Franco Vicariotto

VAGINOME

UNDERSTANDING THE VAGINAL MICROBIOME

QUESTIONS & ANSWERS

Clorofilla

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Graphic design
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This instant book is intended for healthcare professionals only.

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1. Vaginal microbiota: how is it composed?

The different microorganisms that populate the vagina constitute a perfect "ecosystem". The balance between the different species, also known as eubiosis, is crucial both to ensure an adequate state of well-being and a good reproductive function and to protect from infectious gynecological diseases. A state of dysbiosis is reached when this balance is lost.

In healthy women, the vaginal microbiota is made up mainly of lactobacilli and can be divided into 5 "community state types" (CST), also called "vaginotypes", which are clusters dominated by a particular species.

- CST I: *Lactobacillus crispatus*
- CST II: *Lactobacillus gasseri*
- CST III: *Lactobacillus iners*
- CST IVA: *Gardnerella vaginalis* subgroup B
- CST IVC: *G. vaginalis* subgroup A
- CST IVD: *G. vaginalis* subgroup C
- CST V: *Lactobacillus jensenii*

CSTs were identified in a study carried out in 2011 by a group of researchers led by Jacques Ravel. They grouped the vaginal microbial communities after analyzing the 16S RNA profiles of healthy women of reproductive age.

The main lactobacilli of the vaginal microbiome (*L. crispatus*, *L. gasseri*, *L. iners*, *L. jensenii*) are present in 4 out of 5 clusters. The dominance of lactobacilli contributes to keeping both the pH and the Nugent score (derived from the microscopic analysis of a vaginal swab, used for the diagnosis of bacterial vaginosis) values low.
CST I, in particular, is the most protective type of vaginal microbiome. Conversely, CST IV, characterized by a low number of lactobacilli, seems to correlate with the development of vaginal and gynecological disorders. Three subgroups belong to this cluster: CST IVA, CST IVC and CST IVD.
In women with CST IVA the incidence of bacterial vaginosis seems to be modest, while in the other two it's higher.

The lower production of lactate and the resulting increase of the vaginal pH determine an higher risk of bacterial vaginosis.
Moreover, the absence of lactobacilli determines a state of instability the allows one or more anaerobic species to take over the others, causing vaginosis.

2. What are the functions of lactobacilli?

The protective function of lactobacilli is linked to their ability to:

- occupy the bacterial sites of adhesion;
- synthesize hydrogen peroxide (H2O2);
- acidify the vaginal environment by metabolizing glycogen and producing lactic acid;
- produce bacteriocins, substances with bacteriostatic or bactericidal activity on other pathogenic microbial strains.

Furthermore, lactobacilli can form biofilms on the vaginal walls that limit the space and the nourishment for other pathogenic microorganisms.

However, the pathogenic microorganism can do the same with lactobacilli. In particular, the formation of pathogenic biofilms is one of the mechanisms involved in the pathogenesis of chronic or recurrent events. In fact, biofilms allow for a persistent planktonic growth that can elude the immune response and resist the usual antibiotics.

The organization in biofilms facilitates the development of virulence and resistance among the microbial colonies.

For example, the biofilms created by microorganisms such as *Gardnerella vaginalis* persist even after treatment with metradinazole, thus increasing the risk of recurrence.

Unlike antibiotics, the protective biofilms formed by lactobacilli can sometimes penetrate pathogenic biofilms. In case of vaginitis, probiotics could therefore work as a therapeutic intervention to prevent recurrences.

3. How does the vaginal microbiome change during the various stages of life?

During a woman's life, the vaginal microbiome continues to evolve based on the quantity of estrogens produced by the body and on the presence of lactobacilli.

Depending on the ethnicity and on the woman's hormonal status (adolescence, fertile age, pregnancy, menopause) the vaginal microbiome changes and differences between the clusters can also be observed.
The vaginal microbiome is never absolutely stable and the frequent variability can cause a change in the community state types (CST).

During childhood, the vaginal mucosa is populated by many microorganisms with the exception of lactobacilli. When the ovaries start producing estrogens, the mucosa is stimulated to produce glycogen and lactobacilli make their appearance. They convert glycogen into lactic acid, thus leading to a reduction of pH values and to an increase in the host's defenses.

