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# Comparison of Gut Microbiome in Neonates Born by Caesarean Section and Vaginal Seeding with Gut Microbiomes of Neonates Born by Caesarean Section Without Vaginal Seeding and Neonates Born by Vaginal Delivery

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**ABSTRACT**

**Background:** Pregnancy is an admirable biological process, resulting in significant changes in many of the body's normal systems so that they can support the development of the fetus. These changes involve hormonal changes, weight gain, immune system regulation, and others that need to be synchronized to maintain both maternal and fetal health. **Objective:** The purpose of this study was to compare gut microbiome in neonates born by caesarean section and vaginal seeding with gut microbiomes of neonates born by caesarean section without vaginal seeding and neonates born by vaginal delivery. **Methods:** In Democritus University of Thrace, from 2019 to 2022, gut microbiomes were compared for three groups of neonates. Group A included 110 neonates born by CS who underwent vaginal seeding, group B included 85 neonates born by CS without vaginal seeding and group C included 95 neonates born by vaginal delivery. **Results:** Vaginal seeding in neonates born with CS resulted in gut microbiome which was similar to the gut microbiome of neonates born by vaginal delivery (including lactobacillus species and bacteroides). On the contrary, gut microbiome of neonates born by CS without vaginal seeding was "limited". **Conclusion:** According to our findings, vaginal seeding alters the gut microbiome of the neonates born with CS. However, there is a need for further investigation to prove its efficacy and its safety for the neonate.

**Keywords: term pregnancies, caesarean section, vaginal delivery vaginal seeding.**

## 1. BACKGROUND

Pregnancy is an admirable biological process, resulting in significant changes in many of the body's normal systems so that they can support the development of the fetus. These changes involve hormonal changes, weight gain, immune system regulation, and others that need to be synchronized to maintain both maternal and fetal health Although pregnancy-related hormonal and metabolic changes have been known for decades, the dramatic changes became known that take place during pregnancy in the composition of the microbial flora of the pregnant woman recently (1, 2).

When researching the function of the microbiome during pregnancy, it's critical to take into account the point at which the crucial interaction between the host and the germ first manifests, and as a result, the point at which the growing fetus first comes into touch with the microbes. Today there are numerous indications that disprove this hypothesis and suggest that there is already a presence of microbes in the fetal-placental unit. In addition, the type of delivery, whether vaginal delivery or caesarean section, has been shown to affect the newborn's initial microbiome, which then undergoes significant changes as a result of the baby's diet and overall environment during the first 2 years of life (3, 4).

The leading cause of neonatal mortality and morbidity in the US is spontaneous preterm birth, with more than 80% of these resulting from premature rupture of membranes A premature birth may be the result of an inflammatory process, an activation of the hypothalamic-pituitary-adrenal axis, a placental hemorrhage or an excessive dilation of the uterus. Microbial invasion of the pregnancy tissues is the predominant factor of is observed in up to 50% of women with inflammation and intact membranes, while it is much higher in cases of premature rupture of membranes. Although there are many unanswered questions, it is clear that the vaginal microflora is an important intermediary in the adverse effects of pregnancy, as it can be the source of both a microbial colonization and its growth in the fetal tissues of the developing fetus (5-8).

Pregnancy is a unique environment in which the normal microbiological changes that take place during the menstrual cycle are inhibited. Although the vaginal environment can remain healthy and intact during pregnancy, it is also possible for unexpected changes in its microflora that can affect the normalcy of pregnancy. The majority of pregnant women experience both abnormal and normal vaginal discharge. Identifying vaginal infections that are dangerous to pregnancy and the fetus from those that are bothersome but harmless and treatable, as well as determining the proper course of therapy, is difficult for medical experts (8-12). The following study is the result of a retrospective study in order to investigate the effects of vaginitis on pregnancy outcomes.

## 2. OBJECTIVE

The purpose of this study is to investigate the technique of vaginal seeding, to verify its safety, to propose the criteria and conditions for its safe application, and finally to create more data for further study and research.

## 3. MATERIAL AND METHODS

Infants born with a caesarean section lack the benefits of normal childbirth as their microbe community differs significantly from that of neonates born with normal labor. According to studies, neonates born naturally are less likely to develop allergies, asthma, autoimmune diseases and obesity due to exposure of the baby to maternal vaginal fluid. The fetal gastrointestinal tract is aseptic, during birth, breast-feeding, and contact with the mother, the infant acquires microbes that help basically to "build" immune defense in an effort to deal with them. Cesarean section is one of the causes that inhibit this process.

