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Microbiome in Pregnancy and Early Child Development

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ABSTRACT

The maternal and infant microbiomes play pivotal roles in pregnancy outcomes, immune maturation, and early childhood development. Traditionally, the fetus was thought to develop in a sterile environment; however, evidence now suggests that maternal microbial communities influence fetal biology even before birth. Pregnancy induces profound shifts in the gut, vaginal, and oral microbiota, which in turn affect immune tolerance, nutrient metabolism, and fetal growth. Dysbiosis during gestation is associated with adverse outcomes, including preterm birth, low birth weight, and impaired immune development. After birth, maternal transmission of microbes through delivery mode, breastfeeding, and environmental exposure seeds the infant microbiome, which matures over the first three years of life. Early microbial colonization shapes immune function, neurodevelopment, and behavioral outcomes, with disruptions linked to conditions such as asthma, allergies, obesity, autism spectrum disorder, and mood-related disorders. Nutrition, antibiotics, and cesarean delivery are major modulators of microbial assembly. Methodological advances in metagenomics and multi-omics approaches continue to deepen insights into host–microbe interactions. This review summarizes the role of the microbiome in pregnancy and early child development, with emphasis on immune education, neurodevelopment, and potential therapeutic interventions, including probiotics, dietary strategies, and microbiome modulation.

Keywords: Pregnancy microbiome**,** Infant gut colonization**,** Immune system development**,** Gut–brain axis, and early childhood health.

INTRODUCTION

During the last years, our knowledge regarding factors contributing to the development of human biology has dramatically improved, generating a novel aspect to interpret early life events with the microbial world constituted by bacteria, viruses, archaea, and eukaryotic species [1]. Microorganisms began to colonize the human body at birth and, until recent works about microbiota origin, the whole assumption was based on sterile fetus and sterile womb paradigms. However, a microbial community of low but detectable levels has been shown in the fetus developing in the uterus and is finally able to influence fetal biology and the nervous system development through maternal gut or mouth routes [2]. Microorganisms do colonize the human body, and their genes are beyond the cells of the human body, even transcending host species, from bacteria and archaea present in the gut to viruses and fungi in the oral cavity [3]. Microorganisms also produce metabolites capable of regulating the host immunological system and conditioning its resistance to extracellular insult. The term microbiome has been defined by Joshua Lederberg as the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and impact the human being well beyond the simple infection paradigm. In modern human dreaming, the microbiome is currently considered a forgotten organ acting as a barrier charged with a privileged crosstalk to regulate homeostasis in animals and humans, to educate the immune system for tolerance and efficiency, and to produce small molecules affecting the crossing system at multiple levels [1, 2, 3, 4].

The Role of the Microbiome in Pregnancy

Pregnancy involves physiological and hormonal changes that affect parts of the human body with a microbiome, such as the gastrointestinal, vaginal, and oral tracts [2]. Microbial richness appears to decrease during pregnancy,

but microbial composition varies across these tracts. The vaginal microbiome of pregnant women is dominated by Lactobacillus spp., as opposed to non-pregnant or post-menopausal women. Oral and gut microbial compositions during pregnancy also differ from respect of non-pregnant women, and the latter is associated with increased energy production and conversion [2]. The remodeling encompasses an expansion of endotoxin-producing bacteria and a contraction of butyrate-producing bacteria. When the pregnant woman has a balanced microbial composition, she can nurture a healthy child. Unfavourable composition due to infections or malnutrition induces pro-inflammatory and anti-inflammatory cytokine production. These may lead to the disruption of the epithelial barrier and the translocation of bacteria or bacterial products into the amniotic cavity with subsequent poor pregnancy outcomes. The production of cytokines and chemical mediators unbalances the maternal immunological system, which can lead to the arrest of fetal development and eventually pregnancy loss [2].

Maternal Microbiome Composition

A woman's microbiome changes significantly throughout pregnancy. Early studies using culture-based methods of bacteria typically present in pregnant and non-pregnant women showed increases in several species during pregnancy, including aerobic bacteria such as Escherichia coli, Proteus mirabilis, and Staphylococcus aureus, which plateau and return to baseline levels before delivery. Increases in Candida albicans, a fungal species, were also seen late in pregnancy [2]. Later studies utilizing high-throughput methods also show changes in maternal microbiome composition throughout pregnancy. Vaginal samples collected longitudinally from 40 pregnant women showed a notable decrease in diversity with increased stability in the third trimester and a marked increase in Lactobacillus species [2]. Other studies similarly show an increase in the genus Lactobacillus and a decrease in alpha diversity over pregnancy in the vagina, particularly in Caucasian and African American populations. Additionally, biomarkers associated with an increased risk of preterm delivery showed similar characteristics to pregnant women in the first trimester, suggesting that the composition of the vaginal microbiome may be important in healthy pregnancy [2].

