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Microbial transmission, colonisation and succession: from pregnancy to infancy

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ABSTRACT
The microbiome has been proven to be associated with many diseases and has been used as a biomarker and target in disease prevention and intervention. Currently, the vital role of the microbiome in pregnant women and newborns is increasingly emphasised. In this review, we discuss the interplay of the microbiome and the corresponding immune mechanism between mothers and their offspring during the perinatal period. We aim to present a comprehensive picture of microbial transmission and potential immune imprinting before and after delivery. In addition, we discuss the possibility of in utero microbial colonisation during pregnancy, which has been highly debated in recent studies, and highlight the importance of the microbiome in infant development during the first 3 years of life. This holistic view of the role of the microbial interplay between mothers and infants will refine our current understanding of pregnancy complications as well as diseases in early life and will greatly facilitate the microbiome-based prenatal diagnosis and treatment of mother-infant-related diseases.

Key messages

⇒ Microbial interaction between mothers and their offspring occurs frequently during the perinatal period and greatly influences the development of newborns.
⇒ Many determinants, including genetic and environmental factors, exert impacts on microbial transmission and colonisation during gestation, delivery and lactation.
⇒ Women experience great changes in their microbiome from the beginning of pregnancy, and dysbiosis of the maternal microbiome may exert detrimental influences on offspring.
⇒ The prenatal microbiome is highly debated, while increasing evidence suggests its presence and implies its putative effect on the fetal immune system.
⇒ Microbial colonisation and development in early life is very important, and microbes transmitted from mothers help in the normal succession of the microbiome and promote the maturation of the neonatal immune system.

INTRODUCTION

The microbiome is known to be essential to the development of human life and is not only greatly involved in various indispensable physiological activities, such as metabolic processes and immune responses, but also closely associated with the occurrence of multiple diseases.[1–4] Microbiomes across body sites evolve from a very young age, and some of them originate from mothers through vertical transmission during the perinatal period.[5–7] Bacteria transferred from mothers help to shape the initial microbial community of neonates and play a vital role in development in later life.[8–10] Mother–infant microbial transmission takes place from the beginning of pregnancy to a very long time after delivery, has various patterns, durations and locations on the body (figure 1A–E) and is affected by multiple intrinsic and extrinsic factors.[5 9 10] For example, it is now known that not only maternal conditions during pregnancy but also many prepregnancy and postpregnancy factors influence the postnatal development of infants via microbial transmission. These factors include but are not limited to genetics, residential environments, daily diets, lifestyles and other postnatal factors, such as the mode of delivery and feeding patterns (figure 1F–H).[11–16]

In this review, we summarise recent advances in the study of the prenatal and postnatal transmission of the microbiome from mothers to offspring and describe the influence of maternal microbiome alterations on neonates before and during pregnancy. Furthermore, we discuss the possibility of the existence of the prenatal microbiome in utero and summarise recent evidence supporting or opposing the presence of an intrauterine microbiome during pregnancy. Finally, we illustrate the impact of the maternal microbiome and different postpartum factors on the development of the infant microbiome in the early stages.

CHARACTERISTICS OF THE FEMALE MICROBIOME BEFORE PREGNANCY

Although much attention on the female microbiome has been focused on the perinatal period, the prepregnancy microbiome, which may play a vital role in fecundability and pregnancy outcomes, cannot be ignored.[17] The microbiome composition of healthy nonpregnant women varies depending on genetics, ethnicity, age, lifestyle and daily diet.[18–24] In general, microbial diversity decreases with increasing industrialisation.[18 25] With the habitual use of antibiotics and drugs, a high intake of ultraprocessed and high-fat foods, and a sedentary lifestyle, compared with traditional populations, industrialised populations harbour a gut microbiome characterised by a high abundance of *Bacteroides*, *Ruminococcus* and *Blautia* and a low abundance of *Prevotella*, which are associated with a higher risk
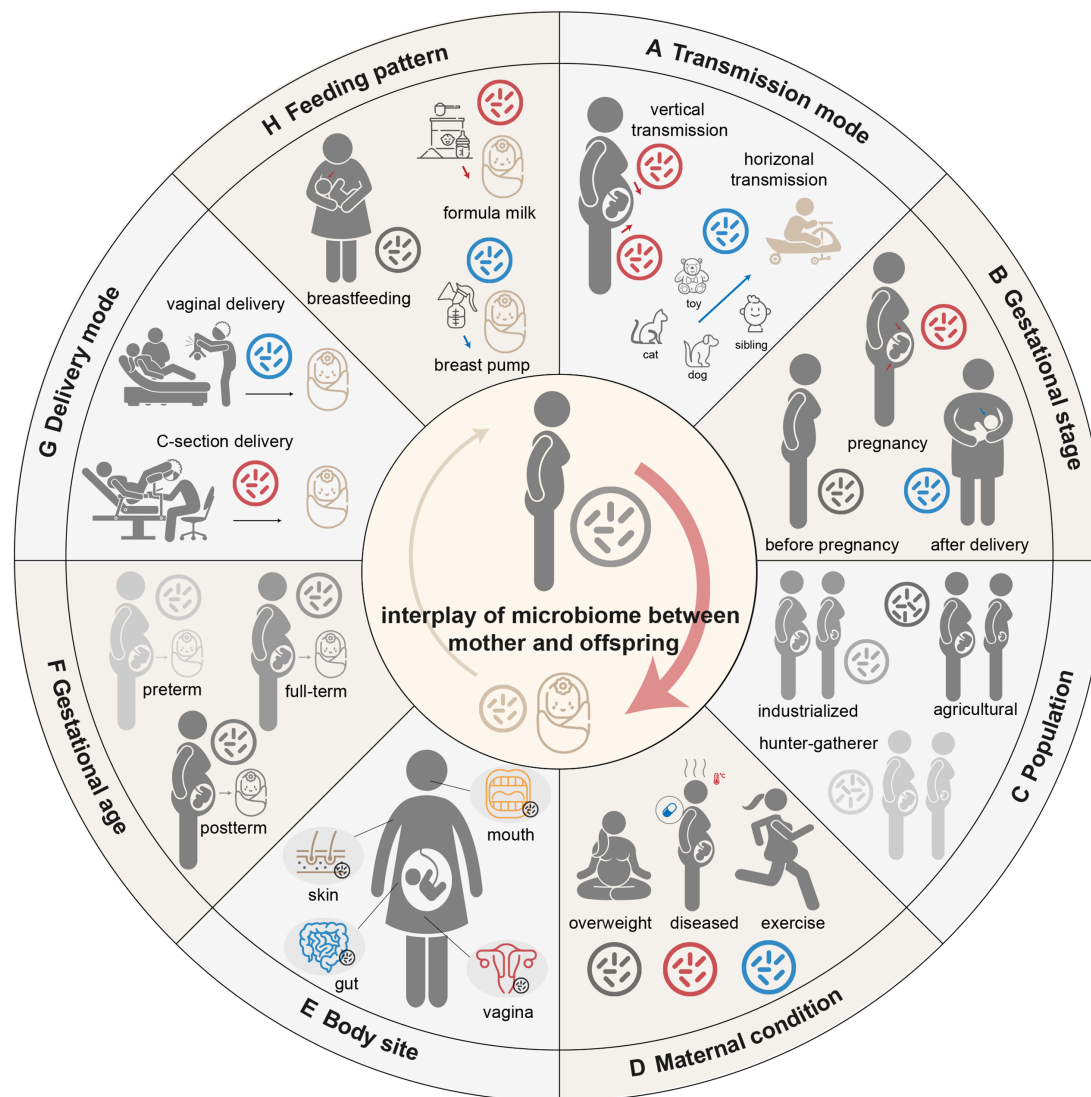
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Figure 1 Interplay of the microbiome between mothers and offspring. Intergenerational transmission of the microbiome in different (A) transmission modes, (B) gestational stages, (C) populations, (D) maternal conditions, (E) body sites, (F) gestational ages, (G) delivery modes and (H) feeding patterns.

of obesity and type 2 diabetes (T2D).[23 26–31] In addition to the gut microbiome, oral and vaginal microbial compositions vary among different regions. Europeans show a higher abundance of Firmicutes and a lower abundance of Proteobacteria in the oral cavity than Africans.[22 32 33] Similarly, White women tend to harbour a vaginal microbiome dominated by *Lactobacillus*, while Black women exhibit a higher microbial diversity, with a microbiome dominated by several non-*Lactobacillus* species, such as *Gardnerella vaginalis* and *Atopobium*.[24 34–39]

It is of great significance to focus on healthy states before conception. Multiple studies have associated prepregnancy BMI with the development of fetuses and infants, indicating that the influences of the prepregnancy microbiome may last into pregnancy.[40–42] Although several studies have emphasised that differences exist between distinct delivery modes,[43 44] there is no escaping the fact that expectant mothers with overweight and obesity (OWOB) have a greater chance of giving birth to obese offspring via transmission of specific bacteria and metabolites.[42 45] Overall, bacteria in the Firmicutes phylum, such as *Ruminococcus*, *Blautia* and *Eubacterium,* are most affected by prepregnancy weight, showing a significantly lower relative abundance in the stools of infants of mothers with OWOB than in the stools of neonates with normal-weight mothers, while *Oscillibacter* and Clostridiales showed an increasing trend.[15 42 45] Similarly, other bacteria, such as *Bifidobacterium, Faecalibacterium* and *Parabacteroides,* have been shown to exhibit differences in the guts of offspring of mothers with OWOB and offspring of normal-weight mothers, leading to abnormal metabolism and an increased risk of disease.[15 42 45] Nevertheless, additional evidence is still required to demonstrate causality between maternal health and the female microbiome before conception.