Transferring microbes from the mother's vagina to the newborn, few minutes after birth by cesarean section is a new and controversial practice that has been discussed and interests several scientists and new parents as the data are limited. This transfer is accomplished by placing a sterile gauze in the mother's vagina a few minutes 15-20 min before the cesarean section and then spreading this gauze in the face, mouth, and skin of the newborn. indications for vaginal seeding are unruptured membranes, term pregnancies, and maternal control for infections and STDs. Then apply this gauze to the newborn's face, mouth and skin. If the strep culture is negative, then it should be compared with the newborn's stool culture after 3-5 days for microbes.

Filing and comparison in caesarean sections with vaginal seeding and caesarean sections with no vaginal seeding. The purpose of this technique is to create in the infant born with caesarian section a human microbiota equal to the infant born with normal labor that is exposed to vaginal fluids. In the Department of Obstetrics and Gynecology of Democritus University of Thrace from in the time from 01.02.2019 to 30.12.2022 vaginal swab specimens were collected from 290 pregnant women and tested for pathogens one to three weeks before their elective CS.

All participants were in pregnancy age more than 37 weeks and divided in three groups, 110 pregnant women, who underwent in vaginal seeding born by elective CS group A, another 85 pregnant women born also by elective CS without vaginal seeding group B and those 95 pregnant women born by vaginal labour. The participants who underwent elective CS after 37th week of gestation the method of vaginal seeding group A were applied by putting a sterile gauze into their vagina 20 minutes before their CS and the gauze applied to the neonate's face, mouth and skin. After 5 days we tested neonatal feces for microbes and compared fecal cultures of neonates born with CS were the method of vaginal seeding were not applied. All participants pregnant had a normal pregnancy course without risk factors and underwent in caesarean section or caesarean Ethical approval for this prospective study was confirmed by the ethics committee of the University Hospital in Alexandroupolis, Democritus University of Thrace, (Alexandroupolis, Greece; reference no. 281/29/03/2018). All patients provided written informed consent gave their written consent for their participation in the present study.

**Statistical analysis**

We'll use the Chi square statistic test in order to identify if caesarean section with vaginal seeding of neonates is equal to vaginal labor in terms of microbes. We'll use the Pearson Chi square test (95% Confidence intervals), but we'll use the Fisher's exact test (95% Confidence intervals), when at least 1 cell has expected count less than 5.

| Case Processing Summary | | | | | | |
|---|---|---|---|---|---|---|
| | Cases | | | | | |
| | Valid | | Missing | | Total | |
| | N | Percent | N | Percent | N | Percent |
| sthominis * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |
| bifidobacterium * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |
| STAERIUS * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |
| STEPIDERM * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |
| ENTFAECUM * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |
| GRANULICATELLA * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |
| ECOLI * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |
| lactobacillus * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |
| NONE * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |
| bacteroides * typeoflabour | 205 | 100,0% | 0 | 0,0% | 205 | 100,0% |

**Table 1. We'll use the Chi-Square statistic test in order to test if there is a statistically significant difference between the ear microbiome of neonates delivered vaginally and neonates delivered with CS and seeding. Group C N=95(VAGINAL), Group A N=110(SC & SEEDING)**

| Case Processing Summary | | | | | | |
|---|---|---|---|---|---|---|
| | Cases | | | | | |
| | Valid | | Missing | | Total | |
| | N | Percent | N | Percent | N | Percent |
| sthominis * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |
| bifidobacterium * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |
| STAERIUS * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |
| STEPIDERM * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |
| ENTFAECUM * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |
| GRANULICATELLA * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |
| ECOLI * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |
| lactobacillus * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |
| NONE * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |
| bacteroides * typeoflabour | 180 | 100,0% | 0 | 0,0% | 180 | 100,0% |

**Table 2. We'll use the Chi-Square statistic test in order to test if there is a statistically significant difference between the ear microbiome of neonates delivered vaginally and neonates delivered with CS NO seeding. Group C N=95(VAGINAL),Group B N=85 (SC NO SEEDING)**

## 4. RESULTS

After their consent 110 pregnant women underwent elective CS, and the method of vaginal seeding were applied group A. 85 neonates that used as reference control group B were delivered by caesarean section who no undergone in vaginal seeding previously to surgical procedure and 95 neonates were delivered by vaginal labour. All neonates were tested with fecal ear, ocular, and umbilical nasal cultures after 5 days from their birth.

Our findings in groups A and C (caesarean section and vaginal seeding versus vaginal labours group C are as following:

In terms of ocular microbes there was no statistically significant difference between Bacteroids, Bifidobacterium, St. Hominis, Enterococcus faecium, Granulicatella, E. Coli & Lactobacillus of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. However, neonates delivered vaginally have statistically significant more often no microbes, while neonates delivered with CS and seeding have statistically significant more often St. aerius and St. Epidermidis.