Impact on Fetal Development

The fetal development stage is a critical period during which the maternal microbiome exerts a significant influence. During pregnancy, maternal microbes colonize the developing fetal gut, contributing to early immune training and the development of physiological systems. By the third trimester, the placenta is naturally enriched with distinct saprophytic microbes that may temporarily colonize the fetus [4]. Elevated maternal progesterone levels can increase the production of short-chain fatty acids such as butyrate and propionate by gut microbes like Bacteroidetes and Firmicutes, which affect the growth of fetal regulatory T cells [5]. Concurrently, a notable decline in Roseburia and Faecalibacterium species correlates with raised proinflammatory cytokines, thereby influencing neonatal immunity. Dysbiotic alterations in the maternal microbiome have been linked to adverse fetal outcomes, including low birth weights, preterm births, and developmental delays. Furthermore, maternal dysbiosis can directly alter the natural microbial colonization of the fetal gut, potentially predisposing offspring to later-life inflammatory and metabolic disorders. Collectively, these findings emphasize the pivotal role of maternal microbial communities in shaping fetal development and highlight the importance of maintaining microbial balance during gestation [4, 5].

Influence on Pregnancy Outcomes

The microbiome plays a major role in regulating pregnancy outcomes. Maternal bacterial composition and various physical parameters positively influence fetal development and well-being. Throughout gestation and into lactation, the gut microbiota is shaped by endogenous factors as well as exogenous cues, predominantly by dietary intake [2]. Numerous studies have examined maternal gut microbiota, revealing substantial changes during pregnancy that correlate with initial body weight, diet, inflammatory markers, and metabolic indices. For instance, antibiotic administration during gestation not only alters bacterial communities and reduces diversity but also promotes maternal fat accumulation and modulates offspring behavior [5]. The composition of the maternal microbiome in pregnancy may thus have implications for the progeny's growth, immunocompetence, and health, although it is difficult to disentangle these effects from those of lactational exposure. Maternal probiotic supplementation is known to affect gene expression patterns at the placental interface as well as within the infant, while microbial exposure during gestation can confer protection against allergic conditions later in life. Beyond direct compositional effects, the maternal microbiota also shapes the developing immune architecture of the offspring; variations in maternal bacterial communities during early pregnancy influence immune-related gene expression signatures and promote the establishment of diverse populations of innate immune cells and macrophages in fetal tissues [2, 5].

Microbiome and Immune System Development

Immune adaptations during gestation facilitate tolerance of the semi-allogeneic fetus and provide defensive support to both mother and offspring [6]. The human microbiome is a critical regulator of immunity and educates

immune system development and responsiveness. Consequently, maternal microbial communities and transplacental transport of microbially processed metabolites significantly influence immune development. After birth, continuous microbiome transfer through breastfeeding and environmental exposures shapes immune maturation [6]. The prenatal immune system is both modulated and supported by microbial signals. In utero, the fetal immune environment is dominated by regulatory T cells but retains responsiveness to microbial antigenic signals. Postnatal microbial colonization further stimulates immune maturation and supports the development of pathogen-specific immunity concurrently with maternal antibody protection. In the absence of microbial exposure, this developmental pattern is disrupted [6]. The infant microbiome also plays a pivotal role in immune education during early development. The infant core microbiome, which includes Bifidobacterium, Enterococcus, Lactobacillus, Escherichia, Bacteroides, and Clostridium, shapes the ontogeny of the innate immune system. Variations in microbial composition affect the trajectory of immune maturation and determine resistance to infectious challenges later in life [7].

Immune System in Pregnancy

Pregnancy is regarded as the process of growth and development of the fetus, which expresses paternal antigens within the maternal womb. Microbial infections during pregnancy, caused by bacteria, fungi, or viruses, constitute significant risk factors for adverse outcomes, including miscarriage, eclampsia, intrauterine growth retardation, premature rupture of membranes, and premature delivery [8]. Maternal immune defenses, therefore, require the capacity to mount rapid and accurate responses to pathogenic microorganisms. A successful pregnancy depends on the coordinated immune regulation of the mother, placenta, and fetus, which in turn responds dynamically to the evolving fetal-placental unit [8]. During gestation, the maternal organism undergoes hormonal, immunological, and metabolic alterations in order to accommodate fetal development. Levels of hormones such as progesterone and estrogens rise markedly, influencing immune responsiveness; the immune system must be modulated to tolerate the allogeneic fetus while simultaneously maintaining protective immunity against infections. Some researchers have labeled pregnancy an anti-inflammatory state, whereas others emphasize its multi-stage character: a pro-inflammatory phase during implantation, anti-inflammatory conditions throughout mid-gestation, and a second pro-inflammatory interval preparing for parturition. Meanwhile, the maternal microbiota undergoes shifts at multiple body sites throughout pregnancy. In particular, the gut microbiota composition changes substantially from the first to the third trimester. Microbial profiles observed in late pregnancy are capable of modulating host immunology and metabolism, producing a constellation of changes that bears resemblance to metabolic syndrome [2]. Gestational stress exerts detrimental influences upon fetal growth, developmental trajectories, immunity, and cognition. Prenatal antibody transfer is impaired, while colonization patterns of Lactobacillus and Bifidobacterium species implicated in gastrointestinal allergies and inflammatory bowel diseases also become altered [2]. Maternal environmental factors and microbiota contribute to immune maturation: exposure to rural surroundings enhances the accumulation of Th17 lymphocytes and decreases the risk of allergic disorders. Dysbiosis of the maternal vaginal microbiota correlates with immunological markers measured in cord blood, further underscoring the impact of maternal microbial communities on fetal immune development [9]. The precise mechanisms through which the fetal immune system detects maternal microbiotal signals remain to be elucidated, although transplacental passages of bacterial antigens and introduction via amniotic fluid have been proposed. Prenatal antigen exposure during late gestation may induce specific immunological tolerance in utero, thereby preventing inflammatory damage. Colonization of pregnant mice with non-pathogenic Escherichia coli has been shown to influence postnatal development of the offspring's intestinal immune cell populations, demonstrating the functional significance of maternal microbiotal perturbations [9].