MICROBIAL TRANSMISSION DURING PREGNANCY
Variation in the microbiome during pregnancy
During pregnancy, the microbiome across maternal body sites is altered tremendously. Significant microbial changes have been observed in the oral cavity, gut, and vagina during pregnancy (figure 2A).[46–48] The richness and diversity of the microbiome in the oral cavity increase during early pregnancy, among which *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* are the most significant species that exhibit higher
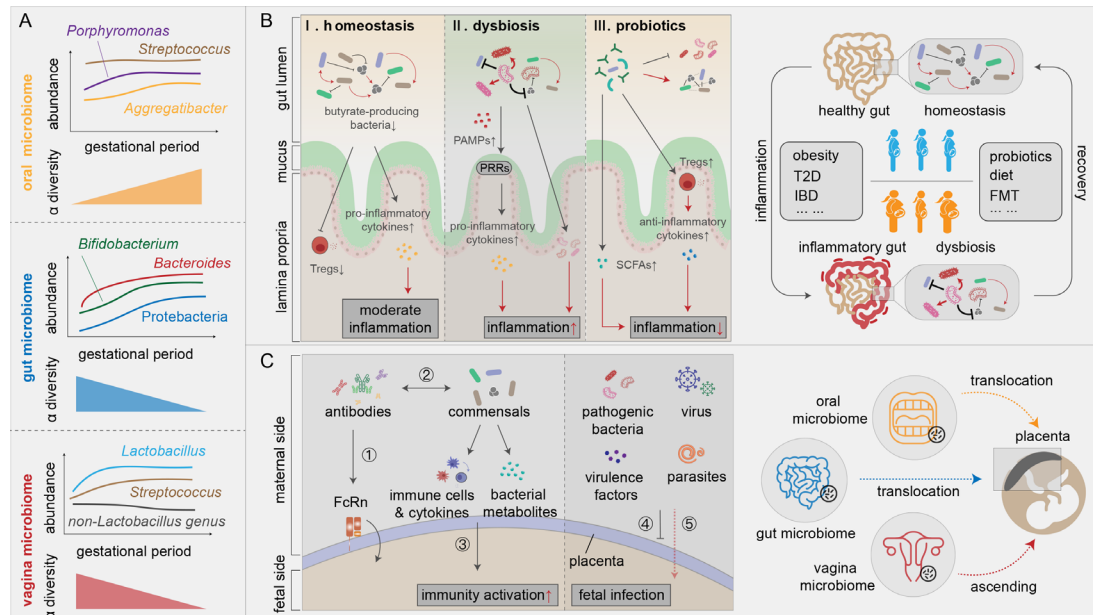
Figure 2 Microbial variation and transmission during pregnancy. (A) Variation in the abundance of representative bacteria in the maternal oral cavity, gut and vagina during pregnancy. (B). Host–microbe interaction in the maternal gut. Under healthy conditions, the gut microbiome and immune response of pregnant women is similar to that in individuals with metabolic syndromes, characterised by decreasing butyrate-producing bacteria and increasing proinflammatory cytokines, inducing moderate inflammation (I). When mothers have conditions such as T2D, obesity or IBD, deviation of the gut microbiome associated with alteration of the immune response is observed during pregnancy, increasing the risk of inflammatory diseases and gut leakage. Increased intestinal permeability allows the entry of bacterial toxins into the systemic circulation and induces multiple diseases (II and the left panel). Some interventions, such as probiotic, anti-inflammatory diet or FMT interventions, may restore dysbiosis of the gut microbiome and reduce inflammatory responses (III and the left panel). (C) Maternal–fetal interface communication during pregnancy. Maternal antibodies such as IgG are transferred to the fetus via FcRn (case 1). Some bacterial molecules are bound to maternal IgG and are transferred to the offspring (case 2). Such vertical transmission provides protection to the fetus. Fetal immune responses can also be activated by bacterial components or bacterial metabolites from mothers (case 3). Other substances, such as viruses, pathogenic bacteria, virulence factors and parasites that are harmful to the fetus are usually unable to cross the placenta (case 4, black lines with flat ends), except in situations of maternal infections (case 5, red dotted arrows). Translocation of the microbiome between maternal body sites (oral cavity, gut and vagina) and the fetus was observed. Whether the detection of microbes in the placenta is derived from contamination is highly debated, and thus, dotted lines are used for microbial translocation (the left panel). FcRn, neonatal Fc receptor; IBD, inflammatory bowel disease; PAMPs, pathogen-associated molecular patterns; PRRs, pattern reorganisation receptors; SCFAs, short-chain fatty acids; Tregs, regulatory T cells; T2D, type 2 diabetes.

abundance during pregnancy.[49] In addition, some species of *Candida* thrive during middle and late pregnancy.[49 50]

Distinct from the variation in the oral microbiome, the microbial diversity in the gut and vagina significantly decrease during pregnancy (figure 2A).[46–48] In women at the beginning of pregnancy, the gut microbiome pattern is similar to that of healthy nonpregnant women, characterised by a high abundance of Bacteroidetes and Firmicutes, such as Clostridiales.[51] From the first to the third trimester, profound alterations are observed in the guts of pregnant women, where butyrate-producing bacteria, such as *Faecalibacterium*, exhibit a significant decrease, while Proteobacteria and some lactic acid-producing bacteria, such as Bifidobacteria, are highly increased.[51 52] Some studies revealed the expansion of members of Enterobacteriaceae and *Streptococcus* in the third trimester,[47 51] which were also early colonisers in the infant gut, indicating potential transmission from the maternal gut to the neonatal gut. Changes in the microbial composition, together with immune and metabolic variation, often induce weight gain in normal pregnant women. In one study, the first-trimester and third-trimester faecal microbiome was transplanted to germ-free mice, and greater weight gain and insulin resistance were observed in the mice that received the third-trimester microbiome compared with those that received the first-trimester microbiome.[51] These findings indicate that the gut microbiome makes a great contribution to healthy pregnancy

and that changes in the gut microbiome potentially lead to changes in host immunology and metabolism.

The between-individual diversity (beta diversity) of the gut microbiome greatly increases during pregnancy.[47 48] In contrast, the vaginal microbial composition of pregnant women exhibits considerable convergence across different populations (figure 2A).[53 54] During pregnancy, *Lactobacillus* becomes the only predominant bacteria in the vagina in most women, leading to a dramatic decrease in alpha diversity (figure 2A).[55 56] Numerous studies have demonstrated that a core microbial pattern exists in the vaginas of healthy reproductive-age women, characterised by the dominance of *Lactobacillus* species, termed community state types. The dominance of *Lactobacillus* spp is very important to the maintenance of a healthy vaginal microenvironment, where the production of lactic acid lowers the vaginal pH and inhibits the growth of other harmful bacteria.[57–60] In contrast, a lower proportion of *Lactobacillus* is frequently associated with adverse pregnancy outcomes, such as miscarriage and preterm birth.[35 61 62]

Deviation of the maternal gut microbiome and interventions

It was demonstrated that the alteration of the gut microbiome and immune responses in late pregnancy resembled that in metabolic syndromes, such as obesity or diabetes, characterised by

low microbial diversity, reduced levels of butyrate-producing bacteria, decreased insulin sensitivity and moderate intestinal inflammation (figure 2B).[63] [64] Short-chain fatty acids (SCFAs, eg, acetate, propionate and butyrate) are often considered to alleviate low-grade inflammation,[65] and the loss of butyrate-producing bacteria is associated with increased production of proinflammatory cytokines and decreased generation of regulatory T cells (Tregs) (figure 2B).[66] Nevertheless, the causal relationship between the gut microbiome and immune responses in pregnant women and the underlying mechanisms need further exploration.[67]

