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The Development of the Human Microbiome Why Moms Matter

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INTRODUCTION

Bacteria and viruses, colloquially referred to as germs, have historically been regarded by the lay public as harmful to human health and thus avoidable at all costs, particularly during pregnancy. Hand sanitizer and antibacterial soaps are ubiquitous in homes and hospitals alike, whereas the media are rife with reports on the next antibiotic-resistant “superbug” and the looming threat of bioterrorism in the form of genetically modified microbes. However, it might frighten even the mildest of germophobes to know that the human body is cohabitated with trillions of commensal bacteria that are essential for our health. Current estimates indicate that the number of microbes that inhabit the entire body roughly equals the total number of cells that comprise the human body.¹ The bulk of this biomass is found in the large and small intestines, although bacteria are known to inhabit nearly every niche throughout the body, including the skin and the vagina.² Together, these bacteria comprise an individual’s microbiota and encode for thousands of metabolic functions known in totality as the microbiome. The community genetic repertoire encoded by both human and microbe is referred to as the metagenome.

Before the microbiome can be attributed to disease risk and pathogenesis, normal acquisition and development of the microbiome must be well understood. For this reason, acquisition of the microbiome in the first few years of life has been intensely studied over the past decade and our laboratory has joined with others to pioneer these efforts. The historical paradigm assumes that neonates are born sterile and are colonized differently depending on mode of delivery (ie, cesarean vs vaginal).^{3,4} However, emerging evidence showing both a low biomass and low abundance of microbes harbored in the uterine decidua and fallopian tubes, as well as in association with the amniotic fluid, placenta, amnion, and chorion, and the developing fetus has challenged this notion, indicating that exposure to microbes (or at least their metagenomes) may begin well before delivery.⁵⁻²⁹

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This article first explores the evidence surrounding in utero microbial exposures and the significance of this exposure in the proper development of the fetal and neonatal microbiome. It then delves into the development of the fetal and neonatal microbiome and its relationship to preterm birth, feeding practices (breast milk vs formula), and mode of delivery. In addition, it evaluates the impact of the maternal diet on the developing fetal and neonatal microbiome.

HOW PURE IS THE WOMB ANYWAY?

Evidence Supporting a Nonsterile Intrauterine Environment

In the past it was thought that the fetus and intrauterine environment is sterile, with the newborn's first contact with microbiota occurring at the time of parturition. However, observations arising from healthy pregnancies and studies within relevant animal models have indicated that the fetus may be first exposed to bacteria during gestation.^{7,11,22,30-38} Recent studies of the reproductive tract in reproductive-age women show a continuum of microbiota from the vagina, cervix, endometrial lining, and fallopian tubes, indicating a nonsterile intrauterine environment before and during the time of conception, implantation, and placental development.^{22,32,33} Other studies using culture-based and polymerase chain reaction (PCR)-based techniques have positively identified bacteria in the fetal membranes, cord blood, and possibly amniotic fluid of healthy, term pregnancies, suggesting that microbiota can inhabit the in utero environment without overtly affecting the pregnancy or the health of the infant.^{6-29,34,35,39} In addition, the use of metagenomics sequencing technologies revealed the diversity of the low-biomass microbial community of the placenta parenchyma and chorionic villus,⁶ which was also historically considered a sterile tissue in the absence of disease. Across the 320 placentae examined in this study, the most common bacterial taxa were Proteobacteria, such as *Escherichia coli*, and other microbiota common to the oral cavity, such as *Fusobacterium* and *Streptococcus* species.⁶ This work has since galvanized efforts to reconsider the fundamental assumptions about when and whence humans first being to acquire microbes in early life. Observations of mother-neonatal pairs by Dong and colleagues⁴⁰ and Collado and colleagues¹¹ showed that the microbiota found within the placenta share significant similarity to that of the neonate's meconium, indicating that microbiota may be additionally transferred across the placenta at the maternal-fetal interface into the fetus, where it would thereafter be presumptively excreted into the amniotic fluid as fetal urine. Of note, in midgestation (17–20 weeks' human gestation), amniotic fluid transitions from being a placental-derived fluid to being composed of fetal urine.