A study led by Roxana J. Hickey, of the University of Idaho in the United States, has shown that, despite what one may think, 10-12 years old girls who have not had sexual intercourse or their first menstrual cycle yet, already have a vaginal microbiome very similar to that of women of childbearing age.
In particular, it appears that most of the girls show a dominance of lactobacilli, but also the presence of *Gardnerella* in some vaginal consortia.
Following the first menstruation, a doubling of the CST I frequency was detected, to the detriment of all the other clusters.

The menstrual cycle can also influence the composition of the vaginal microbiome by reducing its stability during the menstrual flow.
In particular, the abundance of *Gardnerella vaginalis* seems to increase in parallel with a decrease in *Lactobacillus* spp., with the exception of *L. iners*.

Menopause, on the other hand, is one of the most typical examples of a quantitative decrease in the proportion of lactobacilli. In this period, the richness of anaerobic taxa (*Bacteroides*, *Mobiluncus*) and of those implicated in bacterial vaginosis, such as *G. vaginalis*, increases. The absence of estrogens on the vaginal epithelium causes and maintains a real imbalance (dysbiosis) in the vaginal microbiome which leads to possible anaerobic and aerobic vaginitis.
In particular, as far as the CST clusters are concerned, it has been shown that:

- in healthy pre-menopausal women, the most frequent ones are CST I and III;
- during peri-menopause the most frequent ones are CST IVA and CST II;
- during menopause CST VAT are the most frequent ones.

4. How does lifestyle affect the vaginal microbiome?

Apart from the changes induced by a woman's hormonal state there are numerous other external factors that can lead to dysbiosis by modifying the composition of the vaginal microbiome. One of this is, for example, is intimate hygiene that, if excessive, can cause certain disorders. In particular, one should avoid vaginal lavages as they risk to further unbalance the vaginal microbiome.

As far as diet is concerned, the so-called "gut-urogenital axis" explains how any intestinal inflammation, even of low grade, can cause damage to the vagina and bladder, decreasing its defenses and increasing the concentration of pathogenic bacteria.
Indeed, the first nutraceutical therapy is a correct diet that reduces the intestinal inflammation and the risk of urogynecological disorders.

The vaginal microbiome can also be influenced by sexuality. This happens not only because of sexually transmitted infections, but also because of the imbalance that can follow sexual intercourse, as exemplified by the very high number of cases of bacterial vaginosis in American lesbians.

5. What happens in case of bacterial vaginosis?

Bacterial vaginosis is the most common vaginal disorder in women of childbearing age.
However, it is often underestimated or poorly treated despite the discomforts it causes, especially to those suffering from symptomatic and/or recurrent forms.

The characteristic symptoms of bacterial vaginosis are:

• the presence of more or less abundant white-greyish/milky discharges
• an intense and unpleasant odor, despite regular and careful intimate hygiene.

The bad smell tends to be more marked after sexual intercourse (especially if unprotected), during menstrual flow and after intimate hygiene. The inflammatory symptoms, such as burning, itching, pain during sexual intercourse and urination problems (pain, burning and feeling of having to urinate often) are absent or mild.

According to the Amsel diagnostic method, bacterial vaginosis is diagnosed if at least 3 out of 4 of the following conditions are met:

1) white-ish, homogeneous and non-viscous vaginal discharges;
2) cells of the vaginal mucosa with a dotted appearance due to the presence of bacteria adhering to the surface ("clue-cells"> 20%);
3) vaginal pH >4,5;
4) positivity to the fish odor test (called "fish test" or "whiff test"), consisting in the addition of a solution with a basic pH (10% of KOH, potassium hydroxide) to a sample of vaginal secretions: if intense fishy odor is released, it means that bacterial vaginosis is present.

Bacterial vaginosis is caused by a state of dysbiosis induced by the reduction of lactobacilli.
The loss of lactobacilli fractions favors the proliferation of other microorganisms, such as *Gardnerella vaginalis*.

A study published in Frontiers in Bioscience analyzed the vaginal microbial profile of 64 women showing symptoms of vaginal disorders of different degrees. An abundance of *Prevotella bivia*, which can favor the formation of biofilms by *Gardnerella vaginalis*, was found together with the presence of *Streptococcus anginosus*.