Dysbiosis of the gut microbiome refers to the loss of beneficial microbes and the enrichment of pathobionts, which are often associated with unhealthy lifestyles and may disturb the ecological balance in the intestines, leading to undesirable consequences (figure 2B).[68] For example, many studies have emphasised associations between microbial dysbiosis and poor habits, such as smoking, drinking alcohol, consuming a high-fat diet (HFD) and drug abuse.[14] [69–76] Among these habits, consuming an HFD, which is a diet consisting of at least 35% of the total calories consumed from fats, including but not limited to animal fat, chocolate and butter,[77] is one of the most common factors that disrupts the normal gut microbiome.[71] Pregnant women who are accustomed to an HFD often suffer from obesity or gestational diabetes mellitus (GDM), exhibiting lower microbial diversity and persistent perturbation of *Staphylococcus*, *Bacteroides*, *Bifidobacterium* and *Lactobacillus*, as well as considerable variations in insulin secretion and insulin sensitivity.[78] Nonetheless, several studies suggest that various outcomes may occur depending on fat types and compositions. Specifically, fish and unsaturated fatty acids such as vegetable oils and olive oils are suggested to have protective effects, whereas it is recommended that trans fatty acids and saturated fatty acids be consumed as little as possible.[79] [80]

Deviation of the gut microbiome may disrupt the ecological balance in the intestines, leading to an increase in pathogen-associated molecular patterns, which can be recognised by pattern recognition receptors and activate the immune response through downstream signalling pathways (figure 2B).[81–84] In addition, an abnormal gut microbiome is frequently associated with a higher risk of intestinal permeability,[85] which impairs the gut barrier and allows the leakage of pathogens from the gut lumen into the lamina propria, increasing inflammatory responses (figure 2B).[1] [86–88] Persistent inflammation leads to the disruption of immune homeostasis and is associated with many autoimmune disorders and chronic metabolic diseases, such as GDM, T2D, non-alcoholic fatty liver disease, chronic kidney disease and atherosclerosis.[86] Deviation of the gut microbiome not only has a detrimental effect on mothers but also influences offspring.[72] [78] [89] [90] In an intergenerational microbiome study of multiple body sites, Wang *et al* found an obvious concordance of microbial variation between neonates and mothers suffering from GDM. The deviation of the neonatal gut microbiome was associated with metabolic depletion and virus prevalence in the meconium, indicating the importance of microbial inheritance during pregnancy.[91] Other studies also demonstrated that for pregnant women who use antibiotics (eg, ampicillin, penicillin and cefazolin), great microbial alteration is observed in their offspring, with markedly reduced microbial diversity and abnormal microbial composition.[75] [92] [93]

The occurrence of many diseases can be associated with dysbiosis of the gut microbiome.[94] [95] To better improve maternal health and fetal outcomes, it is necessary to restore the maternal gut microbiome via interventions. Recently, probiotic interventions for pregnant women have been introduced in many clinical trials.[96–98] Probiotics are specific live microbial cultures that benefit the host by improving its intestinal microbial balance.[99] Currently, many metabolic syndromes and immune diseases, such as allergies, obesity, GDM and T2D, have been treated with such a strategy, and promising progress has been made.[97] [98] [100] For example, Isolauri *et al* have focused on probiotic interventions for both pregnant women and infants for a long time. In a double-blind, placebo-controlled study including 256 pregnant women, they demonstrated that a probiotic-supplemented diet was an effective method for the prevention of obesity during pregnancy and could significantly reduce the occurrence of GDM for mothers.[101] [102] Another follow-up study from birth to 10 years indicated that a perinatal probiotic intervention helped reprogram the infant gut microbiome and avoid offspring obesity in childhood, especially for children with a parent or sibling who suffered from immune diseases.[103] [104] Such studies suggest the inheritance of the maternal microbiome during pregnancy and emphasise the importance of maintaining homeostasis of the maternal microbiome before and after delivery. Other groups also confirmed the protective effect of probiotic interventions on restoring the maternal gut microbiome and preventing multiple gestation disorders such as GDM, dyslipidaemia, preeclampsia and excessive gestational weight gain.[98] [105]

The most frequently used probiotics include strains from *Bifidobacterium* and *Lactobacillus*. On the one hand, such strains modulate the gut microbiome by competing with and inhibiting pathogen adhesins. On the other hand, they produce antibacterial substances, SCFAs, and anti-inflammatory cytokines, increasing the mucus layer and cell junctions (figure 2B). Despite the low biomass, probiotics help restore homeostasis of the gut microbiome and reduce intestinal inflammation.[106–108] The gut microbiota is a balanced ecosystem, and the occurrence of many diseases is associated with the disturbance of some key species.[109–111] Most recently, Xiao *et al* explored common characteristics of the gut microbiome in multiple diseases. Based on a novel biomarker identification algorithm, they constructed microbial networks related to different diseases and revealed a high prevalence of multirelated bacteria, which exhibited wide associations with multiple diseases, in global populations. The similar disorder pattern of the gut microbiome in different disease networks suggested the driving effects of some keystone microbes in the development of disease.[112] Consequently, identifying keystone species in microbial networks and developing new probiotic interventions to target key taxa can be a promising strategy for disease treatment.

In addition to probiotic interventions, other diet-based interventions and microbiome-based therapies, such as faecal microbiota transplantation (FMT), have also demonstrated successful results for the restoration of the normal microbiome (figure 2B).[113–116] Nonetheless, a recent study implemented FMT in one pregnant woman and reported the detection of donor-derived bacterial strains in the later-born infant, indicating vertical transmission during the perinatal period.[117] No further disorders have been observed in the offspring. This case reminds us that the choice of therapy for pregnant women and neonates must be made very carefully, and the safety of such interventions, especially during pregnancy and at an early age, needs further evaluation.

Vertical transfer from mothers to fetuses

Multiple studies have shown that maternal exposure during gestation may alter the microbiome and immunity of offspring,

indicating the importance of vertical transmission during pregnancy.[69 91 118] The placenta is an organ that is essential during the development of fetuses, providing indispensable nutrients and oxygen to growing babies,[119] and frequent substance exchange occurs at the maternal–fetal interface (figure 2C).[11 120] Many maternal components, such as antibodies and immune cells, are able to traverse the placental cell layers from mothers to fetuses.[121–125] For example, maternal IgG is the dominant antibody that can cross the human placenta via neonatal Fc receptor (FcRn), providing initial protection to the fetus from pathogens (figure 2C).[11 120 126]

In contrast to the direct transfer of antibodies, the maternal microbiome impacts and promotes fetal immune development in an indirect way. Many studies have indicated that bacterial antigens or metabolites can cross the maternal–fetal interface, inducing immune tolerance (figure 2C).[120 126] One elegant experiment, designed by Gomez de Agüero et al, demonstrated the association between the maternal microbiome and the fetal innate immune system.[127] A modified *Escherichia coli* strain was used to colonise germ-free pregnant mice, and before delivery, the dams were returned to sterile conditions. Compared with the offspring of the control groups, the offspring of the strain-colonised group showed altered intestinal transcriptional profiles and increased type 3 innate lymphoid cells and F4/80 mononuclear cells, indicating that the bacteria-induced immune development of infants starts before birth and that the gut microbiota, even transient residents, has the capability to prime fetal immune programming.[127] Intriguingly, this study also showed that maternal IgG antibodies, which were bound to microbial molecules and transmitted to the offspring, played an essential role in postnatal innate immune development.[127]

In addition to the beneficial substances described above, other detrimental substances or microbes, such as viruses, pathogenic bacteria, virulence factors and parasites, may cross the placenta and induce fetal infection (figure 2C).[128] Severe fetal infection may result in adverse pregnancy outcomes, such as preterm labour, miscarriage and maternal and fetal death.[128] Even when no infection occurs, an unfavourable intrauterine environment can contribute to an abnormal gut microbiome and metabolome in offspring. In a large twin study focused on fetal growth restriction (FGR), Yang et al demonstrated that adverse intrauterine environmental factors related to selective FGR-dominated genetics in their effects of altering microbial diversity and composition in the offspring. Dysbiosis of the gut microbiome in early life is not only correlated with pronounced metabolic alterations but also has long-term effects on the neurobehavioural development of infants.[129] Furthermore, previous studies have shown the vertical transmission of antibiotics through the placenta and demonstrated that the overuse of antibiotics during pregnancy may influence the resistome profiles of offspring.[14] Such evidence emphasises the importance of maternal health to unborn babies.

Whether the microbiome already exists in utero before delivery has been highly debated for a long time.[130 131] Recently, an increasing number of studies have shown that certain bacteria exist in the placenta or uterus.[132 133] While some researchers claimed that this could be due to contamination, as the placenta or uterus contains very little biomass,[130 134] others believed that potential transmission of the microbiome from mothers to fetuses may be involved in normal gestation.[132 133] For example, as the closest organ, the maternal vagina is considered to be the most likely source to transfer the microbiome into the uterus (figure 2C). Multiple studies have revealed *Lactobacillus* spp, the dominant bacteria in the vagina, to be one of the most frequent microbes present in placental or endometrial samples, suggesting putative transmission of the microbiome ascending from the vagina to the uterine cavity.[135–140]

In addition to the vagina, the maternal oral cavity is another potential source involved in the seeding of the microbiome during pregnancy (figure 2C).[136] Bacteria residing in the oral mucosa, such as *Streptococcus* and *Fusobacterium*, may translocate to the placenta through the maternal circulation, sometimes leading to preterm birth or miscarriage.[137 138 141–143] With some gut-derived bacteria, such as *Enterobacter* and *Enterococcus*, being observed in placenta or fetal meconium samples, the maternal gut is also thought to be a main contributor to the microbiome of babies (figure 2c).[6 138 144–146] Contrary to the potential adverse outcomes associated with microbial translocation from the maternal vagina and oral cavity to the uterus, the gut-derived microbiome is often identified in samples without any inflammation or infection, indicating the supporting role of the maternal gut microbiome in the development of the fetus.[119 138]

However, most 'source-tracking' studies focused on microbiome profiling between different organs and thus provide only indirect evidence of transmission. As no culture-dependent methods were used, it was difficult to determine whether the detected microbes were live bacteria or bacterial fragments or simply contamination derived from sample processing. Do such transmissions truly exist during pregnancy? What is the mechanism and further impact underlying the transmission? Additional research is required to answer these questions, which will be discussed in the next section.

