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

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
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.* (79.56%) 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-Lactoba-
cillus Dominant; < 90%). The analysis of endometrial micro-
bio 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),
going 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, com-
parison 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 endometritis coexisting with endometrial
polyps, compared with healthy control. By contrast, the
work of Moreno et al., reported that high levels of Lactoba-
cillus (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 assess 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 diag-
nostic 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 classi-
cal 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 fertile patients, likely causing repeated im-
plantation 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 inflamm-
ation 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 tu-
morigenic 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 mainte-
nance of the uterus deserves attention.

In the future, the targeted elimination of cancer — as-
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), endome-
trial microbiome is becoming better characterized and its
importance in gynecologic and reproductive health is increas-
ing. However, researchers have not yet reached a consensus,
whether an altered microbiome is a cause or an effect of up-
per 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 ferti-
лизation. For optimal success, further studies require well-de-
signed experiments and larger patient groups to explain the
interactions between host microbiome and women’s health.

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