Microbiome's Role in Immune Education

The maternal immune system undergoes extensive adaptations during mammalian pregnancy to permit tolerance of the genetically distinct fetus. The education of the maternal immune system by the microbiome encompasses the necessary adaptations of the immune response throughout pregnancy and is linked to fetal and neonatal immune-maturity [10]. Microbial influences on immune-maturation are also evident in early childhood, during which time the infant's immune system undergoes rapid developmental change. Emerging data support the existence of a co-developmental process whereby postnatal immune-maturation occurs in parallel with colonization of body surfaces by a specific repertoire of microorganisms [11].

Microbiome in Early Childhood

In early life, the infant microbiome develops rapidly, maturing over 2 to 3 years to a structure that resembles the adult gut microbiome. The long-term composition of the infant microbiota sets the stage for normal health development and adulthood well beyond early childhood, and a greater understanding of the factors driving infant microbiome development is increasingly valuable for public health and therapeutic improvement [3, 11]. The newborn gut microbiota is characterized by fewer microbial species, but a higher inter-individual variation

compared to adults. The colonization of the gut commences at birth, and microbiota development is modulated substantially by diet, location of residence, antibiotic use, birth mode, and host genetics [3, 11]. Several key studies show that the early-life composition of the infant microbiota has an important role during the development and maturation of the immune system. Although the adult-associated microbiota profile is more stable, external factors such as diet and antibiotics can cause drastic perturbations [3].

Establishment of the Infant Microbiome

The maternal microbiome facilitates the establishment of the infant microbiome in part through the transmission of beneficial microbes collected in utero that seed the fetus. After birth, microbes from maternal milk and the surrounding environment help shape the early-life infant microbial community [1]. As the infant microbiome develops from initial colonization toward a more balanced state (termed eubiosis), it becomes less susceptible to the excessive fluctuations or loss of beneficial taxa that can be harmful to developing organ systems. However, this process is impeded if the infant microbial community is disrupted or rendered poor in microbial diversity, particularly in communities with a low number of key members responsible for broad metabolic capabilities [3].

Factors Influencing Microbiome Development

Many factors influence the development of the infant microbiota. Antibiotic use is common during pregnancy and has been associated with an increased risk of asthma, eczema, and obesity during Childhood. Delivery mode can also affect early microbial colonization [2]. Compared to vaginally delivered infants, infants born via C-section tend to have low gut microbiota diversity and higher numbers of pathogens. Blaut et al. provide an overview of key sampling methods and data analysis techniques used in microbiome studies related to pregnancy and child development [3]. Maternal nutrition influences the maternal and infant microbiomes, partly through the establishment of the infant gut microbiome. Changes in the infant gut microbiome have been associated with feeding mode. Microbial metabolites derived from the gut microbiome and dietary components also contribute to cognitive development [2, 3]. The influence of the microbiome on behavior during early childhood, and whether disruptions in the microbiome may be associated with behavioral disorders such as autism spectrum disorder, are discussed by Stinson et al.

Nutrition and the Microbiome

Maternal nutrition during pregnancy shapes the intestinal microbiome of the mother and thereby potentially affects the microbial population transmitted to the infant during vaginal birth or through breastfeeding. Unhealthy perinatal diets have been found to disrupt early-life microbiome assembly, altering the abundance of key bacterial species in the offspring, such as Akkermansia, Lactobacillus, and Bacteroides [1]. Breastfeeding strongly promotes Bifidobacterium, while formula feeding is associated with reduced Bifidobacteria and increased Clostridium and Enterobacteriaceae [2].

Maternal Nutrition during Pregnancy

From conception to birth, the developmental environment comprises a complex network of interacting systems, including the maternal organs and placenta. Nutritional stimuli, gut microbial metabolites, and immune factors work together to set a homeostatic placental environment that can support a successful pregnancy [12]. Maternal gut microbes strongly influence fetal maturation through metabolite transfer, underscoring the formative importance of nutritional intervention and dietary counselling. Although the Recommended Dietary Intake (RDI) of various macro- and micronutrients for pregnancy was first established over 50 years ago, the status quo plods on with the very same guidelines. However, parallel studies on the microbiome firm the importance of a healthy, balanced diet, whereas a poor nutritional profile (e.g., insufficient vitamin D or zinc, low intake of dietary fibre, or a high level of saturated fat and refined sugar) disrupts the compositional balance of the gut bacterial community [4]. In combination, these studies advocate for the precise form and amount of supplementation that should be given throughout a complicated pregnancy [12, 4].