PRENATAL MICROBIOME DEBATE

Although there is some evidence supporting microbial transmission from mothers to fetuses during pregnancy, the existence of the prenatal microbiome has been highly debated in recent years, leading to increasing research concentrating on this field. There are two main groups regarding this controversial issue. One group includes the faithful followers of the 'sterile womb hypothesis', according to which the uterus of the mother is a completely sterile environment under normal circumstances, and the infant acquires their initial microbiome during birth, either by vaginal or caesarean delivery (figure 3A). On the other hand, proponents of the 'in utero colonisation hypothesis' believe that a certain number of bacteria exist in the mother's uterus and can exert influences on the postnatal development of the newborn (figure 3B).

Sterile womb hypothesis

It has long been thought that the uterus is a sterile environment free of microorganisms. One of the strongest pieces of evidence supporting this hypothesis is the successful cultivation of gnotobiotic animals (figure 3A). Such animals, although they are different from normal animals and along with their immune and neurological deficiencies, can survive for a very long time, corroborating the possibility of a sterile womb environment.

Over the past few decades, a number of researchers have used sequencing-based methods to detect potential bacteria in placentas or fetuses (figure 3A). For example, Lauder et al conducted one of the earliest case–control comparisons using 16S rRNA sequencing and shotgun metagenomic sequencing.[147] In their studies, no difference was observed between placental samples and negative control samples. Subsequently, several studies also denied the existence of the prenatal microbiome based on 16S rRNA sequencing and suggested that the acquisition of bacteria was more likely to come from the contamination
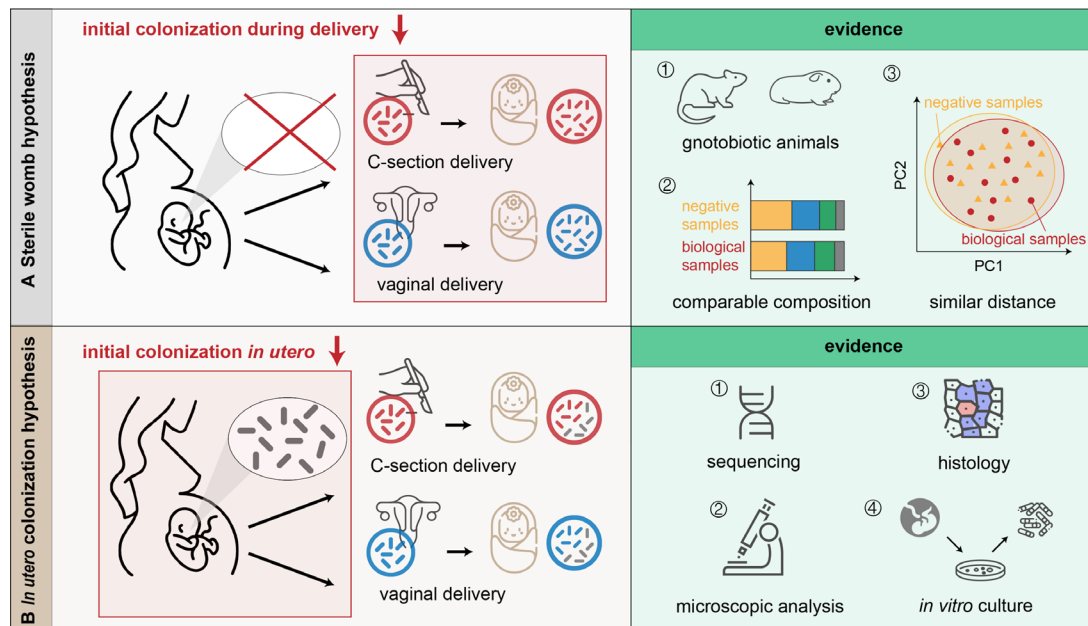
Figure 3 The debate regarding the prenatal microbiome. (A) The sterile womb hypothesis regards that the womb is sterile and that microbial colonisation in infants only starts during delivery. The evidence comprises (1) the generation of gnotobiotic animals; (2) similar microbial composition and (3) insignificant differences between biological samples and negative control samples. (B) The in utero colonisation hypothesis claims that microbial colonisation in infants occurs before birth. The supporting evidence includes (1) the sequencing of bacterial DNA fragments in placental or uterine tissues; (2) the detection of bacteria under a microscope; (3) the identification of bacteria using histological methods and (4) clinical cultures of live bacteria in vitro.

of kits and reagents in the laboratory.[148–152] Similarly, in an analysis among spontaneous preterm-delivered, non-spontaneous preterm-delivered and term-delivered placentas, Leon *et al* found that contamination during delivery contributed greatly to the artificial signals of low-biomass samples.[153] Sterpu *et al* also found that more bacteria could be cultured from vaginally delivered placentas than from caesarean-delivered placentas, enhancing the possibility of delivery-derived contamination.[154]

Apart from detecting the microbiome using 16S rRNA sequencing, Lager *et al* modified 18S sequencing but detected no eukaryotic pathogen signals in placental biopsies either in women with adverse pregnancy outcomes or in healthy controls.[155] Six different methods were used by Kuperman *et al* to identify bacterial signals in placenta samples from C-section deliveries.[156] Except for a small number of bacterial cells detected by immunohistochemistry, none of the other methods supported the presence of microbial colonisation. Recently, Kennedy *et al* collected fetal meconium from 20 term fetuses during caesarean section before birth and compared it with first-pass meconium and infant stool[157] but did not detect any microbial signal that was distinct from the fetal meconium of negative controls. Through aerobic and anaerobic culture, they found that the frequently isolated *Staphylococcus epidermidis* from fetal meconium might result from skin contamination.

In utero colonisation hypothesis
Few doubted the sanctity of the dogma that the womb was sterile until the experiment performed by Stout *et al*, where they found that in nearly one-third of the 195 placenta samples collected using a sterile technique in their study, intracellular bacteria could be histologically visualised in the basal plate.[158] Aagaard *et al* performed a larger microbial analysis of placentas in a 320-subject population. They processed samples and isolated DNA in a strictly controlled, decontaminated and sterile environment

and found that the placenta harboured a unique microbial niche characterised by the phyla Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fusobacteria.[141] Further 16S rRNA sequencing analysis revealed that this bacterial community resembled the human oral microbiome, indicating a trend of microbial transmission from the maternal oral cavity to the fetus during pregnancy. Subsequently, with standard techniques to ensure sterile and clean conditions during sample collection and DNA isolation, Antony *et al* demonstrated that in women with excess gestational weight gain, both the microbial composition and metabolic profiles of the placenta were altered, which was associated with preterm birth.[159] Although no causality was examined in this study, it has opened the door for research on the presence of intrauterine bacteria during pregnancy in recent years.

Since then, a number of studies have identified bacterial signals in placental villi,[160–163] the endometrium,[164] the uterine cervix,[162] decidual tissue,[165] fetal membranes,[160 161] the basal plate,[160] amniotic fluid[161 165 166] and meconium[161 166 167] using 16S rRNA sequencing,[137 160–167] the traditional histological Warthin-Starry and Gram stain methods,[163] fluorescence in situ hybridisation (FISH)[167] and clinical culture methodologies (figure 3B).[163 167] These studies, including both term and preterm populations, all claimed sample processing under clean and sterile conditions and demonstrated that although bacteria existed as low-abundance, low-biomass and sparse populations, in utero bacterial colonisation did occur during healthy pregnancy.