In sum, recent evidence in multiple mammalian systems shows that the female reproductive tract tissues (including the upper vagina and cervix, the uterus, and its endometrial decidua) and placenta are not sterile (Fig. 1). Thus, by definition, the womb (uterus) is not sterile. When an embryo of 8 to 16 cells implants in the uterine decidua, and the trophoblasts comprising one-fourth or more of this cell mass begin their process of differentiation and proliferation, they become intimate with the uterine decidua. The subsequent invasion of the spiral arteries facilitates the basis of the vascular fetal-maternal tissue connection, and is an inherent part of establishing the placenta as more of a conduit than a barrier.^{22,41,42}

Supporting evidence has also arisen from multiple animal models. In an early study, Jimenez and colleagues⁴³ orally administered genetically labeled *Enterococcus faecium* to pregnant mice and sterilely delivered their pups 1 day ahead of anticipated delivery. Interestingly, they could culture and identify the labeled bacterium from the fetal intestine, indicating that microbiota can be transferred from mother to offspring even before delivery occurs.³⁷ However, the precise route of transmission was not examined and to date remains unclear. Work by Han and colleagues⁴⁴ and Fardini and colleagues⁴⁵ has put forth a hematogenous model of placental colonization that potentially explains these observations. In the former study, *Fusobacterium nucleatum* was given to pregnant mice intravenously during late gestation (embryonic day 16–17). Although peripheral organs cleared *F. nucleatum* within 24 hours, this bacterial species persisted in the placenta and could be detected in the amniotic fluid and fetus at 72 hours postinfection.⁴⁵ In the latter study, the investigators intravenously administered commensal bacteria typical of the human oral cavity to pregnant mice late in gestation, and found that they could selectively detect many of these administered microbiota in the placental tissues by PCR.⁴⁵ However, the fetal tissues were not specifically examined in this study and thus a hematogenous route of placental and subsequently fetal colonization remains speculative without more definitive evidence.

Ascending colonization from the vagina has been alternatively hypothesized as a potential origin of intrauterine microbes, largely because of its anatomic proximity to the intrauterine environment and its association with preterm birth.^{46,47} However, as aforementioned, among most of the human population, the vagina is predominately populated by *Lactobacillus* species before pregnancy, and is only further enriched for lactobacilli as the pregnancy progresses.^{48–50} Although *Lactobacillus* species have been detected in the placental membranes in healthy, term pregnancies by metagenomics sequencing methodologies, the overall diversity of commensal species found within the placental parenchyma, amniotic fluid, and neonatal meconium suggests that the vaginal microbiome is unlikely to be the only origin for the full gamut of microbial species found within the intrauterine space.^{6–29} Nevertheless, well-designed animal studies are required to further refine these observations and better define a model of microbial transmission during this period.

It is important to consider a limited number of reports and reviews that have challenged the notion that detected metagenomes represent anything beyond environmental or community contamination.^{51–56} It is, and remains, the view of our team that the evidence to date is inconclusive as to whether the low-abundance, low-biomass microbiome detected metagenomically represents a live or actively colonizing community. However, given the weighted evidence from dozens of laboratories using multiple and varied techniques, including metagenomics and targeted PCR sequencing, cultivation, microscopy, and cross-validation with human and animal models warrants ongoing consideration and experimental testing because the studies suggesting that detected taxa cannot be distinguished from contaminant controls have several inherent limitations. It is outside the intent and scope of this article to further detail these limitations.

In summary, although the womb has traditionally been considered sterile, this notion is no longer uniformly accepted. Bacteria or their metagenomes have been found in not only the reproductive tissues before pregnancy but the intrauterine tissues of pregnancy.^{6–29,57,58}

When combined with several recent studies showing the maturation of the fetal gut immune repertoire in this same midgestational interval, it is highly probable that the development of the human microbiome first begins in utero.⁵⁹⁻⁶⁶ Further evidence for this probability arises from data suggesting that maternal exposures have a lasting impact on the offspring microbiome; this is discussed next.

IS THERE EVIDENCE FOR A ROLE OF MATERNAL EXPOSURES ON THE OFFSPRING'S MICROBIOME?