In terms of rectal microbes there was no statistically significant difference between Bacteroids, Bifidobacterium, St. Hominis, Enterococcus faecium, E. Coli & Lactobacillus of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. Also, neonates delivered vaginally have equally to neonates delivered with caesarean section and seeding no microbes. Neonates delivered with cs and seeding have statistically significant more often St. Epidermidis. (P=0,002)

In terms of ear microbes there was no statistically significant difference between St.Epidermidis, Enterococcus faecium, Lactobacillus & St. Aerius of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. However, neonates delivered with cs and vaginal seeding have more often E. Coli, bacteroids & bifidobacterium than neonates delivered vaginally. Also, neonates delivered vaginally have statistically significant more often no microbes than that delivered with cs and vaginal seeding.

In terms of umbilical microbes there was no statistically significant difference between Bacteroids, Bifidobacterium, St. Hominis, St. Aerius, Enterococcus faecium, E. Coli, Granulicatella & Lactobacillus of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. Also, neonates delivered vaginally have equally to neonates delivered with caesarean section and seeding no microbes. However, neonates delivered with cs and seeding have statistically significant more often St. Epidermidis than neonates delivered vaginally.

In terms of nasal microbes there was no statistically significant difference between bacteroids, St. Hominis, St. Aerius, St. Epidermidis, Enterococcus faecium, Granulicatella & Lactobacillus of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. However, neonates delivered vaginally have statistically significant more often E. Coli (P=0,037) & Bifidobacterium (P=0,015) than that delivered with cs and seeding. Also, neonates delivered with cs and seeding have statistically significant no microbes more often than neonates delivered vaginally. (P=0,006)

The comparison of our findings between Group B (no vaginal seeding and caesarean section ) and vaginal labour are as following:

In terms of ocular microbes there was no statistically significant difference between Enterococcus faecium and E. Coli of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. However, neonates delivered vaginally have statistically significant more often Bacteroids (P=0,007), Lactobacillus (P=0,011) & Bifidobacterium (P=0,015) than neonates delivered with cs and no vaginal seeding. Also, neonates delivered with cs and no vaginal seeding have statistically significant more often no microbes than those delivered vaginally (P=0,00). The presence of Granulicatella (N=1 vaginal group, N=0 cs and no seeding group), St. Epidermidis (N=4 vaginal group, N=0 cs and no seeding group), St. Hominis (N=2 vaginal group, N=0 cs and no seeding group), & St. Aerius (N=3 vaginal group, N=0 cs and no seeding group) was extremely rare (<95% of the vaginal group), so the comparison between the groups would not be reliable and the result would have no clinical interest.

In terms of rectal microbes there was no statistically significant difference between E. Coli of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. However, neonates delivered vaginally have statistically significant more often Lactobacillus (P=0,002), Bacteroids (P=0,000), Bifidobacterium(P=0,000), St. Hominis (P=0,005) & Enterococcus faecium (P=0,007) than neonates delivered with cs and no vaginal seeding. Also, neonates delivered with cs and no vaginal seeding have statistically significant more often no microbes than that delivered vaginally (P=0,00).

In terms of ear microbes there was no statistically significant difference between Lactobacillus of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. However, neonates delivered vaginally has statistically significant more often St.Epidermidis (P=0,00) & Enterococcus faecium (P=0,047) than neonates delivered with cs and no vaginal seeding. Also, neonates delivered with cs and no vaginal seeding have statistically significant more often no microbes than that delivered vaginally (P=0,00). The presence of Bacteroids (N=1 vaginal group, N=0 cs and no seeding group), Bifidobacterium (N=1 vaginal group, N=0 cs and no seeding group) & St. Aerius (N=3 vaginal group, N=0 cs and no seeding group) was extremely rare (<95% of the vaginal group), so the comparison between the groups would not be reliable and the result would have no clinical interest.

In terms of umbilical microbes there was no statistically significant difference between Lactobacillus of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. However, neonates delivered vaginally has statistically significant more often St. Aerius (P=0,015) & E. Coli(P=0,001) than neonates delivered with cs and no vaginal seeding. Also, neonates delivered with cs and no vaginal seeding have statistically significant more often no microbes than that delivered vaginally (P=0,00). The presence of Granulicatella (N=1 vaginal group, N=0 cs and no seeding group), Enterococcus faecium (N=2 vaginal group, N=0 cs and no seeding group) & St.Epidermidis (N=1 vaginal group, N=0 cs and no seeding group), St. Hominis (N=4 vaginal group, N=0 cs and no seeding group), Bifidobacterium (N=2 vaginal group, N=0 cs and no seeding group) was extremely rare (<95% of the vaginal group), so the comparison between the groups would not be reliable and the result would have no clinical interest.