Among these studies, Gomez-Arango *et al* confirmed the oral-derived hypothesis in the development of the fetal microbiome in a study that included 37 women with OWOB, in which they further suggested that the gut microbiome was also one of the main contributors to the microbial seeding of the placenta.[137] Intriguingly, Liu *et al* and Parnell *et al* both found that microbial patterns from the placenta were not altered by delivery mode,

suggesting that contamination from delivery made little difference in the identification of bacterial signals.[160 161] In addition, Parnell *et al* employed 16S rRNA sequencing of multiple variable regions and demonstrated that the microbial community in utero exhibited distinct spatial profiles depending on placental location, implying that the sampling sites also greatly affected the detection of bacteria in utero.[160]

More convincing evidence came from two recent studies conducted by Rackaityte *et al* and Mishra *et al*. In the first study, Rackaityte *et al* examined mid-trimester fetal tissue using both culture-dependent and culture-independent methods.[132] According to the results of 16S rRNA sequencing, scanning electron microscopy (SEM), and FISH, they confirmed the existence of a limited microbiome in human fetal meconium, dominated by Micrococcaceae and *Lactobacillus*. Furthermore, strains of *Micrococcus luteus* were isolated from fetal meconium samples and cultured in a fetal intestinal-like environment with placental steroid hormones or THP1 human monocyte cells. These isolates exhibited heightened expression of immune cell recruitment and promotion to the tolerogenic environment. In the second study, Mishra *et al* further revealed the contribution of microbes to the activation of T cells in the fetus.[133] They collected fetal tissues in the second trimester and detected consistent microbial signals across different fetal organs (fetal gut, lung, skin and placenta) based on sequencing and clinical culture methodology. Specifically, *Lactobacillus* and *Staphylococcus* were identified as the most prevalent bacteria in multiple tissues. In addition to these microbes, the activation of T cells was observed in fetal tissues, implying the presence of antigenic stimuli before birth. In vitro experiments confirmed that bacterial antigens in utero, rather than bacterial contamination from the external environment, induced fetal T-cell expansion and memory activation. These findings strongly suggest the existence of viable microbes in utero and that even such a low-biomass community can educate the fetal immune system, promoting fetal and infant development before and after birth. The two studies provided strong evidence on the presence of the prenatal microbiome; however, contamination in low biomass samples such as placental, uterine, and fetus tissues is still a difficult problem that needs to be addressed.[134] Next, we will discuss the problem and propose available solutions.

Problems & solutions

Despite the accumulating evidence on in utero colonisation, many researchers remain opposed to the presence of the prenatal microbiome. One of the most concerning reasons is potential contamination during experimental operations. Potential sources of contamination include the genital tract or perineum during labour and delivery, laboratory-derived bacterial DNA during biopsy collection, sample processing, library preparation and sequencing.[148 168 169] As the microbes in utero (if present) are usually under the limit of detection, their low biomass impedes the distinction of authentic biological features from false signals. Although nearly all studies claimed that rigorous aseptic procedures were conducted during sample collection, DNA extraction and downstream processes, the contamination of microbes was rarely ruled out.

To address this challenge, positive and negative controls must be considered in the analysis. The source and type of contamination can be greatly determined by examining the bacterial load in case and control samples. de Goffau *et al* highlighted the impact of batch effects between case and control samples in the identification of bacterial colonisation.[131] For this reason, it must be ensured that the experimental procedure is identical for all samples to avoid batch effects.

In addition, multiple methods, including culture-independent and culture-dependent methods, are highly recommended for use in parallel in the detection of microbial signals between case and control groups. Culture-independent techniques include molecular-based methods, such as 16S rRNA or shotgun metagenomic sequencing, and FISH, histological methods (eg, H&E, Warthin-Starry and Gram stains), and image-based methods, such as SEM. Efficiency is one of the advantages of these methods, while a disadvantage is that they cannot determine the biological activity of bacteria. The culture-dependent technique mainly refers to the clinical culture of bacteria directly from biological samples. This method provides solid evidence for the presence of a viable microbiome in the tissue, although with some possibility of contamination. If both culture-independent and culture-dependent methods yield the same result, there is a good chance that the detected signal is biologically significant. Most importantly, all these methods must be applied very carefully during experiments to avoid the additional introduction of bacterial contamination.

The second problem corresponds to the real source of the intrauterine microbiome detected using various methods. Several studies have indicated that diseases such as endometritis and bacterial vaginosis may result in bacteria accessing the uterus, seriously affecting the development of the fetus.[135 148] On the other hand, bacteria in maternal blood and the oral cavity may be transferred into the uterus and fetus through the maternal circulation, which may be caused by serious blood diseases, periodontitis or mild inflammation or infection during pregnancy.[136 152] Various abnormal conditions could result in the detection of bacteria in uterine or fetal tissues. Pathogens from other locations of the maternal body are obviously a poor example of bacterial colonisation, which cannot strongly support a stable community in utero. Further evidence is needed to demonstrate that the colonisation of the microbiome truly occurs in utero rather than via transient resident transfer from other organs or tissues under a diseased or inflammatory condition.

Due to the lack of culture-dependent methods in most studies, there is doubt that the bacteria detected in these experiments are likely to be bacterial fragments rather than viable microbes. Some have questioned whether no differences will be observed in prenatal and postnatal development if such fragments or low biomass microbes neither form a stable community nor perform biological functions. While numerous previous studies have failed to prove the functionality of the detected microbiome, recent studies have demonstrated that the presence of live bacteria in utero is not required for immunomodulatory effects, and some bacterial molecules can be transferred to offspring with maternal antibodies.[120 126 127 133]

Whether bacterial colonisation occurs in utero remains an open question, and the present evidence is not sufficient to completely reject the sterile womb hypothesis. If the uterus is free of a microbiome, how can infants survive and immediately adapt when they leave a completely sterile environment and are exposed to a complex environment filled with bacteria at the time of delivery? If the microbiome truly exists, divides and colonises in utero, what function does it actually have? Where do these bacteria come from and when do they colonise? These are all problems that must be handled with caution.

There is still a long way to go before the debate is completely resolved. On the one hand, new ideas and observations are greatly encouraged to open our minds and reshape our views, pushing the field forwards; at the same time, rigorous and well-designed

experiments are highly required to look for more evidence to support or oppose the hypothesis. On the other hand, novel technologies are urgently needed to improve the sensitivity and accuracy in analysing low biomass samples, as well as to improve bioinformatic strategies based on sequencing data.

MICROBIAL TRANSMISSION DURING AND AFTER DELIVERY

Whether acquired in utero or not, a newborn's microbiome experiences rapid growth after delivery, forming a flexible community and maturing rapidly in the next few years.

Delivery mode

At the beginning of this process, the mode of birth plays a critical role in the establishment and development of the microbiome at an early age. Numerous studies have concentrated on the intergenerational transmission of the microbiome during delivery, and most of them have reached a similar conclusion that the microbial community of vaginally delivered neonates exhibits high diversity, dominated by *Bacteroides*, *Bifidobacterium*, *Parabacteroides* and *Escherichia*. Conversely, infants delivered via caesarean section harboured more species of *Klebsiella*,

Clostridia, *Enterobacter*, *Staphylococcus* and some opportunistic pathogens (figure 4A).[5 12 170–174]

During delivery, infants are exposed to complex communities with abundant bacteria, such as the maternal vagina, faeces, skin and the hospital environment. To further determine the transmission route at this critical time point, Dominguez-Bello *et al* performed the first study to explore microbial transmission patterns.[170] They collected initial microbiome samples from multiple body sites of mother–infant pairs and observed that the microbiome greatly varied between different birth modes. Vaginally delivered infants harboured more maternal vagina-derived microbes, such as *Lactobacillus*, *Prevotella* and *Sneathia* spp. In contrast, the microbiome of caesarean-delivered infants was characterised by *Staphylococcus*, *Corynebacterium* and *Propionibacterium* spp, which were more frequently found on the maternal skin surface.[170]