Impact of Maternal Nutrition in Pregnancy on Offspring Gut Microbiota

The authors have published extensively our findings arising from our nonhuman primate model of maternal high-fat diet feeding, showing that offspring exposed to a high-fat diet in pregnancy show increased anxietylike behaviors, reduced thyroid hormone production, hepatic circadian gene expression, and nonalcoholic fatty liver disease.⁶⁷⁻⁷¹ Changes to the fetal epigenome is a likely driver of these observed phenotypes, because there are extensively documented changes to histone acetylation and promotor occupancy around key genes, as well as altered expression of the deacetylase Sirtuin 1 (SIRT1).^{67,71-75}

However, diet is also known to be a potent modifier of the gut microbiome, favoring microbiota capable of metabolizing the available substrates.⁷⁶ Maternal diet was recently shown to have a long-term impact on the offspring gut microbiome, which may independently contribute to the phenotypes seen or help induce the epigenetic changes previously documented.⁷⁷ Dams were either provided a high-fat diet or a control diet before and during pregnancy and lactation. As with humans, a high-fat diet caused significant weight gain and induced corresponding shifts in the nonpregnant gut microbiome.^{76,77} To isolate the effects of maternal diet, the offspring of both high-fat and control dams were weaned onto a control diet at 6 to 7 months of age. At 1-year of age, the offspring gut microbiota could be discriminated based on whether their mothers consumed a high-fat or control diet, despite the offspring consuming a control diet for several months.⁷⁷ Specifically, a high-fat diet seemed to persistently diminish the relative abundance of commensal *Campylobacter* species in the offspring gut, indicating that maternal diet may play a significant role in shaping the transmission of commensal microbiota that can persist beyond infancy and may extend into adulthood.⁷⁷

The effect of maternal diet seems to extend beyond gestation. Research has shown that a high-fat diet leads to increased milk fat concentration and content compared with a high-carbohydrate diet.^{78,79} However, no differences in milk production or quantity of milk were observed. Therefore, neonates consuming breast milk from mothers with a high-fat diet consume a higher energy intake, which can have effects on the development of their microbiome. Although it has not been studied, differences in the properties of the breast milk likely affect which bacteria flourish in the neonatal microbiome, but further studies are needed to confirm this hypothesis. Along these same lines, the maternal diet may be associated with alterations in the breast milk microbiome. However, there currently are no studies published evaluating this association. Investigations in our laboratory are currently

exploring this hypothesis and will help us understand any substantial impact the maternal diet has on the breast milk microbiome.

Limited evidence to date has similarly indicated that maternal gestational diet may influence offspring adiposity in early life by altering offspring gut microbiota. Independent studies have implicated certain bacterial species, including *E coli*, as a major modifying factor of this phenotype in the mouse,⁸⁰ although it is uncertain whether *E coli* has a similar impact on infant adiposity and growth trajectories in humans or primates. Intriguingly, mitigating the effects of a high-fat diet on the maternal gut microbiota with a prebiotic supplement has been reported to attenuate the impact of maternal diet on the offspring's propensity for adiposity,³⁰ although our recent work in a nonhuman primate model of a maternal high-fat diet has shown that probiotics do not alter gut microbiome structure, nor do they persist in the gut microbiome.³¹ Nevertheless, the development of obesity is an extremely complex pathophysiologic process that may be first programmed in fetal life, but is likely sustained in postnatal life by continued environmental exposure to high-density dietary intake or aberrant microbiota.

In addition to obesity and immunity, recent data suggest that maternal diet may have an impact on offspring behavior by modulating gut microbiota. Bidirectional communication between the brain and the enteric nervous system has long been recognized, but only within the last few years has the impact of gut microbiota been explored in greater detail.⁸¹ By producing neurotransmitters in the gut, such as serotonin or gamma-aminobutyric acid, gut microbiota are hypothesized to contribute to several neurologic and behavioral disorders, including anxiety, depression, and autism, by activating or depressing neural pathways in the enteric and central nervous system.⁸¹ Recent work in the mouse by Buffington and colleagues⁸² has indicated that maternal gestational diet may modify offspring behavior by altering offspring gut microbiota in early life. Offspring whose mothers consumed a high-fat diet in pregnancy showed profound social deficits associated with significant changes to oxytocin levels in the brain and specific alterations of the offspring gut microbiota.⁸² Intriguingly, postnatal reintroduction of *Lactobacillus reuteri*, which was depleted as a result of a maternal high-fat diet, to the affected offspring was found to ameliorate the deficient social behavior and enhance oxytocin levels in the brain, indicating a causal linkage between maternal diet, gut microbiota, and neurologic development.⁸² Thus, future studies examining the impact of maternal gestational diet on offspring gut microbiota will likely continue to refine the understanding of which microbiota are important to neurologic development, how these microbiota are capable of modulating the gut-brain axis, and when these interactions are required.