In terms of nasal microbes there was no statistically significant difference between St. Epidermidis of neonates delivered with caesarean section and seeding and neonates delivered with vaginal labor. However, neonates delivered vaginally has statistically significant more often Granulicatella (P=0,02), Bacteroids (P=0,001), Bifidobacterium (P=0,004) Lactobacillus (P=0,013) & E. Coli (P=0,000) than neonates delivered with cs and no vaginal seeding. Also, neonates delivered with cs and no vaginal seeding have statistically significant more often no microbes than that delivered vaginally (P=0,00). The presence of Enterococcus faecium (N=4 vaginal group, N=0 cs and no seeding group), St. Aerius (N=2 vaginal group, N=0 cs and no seeding group) & St. Hominis (N=4 vaginal group, N=0 cs and no seeding group), was extremely rare (<95% of the vaginal group), so the comparison between the groups would not be reliable and the result would have no clinical interest.

## 5. DISCUSSION

The definition of microbiome refers to a set of microorganisms in a specific area, such as the human intestine, the oral cavity and the vaginal area. In healthy women, the predominant microorganism of the vaginal microflora is Lactobacillus spp., and the most abundant species are Lactobacillus gasseri, Lactobacillus iners, and Lactobacillus jensenii. However, there are other species in smaller proportions, such as Bacteroides, Fusobacterium, Veillonela, Actinomycetes, Bifidobacterium, Peptococcus, Peptostreptococcus, Propionibacterium, Staphylococcus aureus, Staphylococcus epidermis virginus, Streptococcus, Streptococcus, Streptococcus. Despite the fact that in healthy women the vaginal microflora shows a relatively small diversity, it can change the menstrual and sexual cycle. The mechanism by which a healthy vaginal microbial flora shifts to a pathological one can be understood by examining the context of the interaction between host and microbes. Infectious diseases arise both from the effect of microbial invasion and

from the nature of the response of the host organism. Therefore, clinical examination of the vaginal microbial flora should focus on this interaction (13-16).

The vaginal mucosa, cervix and cervical mucus form a natural barrier against the invasion of microorganisms into the endometrial cavity. This is the result of a natural epithelial barrier with tight connections and the presence of a continuous control of inflammation, part of which is the function of the autonomic immune system. The cellular composition of the autonomic immune system, including antigenic dendritic cells, macrophages and natural killer cells, is the first to come into contact with microbial invaders. This part of the immune response recognizes bacteria mainly through pathogen-associated molecular patterns (PAMPs). This recognition allows the immune cells to respond to a wide range of microorganisms, using a limited number of receptors. A major family of PAMP receptors is the Toll-like receptor (TLR) family, which binds different bacterial products and carries an inflammatory signal within cells. After activation of the autonomic immune system, dendritic cells activate T lymphocytes, which are part of the adapted immune system, leading to the differentiation of the original T cells into regulatory and influential T cells. Following activation, T cells respond by secreting soluble mediators, including pro-inflammatory drugs [such as interleukin-1 (interleukin-1, IL-1), tumor necrosis factor-a, TNF- a)] and anti-inflammatory [IL-1 (receptor antagonist IL-1ra), IL-10] cytokines and other immunomodulators (16-20).

Although the transition from a Lactobacillus-dominated to a bacterial vaginal flora is associated with variables such as sexual intercourse with specific male partners, vaginal douching, smoking, intrauterine devices and chronic stress, the factors that trigger this, remain unknown. Some recent researche suggests that this may, in part, be the result of alterations in the immune system. The proposed mechanism includes: 1) decreased TLR activation, 2) increased hsp70 production, and 3) inadequate release and /or function of mannose-binding lectin (MBL), all of which affect controlled inflammation that inhibits bacterial growth in the vagina. This hypothesis is consistent with the observation that most patients with a clinical diagnosis of bacterial vaginosis lack vaginal leukocytosis (19-24). However, there is a group of women with bacterial vaginosis-type microflora that exhibit a local pro-inflammatory response. The cervical specimens of these women include a large number of leukocytes and elevated levels of pro-inflammatory cytokines (IL-1b, IL-6, IL-8). Compared to non-pregnant women, the environment of pre-inflammatory cervical cytokines in pregnant women with bacterial vaginosis is enhanced. Smokers with heavy colonization with anaerobic Gram-negative strains and with Vaginal Hemophilus (Gardnerella vaginalis) show higher levels of pro-inflammatory cytokines. This group of women is at greater risk for premature birth. The risk of preterm birth was three times higher among women with elevated IL-1b (> 10 pg / ml) measured in cervical samples during the third trimester of pregnancy. In almost all cases with elevated IL- 1b had affected vaginal microflora. Similar observations were made by Simhan and Krohn, as in their population, women with a high number of pro-inflammatory (IL-1a, IL-1b, IL-6) and anti-inflammatory (IL-4, IL-10 and IL- 13) cytokines in their vaginal cervical secretions during the first trimester of pregnancy were 7.7 times more likely to give birth prematurely before the 34th week of gestation. In another study, Simhan et al.showed that only women with elevated atrial pH and leukocytosis had an increased risk of premature rupture of fetal membranes in the early third trimester of pregnancy (19-24).