Following this study, several studies further revealed transmission during this process: with partial bacterial transfer from the maternal oral cavity and vagina, the main source of the microbial community in vaginally delivered infants was the mother's gut, where strains of *Bacteroides* and *Bifidobacterium*, as well as
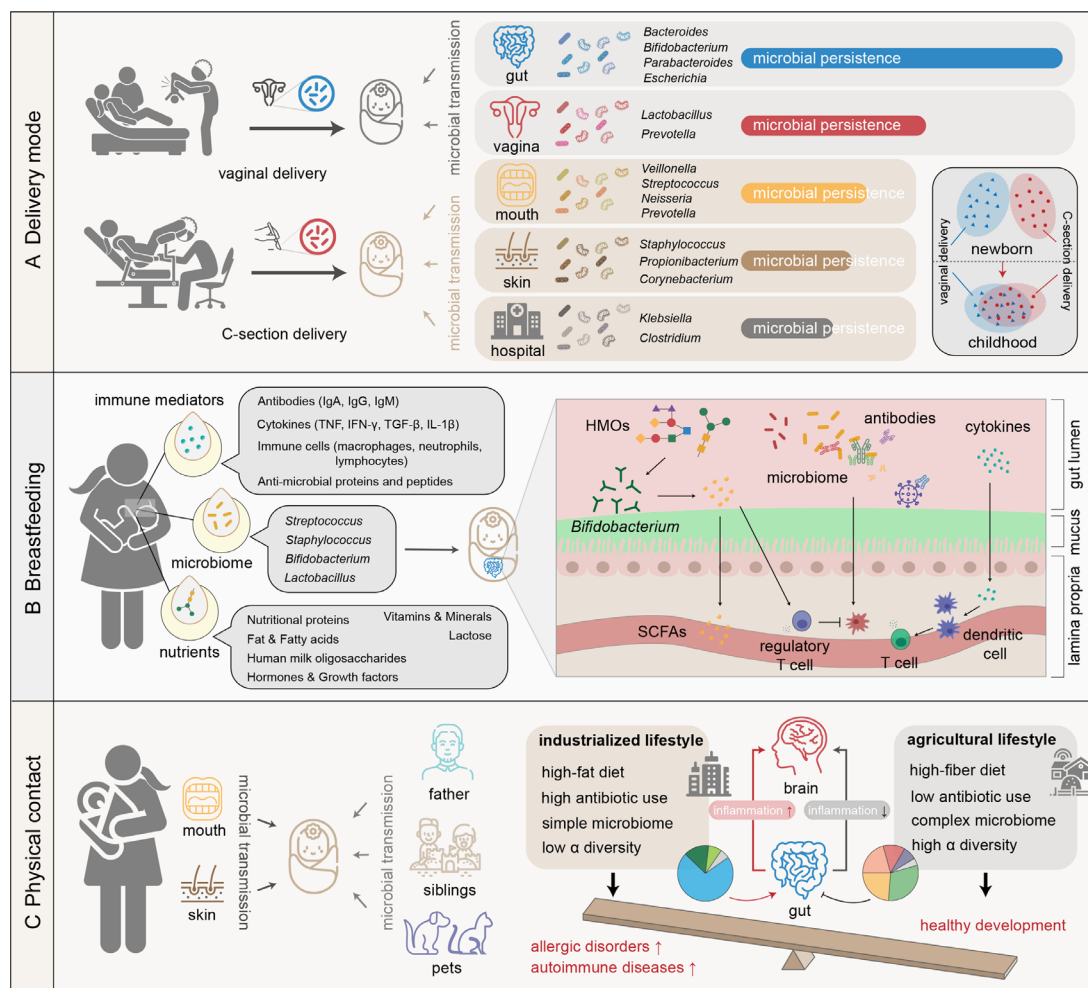

Figure 4 Microbial transmission during and after delivery. (A) Different transmission patterns between vaginal and C-section deliveries. The microbiome from the maternal gut persists much longer in the infant gut than those from other sources. Microbial divergence between different birth modes decreases with the growth of infants. (B) Transmission during breast feeding. Microbes in maternal breast milk benefit the establishment of the infant gut community. Other bioactive components, such as HMOs, antibodies, immune cells and cytokines, are largely involved in the regulation of the neonatal immune system. (C). Transmission during physical contact. Microbes from different sources contribute to the colonisation of the neonatal microbiome in early life. A wider range of microbial exposure (eg, living on farms) is associated with decreased inflammation and a low risk of autoimmune diseases. HMOs, human milk oligosaccharides.

Escherichia, were the bacteria most frequently transmitted from mothers to newborns. In contrast, caesarean-delivered neonates acquired their microbiome mainly from the maternal skin and hospital environment.[5 172 174–176]

Except for differences in initial acquisition in the first few days, persistence patterns of these bacteria also vary between infants delivered vaginally and by caesarean section (figure 4A). Bacteria such as *Bifidobacterium longum, Bifidobacterium bifidum* and *Parabacteroides diastonis,* which are enriched in vaginally delivered infants, may come from the maternal gut and have a better fitness for colonisation, while others such as *Streptococcus salivarius, Staphylococcus hominis, Staphylococcus massiliensis* and *Veillonella parvula* come from external environments (maternal skin and the hospital environment) and tend to be transient passengers.[174 175] One possible explanation is that the bacteria with high abundance that are transmitted from mothers have a better adaptation to the intestinal environment and thus have a stronger competitiveness in the development of the infant microbiome. Despite high variability at the early stage, microbial convergence between vaginal and caesarean deliveries is observed with infant growth (figure 4A).

Feeding

As one of the most important sources providing the initial bacterial community, human breast milk is the optimal food for infants, while the feeding guidelines vary in different regions.[177–179] Previous studies have confirmed the benefits of breast feeding for both mothers and infants.[11 178 180 181] Through breast feeding, a variety of nutrients that are important in early life are transferred to infants (figure 4B). These bioactive components, including human milk oligosaccharides (HMOs), immune cells, lactoferrin, cytokines, antibodies, antimicrobial proteins and peptides, provide strong protection to infants to effectively avoid the occurrence of multiple diseases, such as asthma, obesity, T2D and allergies, in childhood.[11 177 182] Breast milk is the primary source of antibodies and immune cells for newborns who are characterised by an immature immune system.[11] These immune cells are crucial for reducing inflammation and promoting immune tolerance in early life.[177] Antibodies in breast milk include IgA, IgG and IgM, the proportion of which varies in different lactation periods and populations.[11] Among these antibodies, the most abundant is secretory immunoglobulin A (SIgA), which acts as a bridge between microbiome colonisation and immune regulation.[120 180] Many studies have demonstrated that maternal SIgA plays a vital role in bacterial adherence, pathogen clearance and intestinal homeostasis to protect against viral infection and enhance microbial transfer from mothers to infants.[180 183–185] Additionally, breast feeding is suggested to alter the maternal metabolic process, greatly decreasing the risk of hypertension, hyperlipidaemia and cardiovascular diseases.[186] Although many studies have associated breast milk with a decreased risk of allergies in infants, some of them have indicated that the effectiveness of breast milk in allergy prevention is insufficient and is highly related to the timing and type of introduction of solid food.[179 187–189] Such conflicting evidence remains to be further explored.

Infants fed exclusively with breast milk harbour increasing abundances of *Bifidobacterium, Lactobacillus, Streptococcus* and *Staphylococcus.*[190] Among these, strains of *Bifidobacterium* were the most prevalent, with *B. breve, B. longum, B. dentium, B. infantis* and *B. pseudocatenulatum* accounting for over 70% of the whole community.[191–194] The strains of *Bifidobacterium,* especially *B. bifidus* and *B. longum* subsp. *Infantis,* have a high

digestion capacity of HMOs, a group of glycans in human milk that provide a protective effect to intestinal mucosa and promote maturation of the immune system.[195 196] In turn, HMOs also boost the increase in *Bifidobacterium.*[182 196]

Breast milk was once considered a sterile fluid; however, increasing evidence has recently demonstrated that breast milk harbours a complex microbial community, most of which belongs to Firmicutes, Proteobacteria and Actinobacteria.[182 197] Although several studies have suggested that breast milk exhibits great variability among different populations, *Streptococcus* and *Staphylococcus* are identified as the core genera in most lactating women, followed by some other bacteria, such as *Lactobacillus, Bifidobacterium, Propionibacterium, Corynebacterium, Enterococcus* and *Rothia,* depending on geographical location, diet, length of gestation and mode of delivery.[182 197–199] Interestingly, *Streptococcus* and *Staphylococcus* are representative bacteria in the oral mucosa and on the skin surface.[200] Large population analysis also confirmed that at the very beginning of life, these two genera, as well as *Bifidobacterium,* act as pioneer bacteria colonising the gut habitat of most newborns.[194 201] Recently, some studies demonstrated that the consumption of breast milk with a reduced microbial richness in the first month was associated with allergy development, implying the great importance of vertical transmission of the microbiome and organised colonisation in early life.[202 203] In contrast to direct exclusive breast feeding, some studies have indicated that feeding breast milk collected with breast pumps or formula milk leads to the depletion of some important bacteria, interrupting microbial transmission between mothers and infants.[204–206] Specifically, one study reported that sucking on the infant's pacifier before it was given to the infants was associated with a lower risk of eczema and asthma.[207] This evidence implies putative microbiome communication between mothers and infants during lactation, highlighting the importance of direct breast feeding at early ages.