Indeed, bacterial vaginosis is a polymicrobial syndrome: it is not caused by a single microorganism, but rather by more bacterial species. They form a pathogenic biofilm that makes it easier to evade the host immune response and to harbor resistance to antibiotic therapies. This condition also favors the release of short chain fatty acids that can inhibit the chemotactic inflammatory responses of neutrophils and monocytes at the vaginal level.

The antibiotic therapy necessary to counteract the acute infection inhibits the excessive proliferation of pathogenic anaerobic bacteria. On the other hand, this causes an additional stress for the lactobacilli. In order for the cure to be effective in the long term, thus reducing the risk of recurrence, it is important to also provide treatments that favor the rapid recolonization by the lactobacilli, restoring the pH to acidity values capable of protecting the vaginal mucosa.
To this end the administration of probiotics may be useful.

6. What happens to the microbiome during pregnancy?

Pregnancy is a state of relative immune impairment due to a depression in the cell-mediated immunity.
A healthy vaginal microbiome is therefore an important defense especially during this phase of life, while its alteration can represent a risk for the development of disorders in both the pregnancy and the unborn child.

In a pregnant woman, a healthy vaginal microbiome is characterized by a low diversity and a dominance of lactobacilli. The increase of the estrogens' levels during the 9 months of pregnancy seems to stabilize the

vaginal microbiome, with the aim of increasing the relative abundance of lactobacilli and to keep the vaginal pH low.

The main risk associated with vaginal dysbiosis during pregnancy is that the microbiome is no longer able to protect against infections causing premature birth, one of the major causes of infant mortality in the world.

A study carried out on African-American women by researchers at Washington University suggests that changes in the vaginal microbiome of mothers who gave birth prematurely occurred between the first and second trimester. This suggests the importance of a balanced vaginal microbiome from the first months of pregnancy.

The alteration of the vaginal microbiome during pregnancy can also increase the risk of disorders in the unborn child, particularly allergies and eczema.

A study carried out on mice at the University of Maryland School of Medicine in collaboration with the University of Pennsylvania, and published in Nature Neuroscience, suggests that prenatal insults, such as maternal stress, have effects on the progeny that are partially mediated by the maternal vaginal microbiota.
In particular, stress during pregnancy can increase the risk of gastrointestinal disorders and affect the development of the nervous system in children.

Escherichia coli, *Streptococcus acidominimus*, *Peptococcaceae* and *Streptococcus thoraltensis* regulate immunity at the mucosa level and predispose the intestinal niche to colonization by other anaerobes that produce metabolites important for the development of the nervous system. A change in the levels of these species was detected in mice children of mothers subjected to stress who received a vaginal microbiome transplant from stressed mothers.

Diet and the risk of insulin resistance and obesity have a relationship with the vaginal microbiome too.

For all these reasons the supplementation of probiotics should be considered in case of risky pregnancies.

7. Some studies link the vaginal microbiota with idiopathic infertility. What is the rationale?

About 10% of infertility cases are classified as idiopathic infertility, also called "*sine causa*", that is characterized by the lack of pathological anatomical or functional alterations: in men, sperm counts are normal and there are no disorders impeding conception, while in women the cycle is regular and no changes in the reproductive system are observed.

Several studies have analyzed the vaginal microbiota of women with idiopathic infertility. In these patients, who usually refer to the centers for assisted procreation, a state of dysbiosis of the cervico-vaginal microbiome, with a composition similar to that of bacterial vaginosis, is often observed.

One of the first studies on this phenomenon was carried out by the University of Trieste and the IRCCS Burlo Garofolo and published in the Journal of Cellular Physiology.
In particular, using next generation sequencing techniques, the researchers found a link between the presence of some bacterial species in the vagina and infertility. The presence of *Atopobium vaginae* in the vaginal microbiota, for example, was one of the main factors contributing to the failure of IVF and embryo transfer procedures in women with asymptomatic bacterial infections.

It also emerged that some species of lactobacilli can create a favorable environment for the pregnancy. Furthermore, the investigation has revealed that in patients with idiopathic infertility there is a relative abundance of some lactobacillus species, such as *L. gasseri*, while others, such as *L. iners* and *L. crispatus*, are less represented.

It is also known that vaginal dysbiosis can increase the risk of colonization by anaerobic bacteria such as *Atopobium*, *Prevotella*, *Veillonella*, *Ureaplasma* and *Escherichia*, whose presence "mimics" the micro-environment typical of bacterial vaginosis.