Infant Feeding Practices

Nutrition is one of the few environmental factors capable of reshaping microbiome composition, making maternal diet a critical conditioning element that modulates the establishment of the infant's microbiome [4]. During the transition from breastfeeding or formula feeding to a family diet, complementary feeding induces a microbiota shift from Lactobacilli, Bifidobacteria, and Enterobacteriaceae towards Clostridium spp. and Bacteroides spp. The microbiota composition is influenced by the timing of new food introduction and breastfeeding cessation. Consistent microbial changes have been identified irrespective of geographic location, antibiotic use, delivery mode, or feeding practices, including decreases in Bifidobacteriaceae, Enterobacteriaceae, and Clostridiaceae and increases in Ruminococcaceae and Lachnospiraceae following the introduction of solid foods [1]. Infants subjected to mixed or non-exclusive breastfeeding exhibit increased microbiota diversity, reflected in higher species richness compared to exclusively breastfed individuals. Exclusive breastfeeding is associated with elevated levels of Bifidobacterium and reduced abundances of Bacteroidetes and Clostridiales. The commencement of

complementary feeding correlates with a decline in saccharolytic bacteria such as Bifidobacteria and a rise in Lachnospiraceae, consistent with higher protein consumption. Fiber intake is positively linked to Prevotellaceae abundances, a bacterial family regarded as beneficial for long-term health. Introducing various solid foods may enhance early microbiome diversity and stability. The proliferation of probiotic use among infants aims to support digestive health, with studies demonstrating that these supplements can significantly impact gut microbiota composition, contingent upon the infant's diet [1, 2].

Microbiome and Neurodevelopment

The intestinal microbial ecosystem influences homeostasis, and its perturbation has been associated with several human diseases. The initial colonization of the human intestinal tract begins at delivery, and microbiota continues to develop and increase in diversity and richness until 12–36 months of age. This "first 1000 days" window has been postulated as a critical developmental window that influences health, including neurodevelopment, throughout the lifespan. The microbiota and brain development proceed in parallel during early postnatal life, suggesting important microbe–immune interactions during this period. Changes along the microbiome–gut–brain axis have been implicated in neurodevelopmental disorders, and microbial metabolites have been demonstrated to have neuromodulatory properties. Understanding the role of the microbiota in brain development provides an important opportunity to identify novel diagnostic and preventive strategies for neurodevelopmental disorders and associated comorbidities [13]. Within the first 1,000 days of life, from conception to a child's second birthday, the microbiota transitions from rudimentary colonizers to a complex adult arrangement that supports human health throughout the life course. Perturbation of early microbiome development influences developmental trajectories, health outcomes, and disease risk [14]. Investigations of host–microbiota dynamics at the maternal–neonatal interface point to alterations in bacteria found in maternal gut, vaginal, oral, and placental microbiomes with pregnancy. In this critical period for early-life microbiome development, infants are predominantly dominated by Bifidobacterium and Lactobacillus and lack the obligate anaerobes that characterize stool in adults; maturation toward constituent adult taxa occurs after weaning but before 36 months of age. Various maternal and infant factors (that is, maternal age, maternal health, diet, delivery mode, antibiotic usage, breastfeeding status, and timing of introduction to solid food) modulate this transition, and dysbiosis or non-resilience in the infant gut is associated with immune dysfunction (for example, allergy), infection susceptibility, and neurological outcomes. Supplementation with targeted probiotics can correct dysbiosis related to aberrant early-life microbiota maturation and reduce the risk of morbidities associated with preterm birth [13, 14].

Gut-Brain Axis

A complex communication network, termed the gut-brain axis, facilitates bidirectional transfer of signals between the gut and the brain. The vagus nerve is a key neural element of the gut-brain axis, interacting extensively with enteric neurons, while microbial molecules also engage the gut-brain axis via cellular and molecular pathways. Crucial for the development, function, and maintenance of the central nervous system, the gut-brain axis influences cognition and mood [15]. The brain and gut also interact through endocrine, metabolic, and immune mediators, with gut-transported nutrients affecting brain development [15]. This web of communication is often described as the microbiota gut-brain axis, a complex network that both influences and predicts brain health; in germ-free mice, the absence of microbiota is associated with abnormal brain development and function. Experiences early in life are particularly salient for human brain development, with infancy representing a period when the brain is exquisitely sensitive to environmental stimuli and insults [15].