Preterm Birth

Being born preterm, and the underlying factors leading to a preterm birth, have lasting effects on the short-term and long-term outcomes of neonates.⁸³ Preterm neonates are at higher risk for infection and intestinal problems, among other illnesses, owing to the lack of sufficient development of host tissues and immaturity of immune regulation at birth. The neonatal microbiome differs in preterm compared with term infants, and it has been hypothesized that some of the disease morbidity association with prematurity is caused by,

or exacerbated by, changes in the neonatal microbiome that result from the gestational age at delivery, the underlying predisposing factors, and/or extensive postnatal environmental exposures necessary for preterm neonatal care.^{43,84}

Compared with term infants, the gut microbiome of preterm infants tends to be much more sparsely populated. One group of investigators from Spain evaluated 21 premature neonates' intestinal microbiota during the first 3 months of life and compared it with term, exclusively breast-fed, vaginally delivered neonates. Preterm neonates had increased levels of facultative anaerobic microorganisms and decreased levels of strict anaerobes such as *Bifidobacterium*, *Bacteroides*, and *Atopobium*.⁸⁵ However, it is difficult to assess whether the changes they found were caused by lack of exclusive human milk feeding (all preterm infants included in this study received mixed feeding) or other associations with hospitalization and/or premature birth itself, such as antibiotics. Furthering the idea that the microbiome is different among premature neonates compared with term neonates, other investigators have shown that very low birth weight neonates (birth weights <1500 g) have decreased diversity of their microbiota, which may be caused by living in a neonatal intensive care unit (NICU), alongside generally continuous antibiotic therapy, sterile isolators, and receipt of parenteral feeds.⁸⁶⁻⁹⁰

Not only do premature neonates have a delay in the colonization of "healthy" commensal bacteria, such as *Bifidobacterium*, the premature neonate's microbiota contains higher quantities of pathogenic bacteria. *Klebsiella*, *Weissella*, *Clostridium*, Enterobacteriaceae, Enterococcaceae, Streptococcaceae, and Staphylococcaceae have all been found more commonly in premature neonates' microbiota than in neonates born at term.³² Concurrent with these results, other investigators found increased levels of *Klebsiella pneumoniae* in the preterm infant microbiota, and *Clostridium difficile* was detected exclusively in the preterm infants.^{84,91} These observations may be a result of the types of bacteria that tend to exist in the immediate environment, as well as the sparse microbiome that allows opportunistic colonization. Premature neonates are typically treated in NICUs, which have been shown to harbor a wide range of bacteria, many of which are known opportunistic pathogens that contain antibiotic resistance genes.⁹² Many of the neonatal gut microbes can be traced to bacteria found on NICU surfaces, indicating that the environment, in this case, plays a large role in seeding the preterm gut microbiome. Despite this, long-term observations of the preterm gut microbiome have shown that, by 1 to 3 years of age, the preterm microbiome develops similar complexity to term infants, indicating the resilient potential of the gut microbiome.⁹² Nevertheless, considering that early life interactions between the host and its microbes are likely critical for crucial patterning, this has the potential to drastically affect a child's long-term health.

In sum, premature neonates are more prone to foster and harbor pathogenic bacteria rather than beneficial commensals, and the diversity and richness of their microbial communities first seen at birth simplifies days to weeks later and following periods of often intense interventions and isolation, as well as antimicrobial therapy. Harboring pathogenic bacteria with less commensal, protective bacteria may be a key reason why this age group has a higher likelihood of necrotizing enterocolitis and other infectious maladies than term neonates.

Human Breast Milk and Formula Feeding

Human milk is a highly complex nutrient source that has multiple nutritive and bioactive components with potential to affect the developing offspring microbiome. Once thought to be sterile, it is now well established that human milk contains a distinct microbiome consisting of diverse species.^{93,94} These bacteria seed the gastrointestinal tracts of breastfeeding infants, likely contributing to the significant shifts in microbiome composition associated with breastfeeding. Intriguingly, in addition to skin-associated (*Staphylococcus*) and oral-associated (*Streptococcus*) taxa, the breast milk microbiome includes anaerobic bacteria most commonly associated with the gut, such as *Bifidobacterium* and *Enterococcus*.⁹³ The origin of these bacteria has yet to be fully elucidated, but evidence suggests that these bacteria may be translocated from the maternal gut via enteromammary trafficking, a pathway in which bacteria in the gut lumen are engulfed by leukocytes through the process of antigen sampling and translocated intracellularly to the mammary glands via systemic circulation.⁹⁵