Contact and other sources

Apart from transmission via delivery and feeding, daily contact between neonates and family members, including parents, siblings and pets, also contributes to the postnatal transmission of the microbiome, helping to establish the bacterial communities of infants at an early age (figure 4C). Vertical transmission from mothers via physical contact is still the primary source in daily life. Bacteria such as *Parabacteroides distasonis, Alistipes onderdonkii, Bacteroides faecis, Bacteroides caccae* and *Bacteroides salyersiae* from the maternal oral cavity and skin are frequently transferred to infants through kissing, touching and hugging.[208–210] One study focused on early microbial seeding and suggested that fathers also play an essential role in the establishment of the neonatal microbiome.[8] Distinct from maternal seeding at the start of birth, fathers mainly provide novel strains that colonise infants later in life.[8] In addition, infants who grow up with siblings or pets or live on farms exhibit higher diversity and more mature functionality associated with the gut microbiome, where frequent communication of the microbiome, known as horizontal transmission, is observed between cohabitants.[5 16 211 212] The hygiene hypothesis[213 214] or the alternative 'old friends' hypothesis[215 216] explains why environmental exposure to a more abundant microbiome contributes to the maturation of the immune system of infants and thus decreases the risk of early diseases, such as asthma and allergies.[16]
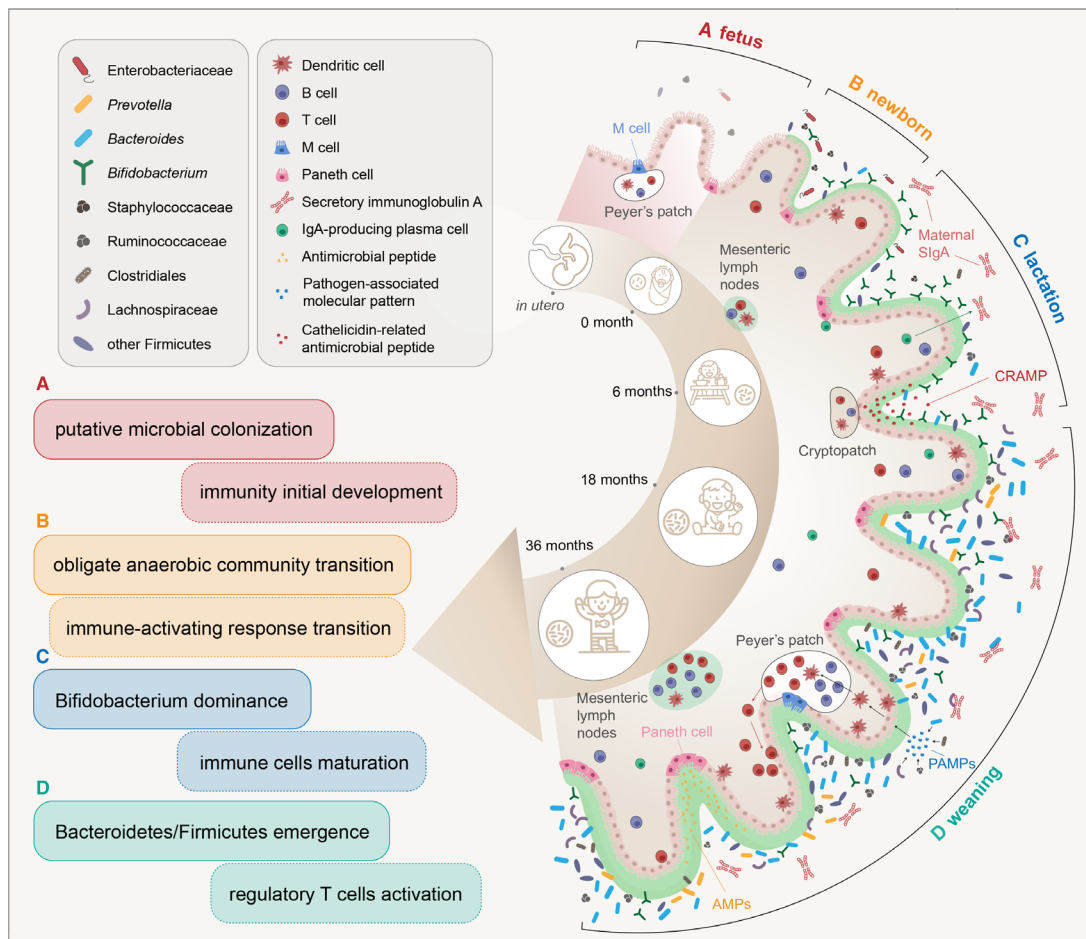
Figure 5 Microbial and immune development during the first 3 years of life. (A) Before birth, the ontogenesis of the immune system begins with stimulation by maternal factors. A few recent studies have indicated that early colonisation occurs during this period. Nevertheless, considering the low biomass in placental or uterine samples, whether the detected signal is associated with contamination needs further exploration. (B) After delivery, the gut microbiome transitions from a facultative anaerobic community to an obligate anaerobic community. Correspondingly, the neonatal immune system switches from a tolerogenic response to an antimicrobial response. (C) *Bifidobacterium* becomes the most abundant taxon during lactation, especially in the guts of infants fed maternal breast milk. Maternal breast milk also stimulates the immune system to mature rapidly and protects the neonatal intestinal mucosa from colonisation by pathogens. (D) At the time of weaning, Bacteroidetes and Firmicutes dominate the gut ecosystem, and bacterial divergence between different individuals starts to decrease. Weaning reactions occur and induce variations in immune cells with the expansion of the intestinal microbiome. AMPs, antimicrobial peptides; CRAMP, cathelicidin-related antimicrobial peptide; PAMPs, pathogen-associated molecular patterns; Tregs, regulatory T cells.

Postnatal development in infants

The first 3 years after birth is the most critical window of early development.[217 218] Recently, we conducted a longitudinal study of infants to explore microbial succession at the early stage.[201] With the analysis of over ten thousand faecal samples of infants from 17 countries, we observed a considerable but predictable change in the gut microbiome from birth to 3 years of age.[201] Such organised microbial assembly in global populations suggests that the development of the microbiome from infancy to adulthood follows a deterministic transition. In addition to microbial succession, the neonatal immune system also develops gradually in this critical period, exhibiting codevelopment between the microbiome and immune system.[219 220]

Microbiome colonisation and immune responses are closely related. Microbiome-mediated immune responses play a very important role in the maintenance of intestinal integrity. Before birth, cryptopatches and lymphoid tissues start to develop, helping the fetus prepare for exposure to the extrauterine world (figure 5A).[221 222] Some studies have indicated that the prenatal microbiome is involved in this essential process.[133 221] After delivery, a wide range of bacteria in the neonatal gut stimulates further development of the immune system (figure 5B).[16] Paneth cells, a major source of antimicrobial substances in adult tissue, are immature in the neonatal mucosa, making the neonatal intestine very sensitive to external perturbation.[221] Instead, cathelicidin-related antimicrobial peptides are expressed during this stage to help prevent bacterial infections (figure 5C).[221]

Various postnatal factors determine immune development in early life. Caesarean delivery is associated with asthma and many immune-related disorders in childhood.[223] Frequently accompanied by preterm birth and the use of antibiotics at birth, infants born via caesarean delivery not only harbour a disordered microbial community but also have a delayed immune system,[16 224] with reduced microbial diversity and significantly lower levels of the Th1-associated chemokines CXCL10 and CXCL11 in blood.[225] Especially for children with a microbial community that has not been restored to normal at the age of 1 year, the level of immune mediators, such as tumour necrosis factor alpha, interleukin 4 (IL-4), IL-3 or IL-1β, is much lower.[226 227] Rodent models also suggested that caesarean delivery might lead to

immune deficiency in progeny, which could be partially restored with prebiotics in the postnatal period.[228 229]

In the first few days after birth, facultative anaerobic bacteria, as pioneer colonisers, dominate the infant gut, where species of Enterobacteriaceae, *Staphylococcus* and *Streptococcus* exhibit the highest abundance (figure 5B).[194 201 230] With the reduction of oxygen, facultative anaerobic microbes are replaced by a group of obligate anaerobic bacteria. Among these microbes, strains of *Bifidobacterium,* which are mainly involved in the metabolism of HMOs, dominate the ecological niche with the start of breast feeding (figure 5B).[194] Infants fed breast milk have a decreased risk of necrotising enterocolitis, which is a devastating and the most common disease in preterm infants, causing high morbidity and mortality.[11 197]

In contrast, infants who are fed cow's milk formula or soy formula harbour more bacteria belonging to Bacteroidetes and Firmicutes, such as *Bacteroides*, *Clostridium* and *Ruminococcus*, showing a higher alpha diversity and more mature microbial pattern compared with exclusively breastfed infants.[197 206] As reduced microbial diversity is associated with the development of allergies and atopic eczema,[231–233] the reason why this difference occurs remains unknown, and the protective effect of breast feeding against autoimmune diseases requires further research.[187]

Most infants were weaned between the sixth month and the eighteenth month of life, when *Bifidobacterium* significantly decreased and *Bacteroides* began to thrive (figure 5C,D).[194 195 201] The period spanning birth to weaning is considered to be a 'window of opportunity', during which host-microbe crosstalk frequently occurs, leading to the development of a balanced immune system and preventing pathological imprinting later in life.[226 234 235] At the time of weaning, the composition of the gut microbiome as well as the population of immune cells are greatly altered, inducing a vigorous immune response called the 'weaning reaction' and promoting the maturation of the immune system.[234]

As infants grow, the gut microbiome enters the transitional stage,[194] characterised by a high abundance of *Bacteroides*, *Lachnoclostridium*, *Faecalibacterium*, Lachnospiraceae and *Ruminococcus* (figure 5D).[230] Following this, around the 18th month of life, *Prevotella*, the dominant taxon in one of the most frequent enterotypes of adults, emerges and continues to increase.[236] By 3 years of age, infants in distinct developmental stages harbour microbial communities dominated by different bacteria, leading to significantly stratified patterns in global populations.[201 230] At this phase, the gut microbiome of infants transforms to an adult-like microbiome and remains stable. Along with maturation of the microbiome, functionalities associated with pathways of complex polysaccharide metabolism are significantly upregulated to better adapt to adult-like lifestyles.[201 217]

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Currently, the maternal microbiome is thought to be the greatest contributor to the colonisation of the initial microbial community of infants. In this review, we summarised vertical microbial transmission between mothers and infants across different gestational periods and different maternal body sites. We highlighted the great importance of the prenatal lifestyle and postnatal care on the development of the neonatal microbiome and immune system. Comprehensive insights into microbial transmission, colonisation and succession from pregnancy to infancy will greatly promote the success of maternal and neonatal microbiome studies.

