 29) and fibromyomas of the uterus (n = 34). 26 of the studied patients were culture-negative for all microorganisms based on the samples from the apex of the vagina and the cervical os. The team has managed to isolate bacteria from the uterine cavity samples in 24 out of 99 analyzed cases. The most common pathological organism isolated from the vagina was *G.vaginalis*. It was found in 45.5% of culture-positive women. Other frequently isolated bacteria were *S.agalactiae* and *Enterobacter spp.* found in in 15% of the cases. Among the 24 patients with a positive culture

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from the uterine cavity *G.vaginalis* was isolated in 11 cases and *S.agalactiae* in 5 cases. The team has concluded that the uterine cavity is contaminated with microorganisms in a significant number of patients admitted for hysterectomies. It has been recommended to send the endometrium biopsy samples for histological and microbiological testing prior to the hysterectomy [9].

The result of this and many other studies have shown, that there is a microbiota continuum along the female reproductive tract. The lower third of vagina, and posterior fornix are dominated by *Lactobacillus spp* .(99.99%). However, samples taken from cervical canal contain lower proportion of *Lactobacillus spp.* (97.56%) than the vaginal samples [9, 10]. According to the study by Chen et al., *Lactobacillus spp.* is not a dominant genus in the endometrial samples (30.6%). Bacteria such as *Acinetobacter* (9.07%), *Pseudomonas* (9.09%), *Sphingobium* (5%) and *Vagococcus* (7.29%) form a large portion of endometrial microbiome. At the openings of the fallopian tubes the proportion of these bacteria increases while the median relative amount of *Lactobacillus spp.* is around 1,69%, and peritoneal fluid from the pouch of Douglas contains little to no *Lactobacillus* genus [10–12].

Next generation sequencing (NGS) has enabled a far more global evaluation of bacterial composition of the uterus as it cannot be measured with culture dependent methods. In the year 2000 Drancourt et al. [13], made several recommendations concerning proposed criteria for 16S rDNA gene sequencing as a reference method for bacterial identification. In further studies however it has been observed that the 16S rDNA is not a perfect target for NGS analysis and bacteria identification. Genomic DNA isolated from a sample contains random fragments of bacterial genomes and can be, potentially, contaminated by host DNA or DNA of other organisms present in this sample [14]. 16S RNA amplicon sequencing can be targeted specifically against bacteria. It also does not require the availability of reference genome sequences. Furthermore it can be used in cases where only trace amount or poor quality bacterial DNA templates are accessible [15, 16]. Therefore 16S rRNA sequencing became a standard method in bacterial community profiling.

It is important to highlight that the differences between the endometrial and vagina microbiome have been observed regardless of the method of collection of endometrial samples, which confirms the existence of indigenous endometrial microbiota and shows that the vaginal – cervical canal is a safe route for sampling the uterine cavity for further microbiome analysis [12, 17, 18].

The role of immune system in uterus colonization cannot be forgotten. Studies have shown that the endometrial fluid and the uterine mucosal surface contain infection-controlling molecules, known as antimicrobial peptides (AMPs), with changing levels during the menstrual cycle [19]. AMPs are contributing to female reproductive tract health with implication for fertility and pregnancy [20]. The secretory leukocyte protease inhibitor, which has antiviral and antifungal properties, is present in the uterus. It acts against gram — negative bacteria such as E.coli and gram-positive bacteria such as S. aureus [21]. Givan et al. [22], has shown presence of the lymphocytes in the mucosal layer, ready to act upon pathogen invasion, throughout all stages of the menstrual cycle. We can, therefore, assume that the uterus could offer a safe niche for symbiotic colonization.

Koedooder et al. [23] has proposed semen to be another possible route of introducing microbiota into female reproductive tract. His studies have shown, that the male and female microbiome are influenced by each other and seem to interact [23]. How the two interact is still unknown. Future research could resolve the question of the existence of temporary female-male microbiome forms during post-coital period and its influence on conception.

Current data suggest that the importance and confirmation of natural presence of healthy uterine microbiota need to be assessed by well-setup large cohort studies [24].

INFLUENCE ON REPRODUCTION AND WOMEN'S HEALTH

There are some indications that uterine microbiome might influence endometrial receptivity. Early prospective studies considering the role of endometrial microbial colonization suggested that positive microbiological endometrial culture, obtained from the tip of the transfer catheter in patients undergoing in vitro fertilization, had negative effects on implantation and pregnancy rates. The transfer catheter tip or cervical smear culture positive for bacteria strains such as: *Enterobacteriaceae spp., Streptococcus spp., Staphylococcus spp., Escherichia coli*, was associated with decreased implantation rate and poor pregnancy outcome [25–27]. For example, Selman et al. have designed prospective clinical trial including 152 patients undergoing IVF procedure. Separate samples for microbial examination, were taken during embryo transfer from the vagina, the cervix and culture medium: prior and post-embryo transfer. Of the 152 patients, 133 tested positive for one or more microorganisms, and the remaining 19 patients tested negative in all samples taken. In the positive group the microorganisms identified were as follows: *Enterobacteriaceae* in 99 patients, *Streptococcus spp.* in 43 patients, *Staphylococcus spp.* in 68 patients, *Lactobacillus* in 19 patients and other species such as: *S.agalactiae, G.vaginalis, Ureaplasma urealyticum* and yeast in 28 patients. Pregnancy rates were significantly lower in patients positive with *Enterobacteriaceae* culture and *Staphylococcus* (in compare with negative culture group (22.2% vs 51% and 17.6% vs 43% respectively) [26].