In support of this hypothesized pathway, a study of mothers given oral *Lactobacillus* probiotics for the treatment of mastitis showed that the *Lactobacillus* strains were detected in the breast milk of 6 out of 10 mothers after oral probiotic administration.⁹⁶ Studies of mother-infant pairs have shown that multiple species of bacteria, including gut-associated anaerobes, are common among maternal stool, breast milk, and infant stool, and that the number of shared species between maternal and infant stool significantly increases with time.^{97,98} Because profiling by sequencing does not necessarily indicate that transferred bacteria are viable, one study showed that a viable strain of *Bifidobacterium breve* was shared among maternal stool, breast milk, and infant stool from a mother-infant pair.⁹⁷ Whatever their origin may be, it is tempting to speculate that these gut-associated bacteria play a key role in establishing the gut microbiome of breastfeeding infants. In addition, because diet is a strong driver of the adult gut microbiome,⁷⁶ enteromammary trafficking may represent a mechanism by which dietary-mediated shifts in enteric bacteria are transferred from mother to infant postnatally. However, the effect of maternal diet on the milk microbiome has not been explored and represents a vitally important focus of future research efforts.

Human milk contains many other components with the potential to transmit maternal dietary influence to the offspring microbiome, including macronutrients, human milk oligosaccharides (HMOs), and immune factors such as maternal immunoglobulins (ie, immunoglobulin A [IgA]). High-fat maternal diet significantly affects fat and energy content in human milk, which may in turn affect proliferation of bacteria in the infant gut.^{78,99} Human milk contains a high abundance of undigestible oligosaccharides (HMOs) that favor proliferation of specific bacteria in the infant gut, such as *Bifidobacterium* spp.¹⁰⁰ The HMO profile of breast milk varies substantially among women, but the effect of maternal diet on HMO composition has not been well characterized.¹⁰⁰ In addition, human milk contains IgA, which protects nursing infants from infections by providing passive immunity.

Although it is presumed that IgA preferentially targets pathogens, its role in molding the infant gut microbiome has not been well explored. Intriguingly, diet has been shown to modulate IgA production in intestinal and extraintestinal mucosal tissues as well as to alter

IgA coating of bacteria in the gut microbiome.¹⁰¹ Further studies are needed to characterize how diet affects maternal IgA content in human milk and the role of maternal IgA in shaping the offspring gut microbiome.

Mode of Delivery

Women can deliver in one of 2 ways: vaginally or via cesarean delivery. Although one is the often considered the more traditional path and the other is surgical, there is a concern that a lack of exposure to the vaginal microbiome may lead to higher rates of certain diseases occurring later in life, such as atopic conditions, inflammatory bowel disease, type I and II diabetes mellitus, and asthma.¹⁰²⁻¹⁰⁵ Given such challenges in establishing a causal relationship between cesarean and later disease, one option is to provide a mechanistic link between the exposure (cesarean) and the outcome (eg, later-life atopic disease). Attempts to do so have led researchers to postulate that absence of vaginal microbes in cases of neonates born via cesarean delivery may be the cause, but how well warranted is this concern?

If the postulate that cesarean-born infants fail to be colonized in their gut (or skin or mouth) by vaginally derived microbes is true, then microbes living in the maternal vaginal niche should (as a rule) establish stable, long-term communities in the infant. However, several lines of evidence have shown this not to be true. First, one of the key tenets of the Human Microbiome Project was the observation of unique body niche speciation. In the vagina, the ecology is dominated by *Lactobacillus* spp, which are highly adept at living at low pH; these same microbes do not dominate the neonatal gut or other body niches. Second, experimental manipulations have not yet provided strong evidence suggesting that vaginal microbes establish long-term community dominance outside the vagina.¹⁰⁶ The reasons to perform a cesarean delivery are often clear and evident medical conditions. For instance, conditions such as placenta previa (in which the placenta overlies the cervix) and vasa previa (in which vessels overlie the cervix) are absolute indications for performing cesarean deliveries because, if a vaginal birth occurred, the fetus would likely exsanguinate from severe blood loss. Cesarean delivery would also be performed in women who have a viable fetus and are found to have significant fetal distress necessitating emergent delivery or otherwise face fetal demise. Thus, the reasons for performing cesarean delivery are significantly different compared with allowing the traditional vaginal delivery to occur.