Impact on Cognitive Development

The microbiome comprises a dynamic collection of microorganisms, bacteria, fungi, viruses, and their diverse gene expressions, colonizing humans from birth and continuing to evolve throughout life. As the neonatal immune system lacks prior antigenic exposure, early, non-pathogenic microbial colonization is crucial for immune maturation in infants [2]. The infant's initial microbial composition is influenced by delivery mode, gestational age, maternal microbiota, diet, antibiotic use, and genetics. Although the microbiome's establishment has been well-characterized, its impact on neurodevelopment remains largely unknown. However, accumulating evidence suggests that gut microbiota and their metabolites regulate various aspects of neurodevelopment and cognitive functioning, with implications for lifelong developmental trajectories [15]. Bi-directional communication between the gut and brain involves microbiota, forming the microbiota-gut-brain axis (MGBA). The MGBA plays a key role in infancy, a critical period for neurodevelopment, especially for preterm infants who face increased risks of dysbiosis and impaired neurological outcomes. Establishing the critical windows during which early-life microbiota colonization affects neurodevelopment represents a vital research avenue, with the prospect of enabling microbiota-based interventions to preserve neurocognitive development [2, 15].

Microbiome and Behavioral Outcomes

Behavioral health during early childhood has already begun to alter the diagnostic criteria of neurodevelopmental disorders. When persistent across an individual's lifetime, behavioral dysfunction can become incapacitating, with symptoms showing throughout development, from childhood to early adulthood [13]. A maternal nutrient-deprived animal model demonstrated that prenatal stress not only altered the microbiota of offspring across their lifespan but also disrupted their stress behavior. Though most research regarding the microbiome and neurodevelopment focuses on broadly cognitive functions, autism spectrum disorders are another manifestation of developmental health affected by perturbations in the early microbial environment [16]. Exploring these possible associations could better elucidate a pivotal relationship within the context of early immune health and nutrient status [2]. Gut microbiota influences neurodevelopmental pathways and the development and persistence of childhood disorders of emotional and behavioral regulation, including anxiety. Since the early childhood microbiome is malleable and highly susceptible to internal and external exposures, it represents an opportune period to intervene and promote optimal neurodevelopmental outcomes. Perturbations of the early microbial environment are a proposed contributing factor to the increasing prevalence of early-onset autism spectrum disorders worldwide [2, 13, 16].

Behavioral Health in Early Childhood

Childhood behavior can be roughly arranged into three main groups: social behavior, problem behavior, and comorbid behavior [17]. Studying early biomarkers and symptomatology is of crucial importance because it could help to understand the underlying etiology of behavioral problems in preschool children. Premature infants are especially vulnerable to microbiome disturbances, and the resulting perturbations in the early developmental trajectory can have repercussions throughout childhood and beyond. Gut microbiota composition showed significant relationships with DSM-based behavioral scales from the Child Behavior Checklist (CBCL): specific amplicon sequence variants analyses were significantly associated with the adjusted CBCL scores, some with positive and some with negative associations. Enterococcus and Veillonella dispar showed significant associations with the CBCL adjusted scales of depression and anxiety; the anxiety CBCL scale was also significantly associated with the presence of Escherichia coli and Ruminococcus. These associations support the relevance of the gut microbiome to mood disorders and mood-related behavioral mechanisms [18]. Similar to autism spectrum disorder (ASD), the relationship between the microbiome and neurobehavioral outcomes such as attention-deficit hyperactivity disorder (ADHD), depression, and anxiety was studied after noting that gastrointestinal symptoms, including food sensitivities, were often comorbid with these outcomes [13]. The early-life gut microbiome is a driver of stress responses across the lifespan: germ-free animal models exhibit altered anxiety and depression-like behaviors, and transplantation of stool from donors with depression into germ-free mice induces depressive behavior [18]. Disturbances in gut metabolism were noted in models of depression. Although animal models provide key mechanistic insights, they are limited in replicating human phenotypes. Cross-sectional studies identified associations between the gut microbiome and anxiety, ADHD, and depression, but the single-timepoint sampling makes causation difficult to establish. Early life microbiome development may be especially important for anxiety and depression: recent longitudinal studies examined microbiome relationships at three early life stages and behavioral symptoms at age three. These findings highlight the potential for early intervention based on microbiome composition in infancy [13].

Links to Autism Spectrum Disorders

Autism spectrum disorders (ASD) encompass a broad range of pediatric developmental conditions with symptoms typically manifesting between the ages of 12 and 24 months. ASD diagnosis is based on core impairments in social communication and behavioral flexibility, resulting in a variety of presentations depending on the relative contribution of these domains [13]. The causes of ASD are unknown, and despite considerable attention, little progress has been made in discovering biomarkers or effective treatment options. Gastrointestinal symptoms commonly accompany ASD, often correlating with behavioral severity and suggesting a potential role for microbiome alterations as either a cause or consequence of the observed neurodevelopmental features [13].

Microbiome Disruptions and Health Implications

Multiple studies demonstrate that antimicrobial exposure during pregnancy lowers microbiota diversity in placental and infant fecal samples. Cesarean birth, either planned or emergency, reduces infant microbiota richness and diversity, affecting health precursors at later stages [2]. Research reveals a connection between early microbiota disruptions and the risk of childhood-onset chronic conditions such as asthma, eczema, and type I diabetes. Antibiotic use during early infancy elevates the risk of developing atopic eczema, higher body mass index (BMI), and juvenile idiopathic arthritis in childhood [2]. These health implications underscore the importance of evaluating clinical practices involving antimicrobial agents and the mode of delivery at birth; further studies are essential to inform public health policies regarding pregnancy and early-life interventions [2].