Those results have been confirmed by Moreno et al. [18] study where patients were divided into two general groups: LD (*Lactobacillus* Dominant; > 90%) and NLD (non-*Lactobacillus* Dominant; < 90%). The analysis of endometrial microbiota showed significant differences in the bacterial diversity in the NLD group. This group, in comparison with the LD group, also had significantly lower implantation (23.1% vs 60.7%, p = 0.02), pregnancy (33.3% vs 70.6%, p = 0.03), ongoing pregnancy (13.3% vs 58.8%, p = 0.02), and live birth (6.7% vs 58.8% p = 0.002) rates.

Genus *Lactobacillus* is a very important component in major part of the uterine microbiome studies. However, comparison of the relative abundance of *Lactobacillus* between sequencing reports underline the inconsistency among reports and needs further investigation [28–30]. Fang et al. [31] described higher levels of *Lactobacillus* in the group of women with endometrial polyps or in women with chronic endometriosis coexisting with endometrial polyps, compared with healthy control. By contrast, the work of Moreno et al., reported that high levels of *Lactobacillus* (over 90% as defined by the group) are significantly associated with growing reproductive success in women undergoing IVF. Nevertheless, it has not been determined, which species of *Lactobacillus* may be capable of conferring this benefit [18].

In other studies, the increased reproductive success in women with high level of *Lactobacillus* may have reflected the composition of the vaginal microbiome at the time of embryo transfer [28]. Haahr et al. have tested 130 patients undergoing IVF treatment. PCR analysis for *G.vaginalis, A.vaginae, L. crispatus, L. jensenii, L.gasseri* and *L.iners* were performed. Dominance of *Lactobacillus spp.* was interpreted as normal, whereas bacterial vaginosis was diagnosed if the *G.vaginalis* and/or *A.vaginae* were dominating. Eighty-four patients completed IVF treatment and overall clinical pregnancy rate was 35% (29/84). Interestingly, only 2 of 22 patients with abnormal vaginal microbiota obtained pregnancy (p = 0.004) [30]. Even though the microbial uterine environment plays a role in the implantation and placentation process, it is mainly tightly regulated by female sex hormones.

Therefore Moreno et al. [18] has evaluated IVF catheter tips at two different time points. One sample was taken at the pre receptive phase and the other at the receptive phase of the same menstrual cycle to asses shift in microbiome composition in IVF patients. This study has indicated, that the uterine microbiome was similar at both time points in 9 out of 13 patients sampled, which is similar to the vaginal microbiome changes in the same time window [18, 32].

Recent reports from Moreno et. al demonstrates, that molecular microbiology is a reliable, fast, and cheap diagnostic tool that allows for the detection of culturable and non-culturable bacteria associated with chronic endometritis and has 77% concordance with a combination of the classical diagnostic methods such as histology, hysteroscopy and microbial culture [10]. This is very important information, as chronic endometritis can be asymptomatic, and is found in about 40% of infertile patients, likely causing repeated implantation failure or even recurrent miscarriage [10]. The study includes a small study group (65 patients), which indicates that more research has to be done to confirm those findings.

Pathological changes in endometrial microbiota may play an important role in carcinogenesis [33, 34]. There are some hypothesis that the pelvic inflammatory disease (PID) may result from pathogenic bacteria ascendance through the cervix into the upper genital tract and cause inflammation of the uterus, fallopian tubes and/or ovaries [34, 35]. Carcinogenesis on the other hand may occur when the tumor-associated loss of bacteria function causes increased commensal penetration and inflammation induction, which in turn result in enhance tumor growth. Other possibility is so called: pathobiont-mediated tumorigenesis, by which potentially pathogenic commensal strains are creating tumorigenic environment by secreting mediators [36].

IMPLICATIONS FOR THE FUTURE

If bacteria are naturally present in the womb, their importance not only in terms of fertility, but also in maintenance of the uterus deserves attention.

In the future, the targeted elimination of cancer — associated with microorganisms might provide a new therapy option. It seems to be a very attractive alternative because of its minimal expected side effects and the possibility of its preventive application. Studying the interactions between host and endometrial microenvironment may open new diagnostic possibilities and help to prevent consequences of serious diseases. It may also help us better understand the role of microbiome in implantation process and suggest routes to achieve positive outcome in infertility treatment. Molecular methods are shown to be a very powerful tool in defining the role of endometrial microbiome in women's health.

CONCLUSIONS

Thanks to next generation sequencing (NGS), endometrial microbiome is becoming better characterized and its importance in gynecologic and reproductive health is increasing. However, researchers have not yet reached a consensus, whether an altered microbiome is a cause or an effect of upper gynecological tract diseases. More research is needed to describe and understand the role of endometrial microbiome in endometrial receptivity and the outcome of in vitro fertilization. For optimal success, further studies require well-designed experiments and larger patient groups to explain the interactions between host microbiome and women's health.

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