Does a lack of exposure to the vaginal microbiome confer risks to neonates as they grow and develop into adulthood? Some studies have found an association between delivery via cesarean and increased rates of atopic disorders, food allergies, metabolic syndrome, and obesity later in life.¹⁰²⁻¹⁰⁵ For instance, a recent large perspective study conducted over 16 years with more than 22,000 participants found that cesarean-delivered infants had a 13% increased risk of obesity later in life.¹⁰² However, how much of that risk can be attributed to the cesarean procedure itself (rather than what led to the cesarean: the maternal indication for cesarean delivery) remains unclear. Investigators studying the human microbiome have attributed these observations to a lack of exposure to the mother's vaginal microbiota (or conversely overt exposure to skin microbiota) during delivery. However, is this assertion confirmed by sound evidence?

Many often-cited studies have not fully elucidated whether it is the mode of delivery versus the underlying indication for delivery that is the culprit. Not all confounders have been thoroughly investigated. For example, in a study by Dominguez-Bello and colleagues,⁴ the neonatal microbiome was found to be altered in neonates born via cesarean delivery compared with vaginal delivery. This landmark study created the foundation that the lack of exposure to the maternal vaginal microbiome during parturition (a lack of vaginal seeding) is the cause for these differences.⁴ Note that their analysis is based on samples within 5 minutes of delivery and the baby's first stool collected within 24 hours, and thus showcases differences in microbial transmission from the mother to the neonate but does not necessarily reflect true colonization. There are several additional aspects to this particular observational study worth mentioning. First, the study enrolled 9 women and their 10 neonates. Four women and their 4 infants make up the vaginal cohort, whereas 5 women and 6 neonates (1 set of twins) represent the cesarean cohort. Except for the twins, the exact weight of each neonate was not provided, but the methods section of the article states that "[a]ll mothers had healthy pregnancies and all babies were born at term, without complications. Babies weighed between 2 and 5.2 kg (the smallest baby was the twin in second order of birth, after his 3-kg brother)."⁴ However, these findings suggest that at least 2 of the pregnancies, likely both cesarean deliveries, were not healthy and uncomplicated." The twins showed significant growth disparity, with a 33% difference in birth weights (2.0 kg vs 3.0 kg). In twins, a discordance of more than 20% is associated with adverse perinatal and postnatal outcomes, and, thus, this is not a healthy and uncomplicated pregnancy. Another neonate in the cohort also had macrosomia, a birth weight of more than 4 kg, which often is found in women who are diabetic. Additional causes include genetic and epigenetic overgrowth disorders, chronic caloric excess, and maternal obesity. Thus, although this study is often cited as showcasing the differences in neonates born via cesarean delivery and those born vaginally, there are at least 3 neonates out of 10 that show signs that the mothers and/or neonates had medical conditions affecting pregnancy.

Our team published one of the first large, prospectively followed cohort of women that includes the maternal indications for delivery along with mode of delivery in evaluating the effects of the development of the neonatal microbiome across multiple body sites.¹⁰⁶ Although neonates born via cesarean delivery have distinct microbiome profiles in the first week of life, this effect did not last past 2 months of age.^{106,107} The neonatal microbiome differentiates at specific body sites by 6 to 8 weeks postnatally.¹⁰⁶ Immediately after birth, the mode of delivery was associated with differences in the neonatal microbiome within the nares, skin, and oral cavity. However, the neonatal meconium microbiome clustered separately among cesarean-born infants, suggesting a distinct maternal origin that separated it from the other body sites studied. By 6 weeks of age, the infant microbiota had diversified with body site specificity, and, most remarkably, no differences were identified between infants born via vaginal delivery and those born via cesarean in any of the body sites examined. When these were further parsed by indication for cesarean, or by labored versus unlabored, cesarean-born neonates without labor were the most dissimilar. In addition, when controlling for the indication for delivery and other clinical factors, only maternal diet and formula feeding seem to have a lasting effect on the neonatal microbiome at 6 weeks of age, and the maternal indication for delivery plays a significant role in this development. Along

these lines, in preterm neonates, other investigators have shown that tracheal aspirates have been evaluated for the evaluation of the microbiome, and there no appreciable differences between those premature neonates born via cesarean delivery versus those born vaginally.¹⁰⁸ The recent The Environmental Determinants of Diabetes in the Young (TEDDY) Study cohort studies further failed to delineate a fundamental role for cesarean delivery in shaping the early developmental microbiome.