Antibiotic Use in Pregnancy

Maternal use of antibiotics can alter the maternal microbiome and the initial inoculum to the neonate during delivery. Another important early-life event influencing the infant microbiome is the mode in which the infant is delivery. Cesarean-section delivery bypasses exposure to vaginal microbiota and complicates the transfer of beneficial microbes to the infants. Numerous studies have highlighted the effects of intrapartum antibiotics and different modes of delivery on the infant gut microbial community [19]. Thus, antibiotic use and delivery mode have a strong influence on the initial microbial community that is capable of labeling the immune system and contributing to health later in life. The effect of prenatal antibiotic exposure on infant microbiome development has been examined together with that of delivery mode on the diversity and composition of the infant meconium microbiome. Gestational antibiotics have been linked to lower diversity of the meconium microbiome. Moreover, meconium microbiota is influenced by both prenatal antibiotic use and delivery mode. High levels of Enterococcus, Streptococcus, and Clostridium were identified in meconium samples exposed to prenatal antibiotics, while delivery mode showed a strong dependence on the relative abundance of Escherichia/Shigella and Streptococcus [20]. These findings are consistent with peripartum antibiotic exposure influencing the infant microbiome, occasionally modification of the signature of cesarean-section-associated dysbiosis. Together with delivery mode, peripartum and postpartum antibiotic exposure can strongly influence the formation of the newborn meconium microenvironment, with further implications for microbial community progression over time [19, 20].

Impact of C-section Delivery

Cesarean section is a surgical procedure conducted when vaginal delivery presents risks for the neonate or mother. The perioperative environment, absence of labour-related stress hormones, and delayed skin-to-skin contact implicate neonates in alterations of microbiome colonization. Delivery mode strongly impacts neonatal gut microbiota establishment [22]. Vaginal delivery confers a microbial assemblage resembling the maternal vagina, and caesarean section results in a microbial profile comparable to maternal oral, skin, and hospital environments [21]. Microbiota differences between delivery modes at 4 days of age persist for at least 21 days. At 2 months, caesarean section neonatal microbiomes more commonly contain Clostridioides difficile, with increased Enterobacteriaceae abundance also observed immediately after birth. Diversification and convergence of microbiota assemblages occur within 4–6 months of delivery. Bifidobacterium and Bacteroides species are depleted in the neonatal microbiome after caesarean section, with opportunistic pathogens such as Klebsiella, Enterococcus, and Enterobacter, more frequently detected [22]. The absence of exposure to vaginal microbes in prelabor scheduled caesarean section produces greater effects than postlabor caesarean section, apparently after exposure to the birth canal. Although Bacteroides increases transiently during the first week of life, it is unstable and undetectable at 2 weeks irrespective of delivery type, suggesting impaired rectal colonization of this species in caesarean section infants broadly [22]. The oral cavity exhibits lower microbial diversity in caesarean section at 6 weeks, with reduced similarity to maternal oral microbiota also observed throughout this time frame [22].To mitigate c-section-associated alterations in sequestration of maternal microbes, direct microbial supplementation may constitute a therapeutic strategy. Offspring of antibiotic-treated dams show disrupted microbial colonization and associated behavioural effects, but treatment with the probiotic Lactobacillus rhamnosus during the perinatal period prevents anxiety-like behaviours in adulthood [21, 22].

Research Methodologies in Microbiome Studies

Sampling methods commonly involve collecting fecal specimens to elucidate gastrointestinal microbiota composition [2]. Data analysis incorporates techniques such as latent class analysis to identify factors shaping the infant microbiome [23].

Sampling Techniques

The human microbiome describes the ecological community of commensal, symbiotic, and pathogenic microorganisms that share a body space [24]. The microbiome contributes numerous physiological functions that are not encoded by the human genome but are essential to human development, health, and disease. For example, the human digestive tract provides a nutrient-rich, protective environment inhabited by a diverse microbial community that ferments undigested polysaccharides, resulting in larger pools of short-chain fatty acids, which not only serve as important energy sources but also physiologically influence gut health and metabolism. In pregnancy and early childhood, the microbiome is connected to a significant number of immunological, developmental, and neurodegenerative disorders [25]. These in turn could affect the physiology, metabolism, immunity, and even neurodevelopment and behaviour of pregnant women and children. Cancer, diabetes, arthritis, and inflammatory bowel disorder have been identified as only some examples of microbiome-associated diseases [24, 25].