The above studies indicate that there is individual differentiation in the host-microbial interaction in the lower genital system. Women who fail to control bacterial overgrowth (especially anaerobic Gram-negative and vaginal Haemophilus bacteria) but have an extensive pro-inflammatory response are at risk for both premature birth and premature rupture of membranes. It has not been clarified whether the markers of inflammation in the cervical fluid are related to those in the intrauterine environment or merely represent a feature of the overall hyperresponsiveness to specific infectious agents. However, it is undeniable that these factors that influence the variation of the microbial-host interaction play an important role in individual sensitivity and adverse pregnancy outcomes (25-30).

The genus Lactobacillus includes more than 130 species that produce lactic acid and are endemic to different environments, with more than 20 species being found in the vaginal flora. Unlike most other parts of the body, healthy vaginal communities are those dominated by only one or two species, the most common of which are Lactobacillus iners, Lactobacillus crispatus, Lactobacillus jensenii and Lactobacillus gasseri. Lactobacilli activate a variety of mechanisms to inhibit colonization by other bacteria, including pathogens. Vaginal epithelial cells produce glycogen, with which lactobacilli enzymes produce D- and L-lactic acid. Some of these produce hydrogen peroxide in vitro. However, recent studies have shown that in these peroxide conditions of the vagina, concentrations may never reach levels that are inhibitory to the action of other bacteria. Inside the vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid, but not with hydrogen peroxide. In addition, some species can also produce bactericides, which can directly kill other bacterial species. Lactobacilli may also compete with other organisms for nutrients or receptors on the surface of epithelial cells. Such inhibitory mechanisms differ between species of Lactobacillus. Comparative genomic analyzes of L crispatus, L gasseri, L iners and L jensenii have provided evidence that each Lactobacillus species has a unique set of protein families, suggesting that these changes may reflect specific community adaptations. Future research aimed at characterizing the functional roles of these species-specific protein families and genes is likely to provide important information on how these common vaginal bacteria affect women's health.

Lactobacilli are also able to inhibit the colonization of

pathogenic bacteria through competition for host receptor cells using urogenital pathogens such as Gardnerella vaginalis, Neisseria gonorrhoeae, Candida albicans, Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli and Prevotella bivia. Consequently, lactobacilli having a higher affinity for host cell receptors can displace adherent G. vaginalis and N. gonorrhoeae. In addition, some lactobacilli are believed to embody with pathogens, such as G vaginalis, C albicans and E coli, thus inhibiting their binding to host cells and allowing a more efficient clearance of them (30-32).

Regarding the host, a number of factors, including the intermittent hormonal cyclicity that promotes the release of glycogen into the vaginal environment and the continuous removal of bacterial epithelial cells, contribute to the innate defenses of the immune system. Presumably, the collective activity of the host, in conjunction with the inhibitory mechanisms of Lactobacillus, contribute to the maintenance of a healthy vaginal ecosystem (29-33).

Although the predominance of Lactobacillus species is the most common representation of a healthy vaginal microbiome, there is a significant proportion of seemingly healthy women who have vaginal bacterial communities that lack a significant number of Lactobacillus species but include a wide range of optional or severely inactive anaerobes associated with a slightly elevated pH. These microbiomes include members of the bacterial genus Atopobium, Corynebacterium, Anaerococcus, Peptoniphilus, Prevotella, Mobiluncus, Gardnerella and Sneathia, which are commonly associated with dysbiotic or pathological conditions. Some of these bacteria, such as Atopobium, can also produce lactic acid. Thus, the question arises as to whether specific bacterial classifications, in conjunction with other factors, could play a role in characterizing these microbiomes as either healthy or pathogenic (29-33).

Bacterial vaginosis is characterized by the disappearance of hydrogen peroxide-producing lactobacilli and the massive growth of anaerobic species, without specifying the order in which these processes take place, as well as the finding of a single organism responsible for its pathogenesis. Although Gardnerella vaginalis is found in almost all women with bacterial vaginosis, it is also present in 50% of women with a healthy vaginal microbiome. Mobiluncus spp. is a highly motile bacillus, found only in the soil of bacterial vaginosis, but only in 50% of these cases. Atopobium vaginae, a gram-positive anaerobic, like G vaginalis is found in more than 95% of cases of bacterial vaginosis, as well as in healthy vaginas. Both Mobiluncus spp and A vaginae have high levels of metronidazole tolerance and have been reported in many treatment failure cases (29-33).