In sum, although others have previously observed that neonates born vaginally or vaginally seeded showed increased *Lactobacillus* and *Bacteroides*, Chu and colleagues^{106,107} found low levels of *Bacteroides* and/or high levels of *Lactobacillus* to be equally probable in vaginally and cesarean-delivered neonates and infants, but their variation depended on several other collinear factors, such as the percentage of fat in the maternal diet, formula feeding, labored versus unlabored delivery, and gestational age at delivery. These observations were consistent with much earlier studies showing that vaginal delivery rarely transfers *Lactobacillus* to the neonate, and have hence been recapitulated by multiple groups that collectively failed to show a significant or lasting distinction in the gut microbiota of vaginal-delivered versus cesarean-delivered infants or children. Based on the emerging data in well-designed, prospective, and population-based cohorts, the authors conclude that it is highly probable that the maternal indication for cesarean delivery plays a larger role than the mode of delivery itself. This possibility renders the alternate view that a lack of vaginal seeding^{109,110} by the maternal vaginal microbiome during parturition is unlikely to be the key the culprit in any subsequent association between cesarean birth and disease later in life. The origin of the risk lies in the maternal “soil” from which the neonate originated (ie, the maternal and intrauterine milieu). Although these may or may not be the indications that led to the decision to perform a cesarean delivery, parsing cause from surgery is of fundamental importance because the nature and timing of any corrective interventions are widely different.

FUTURE DIRECTIONS AND SUMMARY

Future Directions

Future studies expanding on the work presented in this article should focus on 3 major directions: (1) determining the impact of a maternal high-fat diet–associated gut microbiome on the immune and behavioral phenotype of the offspring, (2) identifying the corresponding effect of maternal diet on the offspring gut microbiome during breastfeeding, and (3) further characterizing and identifying the major bacterial and host mediators of maternal-fetal bacterial transmission. Given that any initial differences in the infant microbiota between vaginal and cesarean delivery become less profound over time, it remains unclear how birth mode influences the risk factors for disease by modulation of the microbiota. This question is of paramount importance for mothers to make informed decisions regarding the desired birth mode.

It is similarly crucially important to understand the impact the maternal diet (independent of obesity and weight gain or loss in pregnancy) has on her developing offspring, its microbiome, and its lifelong risk of metabolic disease. Although clinicians cannot merely blame the mothers for any adversity their children experience in life, they can and must

provide sound evidence on those factors that the mothers can control and modify during their pregnancies. Pregnant woman cannot safely lose dozens of kilograms of weight necessary to transition from being obese to normal weight in the span of 10, 20, or even 40 weeks. However, they can make conscious decision regarding the quality of their diets. More importantly, given the association between social disparities, diet, and health outcomes, clinicians can make informed public health decisions regarding the importance of providing high-quality and nutritional food during pregnancy.

As one such example, the authors have previously established within our nonhuman primate model that a maternal high-fat gestational diet (but not maternal obesity per se) results in changes to the metabolic profile and epigenome of the offspring.^{67,70,71} Ongoing work within this model has focused on characterizing additional phenotypes within these animals likely mediated by an altered gut microbiome. This work includes prior and continuing studies that have documented abnormal behavioral tendencies within offspring exposed to a maternal high-fat gestational diet.^{73,75} Future studies examining key gut bacteria and their metabolites altered by a maternal high-fat diet will be required to understand how gut microbiota can influence behavior by acting on the gut-brain-axis.⁸¹ It is possible that gut microbiota either produce key regulators that alter neuronal transmission or indirectly influence the production of key neuropeptides. Therefore, multiomics using relevant dyad models will be crucially important in the future. Along these lines, prior evidence has indicated that microbes are essential for patterning methylation and histone modifications of key components of innate and adaptive immunity, including TLR4 and Th17 balance.¹¹¹⁻¹¹⁴ Studying host responses to the presence or absence of differentially associated bacteria within this ex vivo condition may reveal key host-microbial interactions essential for proper immune development within the gut.