Data Analysis Approaches

Effective analysis of human microbiome data requires specialized tools and approaches [24]. Datasets vary widely in size, sequencing depth, and taxon counts, and often exhibit zero inflation due to true biological absences or insufficient sampling. Bacterial interactions and metabolic dependencies induce correlations between species or taxa that vary across subjects and conditions. Standard statistical methods tend to violate the underlying assumptions of microbiome studies. Appropriate statistical approaches must account for data characteristics by employing nonparametric tests, modelling zero inflation, handling over-dispersion, and adjusting for compositionality [24]. The earliest method for microbial identification was Sanger sequencing, which used chain termination to determine the DNA base order. Sanger sequencing requires a single isolate, but many gut microbes cannot be cultured. The 16S rRNA gene overcame this limitation because (1) it contains variable regions whose nucleotide content varies across species and (2) universal PCR primers were available to amplify the gene. Many different species can be isolated by amplifying the 16S gene in a mixed sample and grouping DNA strands based on similarity. 16S sequencing has known shortcomings [24]. For example, it cannot easily discriminate between some species or strains, and sequencing the gene does not provide direct insights into the microbial functional capacity. Targeted metagenomics uses specific marker genes, such as 16S and 18S, to classify the microbiota and address some issues of 16S sequencing. Most methods describe the microbial communities based on 16S sequences without reconstructing the genes or genomes of the different microorganisms present. In metagenomics, the complete pool of genes of the microbiota is sequenced without using any marker gene; therefore, the sequences are from all these genes in the pool. The sequences are then compared to known genes to predict the genes present in a sample. Shotgun metagenomics generates a vast dataset that is highly heterogeneous depending on the sample DNA composition; taxa present at low abundance might not be sequenced, and homologous regions of different taxa can introduce ambiguity during taxonomic assignment [23, 24].

Future Directions in Microbiome Research

Numerous promising therapeutic approaches for microbiome modification are under intensive investigation: through antimicrobials, probiotics, prebiotics, fecal transplantation, and dietary changes [26]. Along with these strategies, longitudinal studies of pregnant women and infants will permit the collection of dynamic microbiomic datasets, allowing the application of longitudinal data analysis techniques [2].

Potential Therapeutic Interventions

Therapeutic interventions targeted at the microbiome during pregnancy and early childhood hold considerable promise. Antibiotics and other medical treatments with known effects on microbiome composition should be considered carefully during pregnancy due to the potential consequences for both maternal health and fetal development [25]. Probiotic and prebiotic interventions represent another avenue worthy of investigation in the context of clinical outcomes and interventions. Nutritional status of both mother and child has a profound impact on microbiome composition with implications for subsequent development, as diet is one of the most important modifiers of microbiome composition. An educational dietary intervention might help mothers increase their intake of fibre, prebiotic, and probiotic foods so as to positively influence gut microbiota diversity 27. Extensive surveys of population characteristics could shed light on how deposition of microbiota in the infant gut feeds into developmental modelling at later stages [1].

Longitudinal Studies

Longitudinal designs provide robust evidence for significantly altering the trajectories of mental health after natural disasters. Longitudinal epidemiological studies contribute important insights into the pathways to poor mental health in the disaster context. Although risk factors and mechanisms of symptoms such as PTSD overlap with symptoms linked to other mental health disorders associated with disaster exposure, such as depression and grief, these additional pathways require further study [28]. Longitudinal designs are also central to early child development and the circumstances under which children thrive or fail to thrive. Longitudinal researchers accumulate data on the same group of children repeatedly to chart individual patterns of change, reveal widespread patterns and variations, and identify critical elements linked to particular outcomes. When such studies commence early in life, they uncover subtle effects of experience that evade detection at later ages, illustrating the emergence of adaptive developmental pathways [29]. A major goal is to determine the predictive value of early postnatal experiences by linking them to emotional, cognitive, social, and physical outcomes 24. Longitudinal designs addressing the bidirectional flow of influence between parent and child capture the transactional processes characterizing human development [28, 29].

Ethical Considerations in Microbiome Research

Two major points of concern within microbiome studies involving pregnancy and infant development are informed consent and data privacy. Informed consent encompasses the information provided to a research participant before the individual agrees to take part [2]. In the context of microbiome research spanning maternal-infant pairs, the

matter becomes more complicated. What parents decide for themselves can negatively impact the infant, who did not provide consent to enter the study. Finding a balance between the ethical principle of nonmaleficence (not harm) and the rigour necessary for research becomes a necessity [2]. Data privacy becomes another important consideration surrounding microbiome work in this area. Taking into account that microbiome research has been intertwined with genetic and epidemiological studies in the past, it is important to implement data protection methods when handling this type of information [2, 1]. Every precaution should be taken to ensure that the study participants feel comfortable with home visits carried out by the study staff, and that their home environment and biological samples obtained are treated with the confidentiality required.

Informed Consent

Because the microbiome is natural, evolving, and impossible to perfectly analyse, we lack a true standard, especially at the beginning of studies, when investigators are still resolving how prevalence scales with abundance, what variability is normal, what changes, and which time periods are critical [30]. The usual solution to these problems arises at experiment design: amplify the signal of interest, reduce non-biological variation; then measure all sources of real biological variability, classify them according to experiment design, and finally isolate the residual design-dependent perturbations and interpret them as biologically meaningful changes. Local systems do not consistently provide useful signals when analysed in isolation. Limited sequencing depth inevitably estimates prevalence by looking at a single ecological niche, whereas true prevalence includes all sources. Thus, the observed local variation in prevalence across cohorts is unbiological: study-specific sequencing depth differences together with the changing relative contribution of other sites to the observed ecological niche determine the measured local prevalence [30]. Microbiome experimental and bioinformatics details are complemented by a survey that is appropriate for several design types [29]. The information is contextualized by relating a community ecology model linking ecology and evolution in a way consistent with artificial life studies, the observed power laws for stability and variability, Elton's suggestion of a unified science of ecology and evolution, and the utmost importance of open, interoperable data to the reusability of metagenomic material. Unreported occupancy is identified as an important component of missing species and genes [28].