There are innumerable anaerobic microorganisms, such as Prevotella spp., and various anaerobic streptococci, which are involved in causing bacterial vaginosis. Fredricks et al., provided one of the most enlightening works of bacterial vaginosis organisms. Using nucleic acid amplification techniques for the bacterial 16S rDNA, in women with bacterial vaginosis they identified 9 to 17 anaerobic phyllotypes (mean, 12.6) per vaginal specimen, with 58% of them being new (previously uncultivable) organisms in unknown genera such as Megasphaera and Sneathia. In addition, 3 of the newly identified species of the order Clostridiales were the most specific species in bacterial vaginosis. A common finding in bacterial vaginosis was L iners, a non-hydrogen hydroxide derivative. In another study, using similar techniques, Ferris et al. concluded that each case of bacterial vaginosis has its own unique group of anaerobic species. To date, the role of Mycoplasma spp in bacterial vaginosis remains unclear (29-33).

In bacterial vaginosis a number of biochemical and microenvironmental changes have been described. The normal vaginal epithelium is covered by a thin layer of mucus. In bacterial vaginosis, this so-called protective layer is replaced by a special G vaginalis biofilm. In addition, □-defensin-1 and -2 mRNA concentrations and leukocyte protease secretion inhibitors are reduced. At the same time, interleukins IL-1□, IL-1□ and the receptor-1 agonist increase and IL-8 levels fall. It also increases 70 kD heat shock proteins, lytic enzymes (sialidase, intracellular metalloprotease 8, phospholipase A2), nitric acids and endotoxins. Taken together, these events interfere with the normal defense mechanisms of the vagina and increase its destructive and inflammatory effects (32-35).

During pregnancy, the female body undergoes various hormonal, immune and metabolic changes to support the development of the fetus. During this period, the levels of secreted hormones, and in particular progesterone and estrogen, increase dramatically with large differences in immune responses. Immune system changes are complex, and are often referred to as immune modifications, as on the one hand they require some degree of immunosuppression to accept the developing fetus that already has its own immune system and on the other hand require the stay of a strict immunity to protect the mother and fetus from infections. Although pregnancy is considered by many to be an anti-inflammatory condition, others view it as a multistage process that includes inflammatory stages during implantation and childbirth and anti-inflammatory stages during mid-pregnancy, when the fetus is developing.

Pregnancy-related metabolic disorders are variously related to those that occur in metabolic syndromes, including weight gain, elevated blood glucose levels, insulin resistance, glucose intolerance, low-grade inflammation, and changes in hormone levels. As a result of the endocrine, metabolic and immune modifications, during pregnancy there are also significant changes in the microbiota of the different parts of the body (29-33).

The human vaginal microflora is a key component of the immune system in the fight against microbial and viral infections. The vaginal microbiome is made up of many different species, including Lactobacillus and members of Clostridiales, Bacteriodales, and Actinomycetales. The most common species of Lactobacillus genus are Lactobacillus gasseri, Lactobacillus crispatus, Lactobacillus jensenii, and Lactobacillus iners, which

provide various aspects of vaginal health. In particular, their lactic acids produce bacteria that can and do act as a barrier to the penetration of pathogenic microorganisms by maintaining a low pH (<4.5) and secreting metabolites that play a key role in inhibiting bacterial and viral urinary tract infections. On the contrary, it has been found that the presence of pH values close to 5.0 is associated with the presence of vaginitis (29-33).

During pregnancy, the vaginal microbiome experiences considerable changes, such as a large decline in total diversity, an increase in stability (the flora's composition changes gradually), and an enrichment of Lactobacillus species. These changes are associated with a reduction in an increase in vaginal secretion. It has been found that the composition of the vaginal microbiome varies with gestational age, with its composition in the later stages of pregnancy resembling that of non-pregnant women.

The predominant species of Lactobacillus during pregnancy vary according to ethnicity, with L. Jensenii predominating in Asian and Caucasian women and L. Gasseri being absent in Black women. A study by Romero et al. on the characterization of vaginal microbiota in pregnant and non-pregnant African-American women showed that one of the observed changes during pregnancy is the predominance of one species of Lactobacillus over others (29-35).

This effect may be due to the fact that some species of Lactobacillus have bactericidal activity against other species, ensuring dominance and low variability, which may help protect against infections during pregnancy.