The analysis of the vaginal microbiome composition could become an important diagnostic tool, as it could lead to the identification of the causes of female infertility in cases so far classified as idiopathic. Moreover, it could be useful to start developing personalized therapeutic interventions based on probiotics that can restore the balance of the vaginal environment thus promoting conception.

8. Natural vs. cesarean. What impact does the delivery method have on the health of the unborn child?

The quality of life, the diet and some obstetric diseases all influence a woman's vaginal microbiome during pregnancy leading to either risks or benefits for the fetus.

The type of delivery is also important.
In fact, by passing through the vaginal canal, the child takes on the mother's vaginal microbiome, while with a caesarean section, by passing through the abdomen, the newborn comes into contact with the maternal cutaneous microbiome and the microorganisms in the operating theater environment.

The correlation between the mode of delivery and the composition and degree of maturation of the microbiome in early childhood has been confirmed in a study conducted at the University of Alberta, Edmonton (Canada), on 166 newborns. The researchers examined faecal samples at 3 months and one year of age. The results indicate that those born by cesarean section and exposed to antibiotics show a slowed Proteobacteria decrease compared to the physiological trend at three months.

Furthermore, the microbiomes of children born by cesarean section and fed with formula are the most stable over time and therefore less subject to the physiological maturation process, with the possibile exception of

the *Rominococcus* species that increase especially in children born by cesarean section, fed with formula and exposed to antibiotics.

To safeguard the well-being of the newborn, it is therefore important both to reduce the rates of caesarean section and to keep the maternal vaginal microbiome balanced, so that the newborn acquires a healthy microbiome and good defenses with natural childbirth.

In case of caesarean section, the practice of "vaginal seeding" is not indicated. It involves incubating a gauze in the vaginal canal of the mother an hour before surgery, then rubbing it on the mouth, face and the rest of the body of the child after birth, with the aim of contributing to a more natural formation of the microbiome of the child. However, this is a potentially risky procedure due to the danger of infections and it is not recommended according to several international position statements.

9. What is the evidence on the axis intestine-vagina?

In addition to the intestine-brain axis, there is increasing evidence of a relationship between the intestine and the urogenital apparatus.

This is due both to the anatomical proximity and to the possibility that pathological metabolites typical of intestinal dysbiosis pass from the intestine to the vagina and bladder. This can cause inflammation and create an imbalance in the microbiome leading to the development of vaginitis.
The same happens in the bladder in case of recurrent cystitis caused by the abuse of antibiotics and by the passage of bacteria such as *Escherichia coli* from the intestine into the bladder.

Thus, for patients affected with gynecological and bladder disorders, gynecologists and urologists must not focus only on their own specialty, but also also pay attention to the intestine.

10. What are the future prospects for using probiotics in obstetrics and gynecology?

In Asian countries, a very high percentage of women use probiotics throughout the pregnancy but pregnant women should only take some basic micronutrients (iron, folic acid, iodine). Other supplements, such as probiotics, should only be prescribed in specific cases, for example if there is a risk of premature birth, or in case of vaginal infections or to get to the moment of birth with a balanced microbiome to be transmitted to the unborn child during the passage in the vaginal canal.

The efficacy of probiotics, both oral and vaginal, to treat vaginitis caused by dysbiosis is very high and their use is now compared to that of a drug. The efficacy of probiotics does not depend exclusively on the possibility of increasing the presence of "good" bacteria, but also on their specificity or on the ability, demonstrated in vitro and in vivo, of some bacterial strains to restore the balance lost by the vaginal microbiome or to act as specific antibacterials.

However, probiotic-based medical devices will be banned from the EU market starting in May 2020. The new regulation on medical devices, *Medical Device Regulation* (EU) 2017/745, repeals the previous European directives and clearly states that products containing living organisms no longer fall within the realm of medical devices (Art.1, pt. 6).
New opportunities arise for probiotics: they could become full-blown drugs, but there are still several difficulties in demonstrating the dose-response relationship, although research on the human microbiome is yielding promising results.
Alternatively, one could insist on the oral use of probiotics, given their ability to actively modulate the composition of the microbiota, but we need to demonstrate both fecal and vaginal colonization.

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