Data Privacy Issues

During data collection, unique to South Australia, participants are offered the choice of donating samples to a biobank repository for future studies. The associated ethics approval permits the storage of samples for up to 30 years, although efforts are made to re-consent participants after 5 years [28]. Moreover, samples may be shared with third parties, exclusively for research purposes. Upholding privacy and confidentiality remains a paramount concern, and both written and electronic information is managed in alignment with the National Statement on Ethical Conduct in Human Research and Australian data protection law [28].

Public Health Implications

Pregnancy is a phase of permanent physiological adaptation aiming to ensure the homeostatic balance of maternal–foetal well-being and gestational success [2]. During pregnancy, several clinical and subclinical else described disorders are strongly related to profound alteration of the maternal microbiota, with possible repercussions at the placental level [1, 3]. Therefore, the identification of a placental microbial community able to govern the danger signals which circulate at the foetal–maternal interface offers a powerful tool to develop efficient probiotics for the treatment of adverse pregnancy outcomes potentially related to an unbalanced maternal microbial consortium. An integrative approach combining microbiology, immunology, and obstetrics toward a better knowledge of the placental microbiota composition and function, as well as the cellular and molecular strategy adopted for its maintenance, may thus have a practicable clinical impact and enable the development of novel therapeutic tools useful for the management of adverse pregnancy outcomes. Pregnancy outcomes and their association with microbiota remain a crucial public-health topic. Apparently robust individuals collectively live in a metastable balance that preserves the equanimity of the microbiome throughout pregnancy. During this period of immune tolerance, public health guidelines should limit antibiotic use to cases of necessity, considering their negative effect on the microbiota and also their lack of demonstrable benefit on preterm delivery in well-informed populations. A targeted modulation of the microbiome at different body sites, but especially in the vagina, may represent a promising therapeutic approach for the prevention of adverse pregnancy outcomes [1]. Guidelines on infant feeding, delivery mode, and possible times of antibiotic exposure should include in their analysis the long-term impact that an altered microbiota might have on our global health [4].

Guidelines for Pregnant Women

Pregnant women can help optimize their infants' microbiome assembly, with potential lifelong benefits, by maintaining a healthy microbiome during pregnancy and the first years of life. Additional research and trials will increase the options available for interventions. Increased awareness of the importance of microbiome factors may help reduce the incidence of allergies, obesity, and behavioral problems [2]. Various organizations have addressed

public and research concerns [2]. Although the American College of Obstetricians and Gynecologists endorses the use of probiotics, it does not recommend them universally because of limited evidence and the potential for risks, according to the Dosage and Administration section of the guidance. Lactobacilli are generally considered safe during pregnancy and might prevent pregnancy complications [2]. They are also under evaluation for potential to reduce morbidity in pregnancy-related bacterial infections and may protect offspring from inflammatory diseases. The lack of safety data concerning live probiotic administration during pregnancy suggests that industry studies should include pregnant women among other at-risk groups, expanding the population currently considered in clinical trials. Extensive randomized clinical trials are also necessary because many observed microbiome changes and related physiological adaptations remain elusive [2].

Policy Recommendations

Maternal health is paramount for a successful pregnancy as well as a key foundation for the long-term health of the offspring. The microbiome, a cornerstone of health, is a biological system with a determinant role in sustaining human and planetary health. Guidelines and protocols should be established and implemented to improve maternal health and newborn outcomes through a better understanding of the microbiome. Key recommendations rely on the significance of the intestinal microbiota in preterm neonates and during prenatal and postnatal life [31-36].

CONCLUSION

The maternal and infant microbiomes are central to healthy pregnancy and early life development. During gestation, maternal microbial shifts in the gut, vagina, and oral cavity support immune tolerance and fetal growth, while dysbiosis contributes to complications such as preterm birth and growth restriction. After delivery, colonization of the infant microbiome through birth mode, breastfeeding, and environmental exposures establishes the foundation for immune education, metabolic regulation, and neurodevelopment. Perturbations in early-life microbial assembly, driven by antibiotics, cesarean section, or poor maternal nutrition, increase the risk of chronic conditions including allergies, asthma, obesity, and neurodevelopmental disorders. The gut–brain axis represents a critical pathway linking microbial composition to cognitive and behavioral outcomes in early childhood. Advances in sequencing, metagenomics, and systems biology now allow deeper exploration of maternal–infant microbial networks, although methodological and analytical challenges remain. Looking forward, microbiome-targeted interventions such as probiotics, prebiotics, fecal microbiota transplantation, and precision nutrition hold promise for improving maternal and child health. Longitudinal studies and carefully designed clinical trials will be essential to validate these strategies and guide their integration into clinical practice. Harnessing the microbiome's potential during pregnancy and early childhood offers a transformative opportunity to optimize health trajectories across the lifespan.

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