In contrast, a more recent study found no significant change in the vaginal microbiome during pregnancy. However, the same study showed distinct differences in the postpartum period, with the vaginal microbial communities resembling those of intestinal microbiome, with Lactobacillus being replaced by various anaerobic bacteria, including Peptoniphilus, Prevotella, and Anaerococcus. These variations may persist for a period of up to one year after delivery. Similarly, in 40% of women after 6 weeks postpartum, the vaginal microbiome was characterized by a gradual decrease in Lactobacillus species, from an increased -diversity and enrichment with vaginitis-related bacteria, such as Actinobacteria (as opposed to only 2% of women during pregnancy). (29-33)Although there is evidence to support a difference between the microbiome of a newborn born by caesarean section versus that of a vaginal delivery, evidence that this is due to the type of delivery (eg differences in vaginal microbiome exposure during of childbirth) is not convincing and lacks critical data. However, there is a perception among health professionals that a caesarean section deprives the newborn of exposure to the maternal vaginal microbiome and this results in a neonatal dysbiosis and an increased risk for poorer health outcomes. A consequence of this perception is the efforts to correct this problem, even if this "problem" does not exist and the benefits of exposure to any single bacterium have not been documented (30-36).

This perception was greatly influenced by the research of Dominguez-Bello et al. . This practice involves the use of a gauze to transfer uterine vaginal fluid, and its microbiome, to the newborn immediately after the Caesarean section. The researchers, in an effort to test the effectiveness of this practice, recruited 18 mothers who gave birth either vaginally (n = 7) or by caesarean section (n = 11). Four of the newborns born by caesarean section were exposed to uterine vaginal fluids immediately after birth. The researchers reported a gradual recovery of the neonatal microbiome (mainly in the skin and mouth and less in the intestine) of these 4 newborns, with their microbiome resembling that of the corresponding uterine vaginal fluid (30-36).

The above research had many serious limitations. The most important of these was that all mothers who gave birth by caesarean section had received antibiotics, while only one of the women with vaginal delivery had been exposed to antibiotics. The authors also did not take into account that all cesarean sections were selective and did not take into account the differences in the BMI of women before pregnancy and the weight gained during pregnancy. The limitation of this small sample becomes more serious than the fact that several samples are absent at any time during the study. In addition, perceived difference in exposed vaginal fluid can be attributed either to chance or to insufficient data. In fact, a sample of the newborns' hands taken right after birth revealed that only 3 out of every 7 vaginally delivered neonates and 1 out of every 2 caesarean-section neonates had a skin microbiome dominated by Lactobacillus. These results support earlier research by Martin et al. that found Lactobacillus is extremely seldom transmitted to the infant through the vagina (32-36).

This research was based on the assumption that the health consequences and microbial disorders of newborns by caesarean section are a consequence of the absence of exposure to the vaginal microbiome during childbirth. However, there are a variety of factors that may contribute to the phenotype of the neonatal microbiome by caesarean section, and there is very little data to support the theory of the importance of exposure to maternal vaginal microbiome during childbirth. Despite the lack of sufficient data, the notion that reproducing the vaginal seeding process will help reduce the risk of cesarean section by restoring the newborn's natural microbial homeostasis has attracted considerable attention. It is worrying that the practice of vaginal seeding has become basic in some areas and is very often performed without the knowledge or guidance of health professionals. This practice carries serious risks for the transmission of opportunistic pathogens (including viruses and fungi) to the newborn, which may be asymptomatic in the mother (32-36).

From the perspective of innate immunity, if the importance of the mother's vaginal microbiome for the healthy colonization of the newborn is accepted, the question arises as to whether it is possible to successfully imitate vaginal delivery. Amniotic fluid is rich in antimicrobial peptides (AMPs) that prevent infections. Therefore, the impact of the maternal microbiome on neonatal colonization during childbirth may be altered

due to factors such as AMPs and amniotic fluid dilution. In addition, neonatal sebum, a white substance that covers the skin of the newborn at birth, is also rich in AMPs that could prevent any attempt to transfer the maternal microbiome. Such views should be further explored before the therapeutic process of microbial transmission can be accepted (32-36).

The existence of a normal vaginal microflora is crucial both for the woman herself and for the conception and successful outcome of a pregnancy. The ability of Lactobacillus species to block the invasion and colonization of the vaginal mucosa with pathogens without activating an obvious inflammatory response is called "Tolerance" and is particularly valuable for reproduction. Other gynecological conditions, including intraepithelial neoplasia and cervical cancer, a dysbiotic vaginal microenvironment, with reduced tolerance and anti-inflammatory mechanisms, can affect the course and outcome of pregnancy. In essence, the presence and value of Lactobacillus species are invaluable in maintaining a homeostasis of the vaginal microenvironment.