Additional studies are required to determine the relative impact of maternal diet during breastfeeding, and to tease apart the intrauterine exposure risks from the early ex utero risks. It is challenging, if not impossible, to cross-foster primate offspring because of high-rates of infanticide, and generally unacceptable in human clinical studies. As a result, it is not possible to distinguish between the gestational and lactational periods. Breast milk harbors a unique microbiome that changes through time postnatally. Notably, the composition of breast milk has been shown to change with maternal diet,⁷⁸ and ongoing work within our laboratory has shown additional changes in the microbiome composition. It is therefore possible that maternal diet in lactation has an additional or synergistic effect on the offspring gut microbiome. Innovative paired study designs in human trials may be beneficial in parsing out the relative impacts of gestational versus lactational exposures over time.

SUMMARY

Although parturition was traditionally assumed to be the first point at which neonates are exposed to microbes, emerging evidence indicates that this is unlikely to be true. The presence of microbes and low-biomass microbial communities within the intrauterine space (the uterine decidua, the placenta and the amnion and chorionic membranes, and amniotic fluid) has now been consistently documented in a growing multitude of mammalian species. Right at birth, distinct microbial communities in the meconium among preterm and healthy-

term neonates have been detected and described, and these impressively expand in the first days to weeks of life to readily show discrete body niche communities long before that same infant alters its diet or engages in meaningful contact with the outside world (Fig. 2). In addition, although it has been appreciated for more than a century that congenital viruses are vertically transmitted from mother to fetus, it remains a consistent observation from human immunodeficiency virus to Zika that only a small fraction become infected in utero. In recent months, it has been suggested that bacteria may play a role in regulating intrauterine viral transmission, although the mechanisms by which this might occur are presently unknown.

Although some key questions have been answered regarding the development of the human microbiome and its impact on human disease, much still remains unknown. Physicians and scientist alike must seek answers to these vital questions in order to elucidate potential causes for disease states, including those that arise because of aberrant development of the human microbiome and thus can be amenable to therapies early in life. Moreover, continuing to operate under age-old paradigms of “purity at birth,” which ignore mounting evidence for low-biomass metagenomes (be they of sparse abundance or not) in favor of a priori assumptions, will limit progress. Although our laboratory presently remains agnostic as to the issue of whether these consistently observed low-biomass communities are alive and colonize the fetus, or alternately enable later colonization through a process of immune tolerance, we look forward to future and bold science that will help elucidate one of the most significant questions of our time. In the interval until that occurs, clinicians must remain both open-minded and constructively critical of the available evidence.

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KEY POINTS

- The intrauterine environment retains a low-abundance, low-biomass microbiome that may be important for establishing tolerance to commensal organisms in utero.
- Multiple perinatal factors beyond whether the infant was born via cesarean delivery show a lasting impact on the developing human microbiome.
- Prematurely delivered neonates are more likely to harbor pathobionts, which may explain why these neonates have higher rates of necrotizing enterocolitis and other conditions associated with prematurity. Whether these pathobionts are causally related to preterm labor and birth remains unknown.

Fig. 1.

The female reproductive tract and its associated microbiota. Distinct microbial communities reside in specific sites within the vagina and uterus during pregnancy. These findings indicate a nonsterile environment long before pregnancy and implantation, and thus argue against the sterile-womb theory. For the womb to be sterile during pregnancy, the intimately connected placental villi, parenchyma, and the amnion and chorion would need to exert antimicrobial properties ridding the decidua and tract tissues of their resident communities. It is worth considering the evident constituent and functional overlap between those metagenomes observed in the female reproductive tract and the placenta, chorion-amnion, and amniotic fluid. Emerging themes include what is present, their sparseness and low biomass, as well as their functional capacity. CL, Lower Third of Vagina; CU, Posterior Fornix; CV, Cervical Canal; ET, Endometrium; FL, Fallopian Tube; PF, Peritoneal Fluid. (From Chen C, Song X, Wei W, et al. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nature communications*. Oct 17 2017;8(1):875; with permission.)

Factors Affecting Development of the Human Microbiome

Fig. 2.

Factors reported to affect the acquisition and development of the gut microbiome. Numerous reports have observed an effect of both internal characteristics and external factors on the characteristics of the neonatal, infant, and early childhood microbiome. Adulthood and infancy, which encompasses the first year of life, have been the 2 predominately studied time periods. More recent reports have shown an effect of gestational exposures, including maternal obesity status and gestational diabetes. In the future, fundamentally crucial developmental windows to study will include the period of acquiring reproductive competence (periadolescence, adolescence, and early reproductive age) as well as the intervals immediately before and including pregnancy.