In a study by Romero et al, in relation to the vaginal microbiome, no correlation was found between the degree of differentiation and the outcome of pregnancy, while in two other studies the existence of a relationship between high differentiation and premature birth was found (36-38). Early vaginal microbiomes of pregnancy were found by DiGiulio et al. to be strongly related with a higher rate of preterm birth (36-38). These microbiomes included higher colonization by Gardnerella and Ureaplasma, lower colonization by Lactobacillus sp. and higher alpha differentiation. Functionally, abnormal changes in the vaginal microflora during pregnancy, such as reduced levels of Lactobacillus sp., can lead to infections and the production of pro-inflammatory cytokines and prostaglandins, which can cause contractions. and weakening of the fetal membranes. In addition, the presence of specific fungi, such as vaginal colonization with Candida albicans, even if asymptomatic, is associated with higher rates of preterm birth.

According to the American College of Obstetricians and Gynecologists issued in 2017 based on the lack of sufficient data on vaginal seeding underscores the need for further research on the safety and benefits of the practice of vaginal seeding of newborns immediately after Caesarean section (39). As observed from the results of our cultures gut of neonates, who are in concordance to international literature, where the method of vaginal seeding group A and group C were applied their gut were colonized with lactobacillus species and Bacteroides, in contrast with our reference group where the colonization of these microbes were limited (Table 1 and 2) (36-40). The findings of this study on neonatal microbial colonization in relation to the mode of delivery and vaginal seeding have important implications and potential clinical relevance. Understanding the impact of delivery mode on neonatal microbial colonization is crucial as it may influence the infant's health and development, especially in the early stages of life (40).

The study suggests that neonates delivered via cesarean section with vaginal seeding have microbial colonization patterns more similar to those born vaginally in some cases. This finding has potential clinical relevance as it highlights the possibility of using vaginal seeding as a means to transfer maternal vaginal microbes to cesarean-born infants. However, it is important to note that the use of vaginal seeding is still a controversial and investigational practice, and further research is needed to determine its safety and efficacy. The differences in microbial colonization between vaginally delivered infants and cesarean-born infants without seeding, particularly in the gut, are consistent with existing evidence linking microbial diversity to health outcomes. Vaginally delivered infants tend to have a more diverse microbiome, which has been associated with improved immune development and reduced risk of certain health conditions later in life. These findings underscore the potential importance of promoting vaginal delivery when it is safe and appropriate.

While this study provides valuable insights into neonatal microbial colonization, it is crucial to recognize that the long-term health implications of these differences are not fully understood. The early-life microbiome is believed to play a role in various health conditions, including allergies, asthma, obesity, and autoimmune diseases. Further research is needed to determine whether the observed differences in microbial colonization have long-term effects on the health and well-being of the infants. The presence of specific bacteria, such as Staphylococcus epidermidis and Enterococcus faecium, more frequently in cesarean-born infants without seeding raises potential concerns about infection risk. Staphylococcus epidermidis, for example, is known to be a common skin commensal but can become opportunistic pathogens, particularly in hospital settings. Understanding the colonization patterns of potentially pathogenic bacteria can inform infection prevention and control strategies in neonatal care units. The findings of this study highlight the individualized nature of neonatal microbial colonization. Each baby's microbiome is unique, influenced by multiple factors, including delivery mode, maternal health, and early-life exposures. As a result, neonatal care may benefit from a more personalized approach that considers each baby's specific microbial profile and factors influencing colonization. The study's results suggest that certain bacterial species are more prevalent in vaginally delivered infants, while others are more common in cesarean-born infants without seeding. This information may guide the development of targeted intervention strategies to promote a balanced and diverse microbial colonization in neonates born via cesarean section.

## 6. CONSLUSION

The findings of this study provide valuable insights into the impact of delivery mode and vaginal seeding on neonatal microbial colonization. These results have potential clinical relevance, particularly in understanding the role of the early-life microbiome in health and development. However, further research is needed to fully

elucidate the long-term implications of these microbial differences and to determine the safety and efficacy of vaginal seeding as a potential intervention strategy. The individualized nature of neonatal microbial colonization calls for personalized approaches to neonatal care and highlights the importance of ongoing research in this field. More future multicentric studies are necessary to confirm our results and established guidelines for daily clinical activities and prevent premature birth.


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- **Ethical statements:** Authors must state that the protocol for the research project has been approved by a suitably constituted Ethics Committee of the institution within which the work was undertaken and that it conforms to the provisions of the Declaration of Helsinki (as revised in Tokyo 2004). Authors must state their study does not violate the policies and/or procedures established by journal such as those described in "Specific Inappropriate Acts in Publication Process".
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