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REFERENCES

1. Sorbara MT, Pamer EG. Microbiome-based therapeutics. *Nat Rev Microbiol* 2022;20:365–80.
2. Hall AB, Tolonen AC, Xavier RJ. Human genetic variation and the gut microbiome in disease. *Nat Rev Genet* 2017;18:690–9.
3. Wang J, Jia Z, Zhang B, et al. Tracing the accumulation of in vivo human oral microbiota elucidates microbial community dynamics at the gateway to the Gi tract. *Gut* 2020;69:1355–6.
4. Yu Y, Zhang B, Ji P, et al. Changes to gut amino acid transporters and microbiome associated with increased E/I ratio in chd8$^{+/-}$ mouse model of ASD-like behavior. *Nat Commun* 2022;13:1151.
5. Enav H, Bäckhed F, Ley RE. The developing infant gut microbiome: a strain-level view. *Cell Host Microbe* 2022;30:S1931-3128(22)00215-3:627–38.:
6. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* 2015;17:S1931-3128(15)00162-6:690–703.:
7. Yassour M, Jason E, Hogstrom LJ, et al. Strain-level analysis of mother-to-child bacterial transmission during the first few months of life. *Cell Host & Microbe* 2018;24:e4:146–154.
8. Korpela K, Costea P, Coelho LP, et al. Selective maternal seeding and environment shape the human gut microbiome. *Genome Res* 2018;28:561–8.
9. Yang L, Sakandar HA, Sun Z, et al. Recent advances of intestinal microbiota transmission from mother to infant. *Journal of Functional Foods* 2021;87:104719.
10. Wang S, Ryan CA, Boyaval P, et al. Maternal vertical transmission affecting early-life microbiota development. *Trends Microbiol* 2020;28:S0966-842X(19)30208-2:28–45.:
11. Langel SN, Blasi M, Permar SR. Maternal immune protection against infectious diseases. *Cell Host Microbe* 2022;30:S1931-3128(22)00213-X:660–74.:
12. Bolte EE, Moorshead D, Aagaard KM. Maternal and early life exposures and their potential to influence development of the microbiome. *Genome Med* 2022;14:4.
13. Hourigan SK, Dominguez-Bello MG, Mueller NT. Can maternal-child microbial seeding interventions improve the health of infants delivered by cesarean section? *Cell Host Microbe* 2022;30:S1931-3128(22)00100-7:607–11.:
14. Patangia DV, Ryan CA, Dempsey E, et al. Vertical transfer of antibiotics and antibiotic resistant strains across the mother/baby axis. *Trends Microbiol* 2022;30:S0966-842X(21)00128-1:47–56.:
15. Guzzardi MA, Ederveen THA, Rizzo F, et al. Maternal pre-pregnancy overweight and neonatal gut bacterial colonization are associated with cognitive development and gut microbiota composition in pre-school-age offspring. *Brain Behav Immun* 2022;100:S0889-1591(21)00640-1:311–20.:.
16. Brodin P. Immune-microbe interactions early in life: A determinant of health and disease long term. *Science* 2022;376:945–50.
17. Hong X, Zhao J, Yin J, et al. The association between the pre-pregnancy vaginal microbiome and time-to-pregnancy: a chinese pregnancy-planning cohort study. *BMC Med* 2022;20:246.
18. Gupta VK, Paul S, Dutta C. Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity. *Front Microbiol* 2017;8:1162.
19. Bressa C, Bailén-Andrino M, Pérez-Santiago J, et al. Differences in gut microbiota profile between women with active lifestyle and sedentary women. *PLoS ONE* 2017;12:e0171352.
20. Gacesa R, Kurilshikov A, Vich Vila A, et al. Environmental factors shaping the gut microbiome in a dutch population. *Nature* 2022;604:732–9.
21. Dwiyanto J, Hussain MH, Reidpath D, et al. Ethnicity influences the gut microbiota of individuals sharing a geographical location: a cross-sectional study from a middle-income country. *Sci Rep* 2021;11:2618.

22 Blekhman R, Goodrich JK, Huang K, *et al*. Host genetic variation impacts microbiome composition across human body sites. *Genome Biol* 2015;16:191:191.:

23 Shin J-H, Sim M, Lee J-Y, *et al*. Lifestyle and geographic insights into the distinct gut microbiota in elderly women from two different geographic locations. *J Physiol Anthropol* 2016;35:31.

24 Lennard K, Dabee S, Barnabas SL, *et al*. Vaginal microbiota varies by geographical location in south african women. *South African Journal for Science and Technology* 2019;38

25 Pasolli E, Asnicar F, Manara S, *et al*. Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. *Cell* 2019;176:S0092-8674(19)30001-7:649–662.

26 De Filippo C, Cavalieri D, Di Paola M, *et al*. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* 2010;107:14691–6.

27 Ou J, Carbonero F, Zoetendal EG, *et al*. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am J Clin Nutr* 2013;98:111–20.

28 Schnorr SL, Candela M, Rampelli S, *et al*. Gut microbiome of the hadza hunter-gatherers. *Nat Commun* 2014;5:3654.

29 Suzuki TA, Worobey M. Geographical variation of human gut microbial composition. *Biol Lett* 2014;10

30 Tett A, Huang KD, Asnicar F, *et al*. The Prevotella copri complex comprises four distinct clades underrepresented in westernized populations. *Cell Host & Microbe* 2019;26:e7:666–679.

31 Tyakht AV, Alexeev DG, Popenko AS, *et al*. Rural and urban microbiota: to be or not to be? *Gut Microbes* 2014;5:351–6.

32 Li J, Quinque D, Horz H-P, *et al*. Comparative analysis of the human saliva microbiome from different climate zones: Alaska, Germany, and Africa. *BMC Microbiol* 2014;14

33 Mason MR, Nagaraja HN, Camerlengo T, *et al*. Deep sequencing identifies ethnicity-specific bacterial signatures in the oral microbiome. *PLoS One* 2013;8:e77287

34 Zhou X, Hansmann MA, Davis CC, *et al*. The vaginal bacterial communities of Japanese women resemble those of women in other racial groups. *FEMS Immunol Med Microbiol* 2010;58:169–81.

35 Ravel J, Gajer P, Abdo Z, *et al*. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 2011;108 Suppl 1:4680–7.

36 Verstraelen H, Vilchez-Vargas R, Desimpel F, *et al*. Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. *PeerJ* 2016;4:e1602

37 Zhou X, Brown CJ, Abdo Z, *et al*. Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. *ISME J* 2007;1:121–33.

38 Hyman RW, Fukushima M, Diamond L, *et al*. Microbes on the human vaginal epithelium. *Proc Natl Acad Sci USA* 2005;102:7952–7.

39 Fettweis JM, Brooks JP, Serrano MG, *et al*. Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology (Reading)* 2014;160(Pt 10):2272–82.

40 Dreisbach C, Prescott S, Alhusen J. Influence of maternal prepregnancy obesity and excessive gestational weight gain on maternal and child gastrointestinal microbiome composition: a systematic review. *Biol Res Nurs* 2020;22:114–25.

41 Dunlop AL, Mulle JG, Ferranti EP, *et al*. Maternal microbiome and pregnancy outcomes that impact infant health: a review. *Adv Neonatal Care* 2015;15:377–85.

42 Stanislawski MA, Dabelea D, Wagner BD, *et al*. Pre-pregnancy weight, gestational weight gain, and the gut microbiota of mothers and their infants. *Microbiome* 2017;5:113:113.:

43 Mueller NT, Shin H, Pizoni A, *et al*. Birth mode-dependent association between pre-pregnancy maternal weight status and the neonatal intestinal microbiome. *Sci Rep* 2016;6:23133:23133.:

44 Singh SB, Madan J, Coker M, *et al*. Does birth mode modify associations of maternal pre-pregnancy BMI and gestational weight gain with the infant gut microbiome? *Int J Obes (Lond)* 2020;44:23–32.

45 Galley JD, Bailey M, Kamp Dush C, *et al*. Maternal obesity is associated with alterations in the gut microbiome in toddlers. *PLoS One* 2014;9:e113026

46 Nuriel-Ohayon M, Neuman H, Koren O. Microbial changes during pregnancy, birth, and infancy. *Front Microbiol* 2016;7:1031:1031.:

47 Amir M, Brown JA, Rager SL, *et al*. Maternal microbiome and infections in pregnancy. *Microorganisms* 2020;8:1996.

48 Mesa MD, Loureiro B, Iglesia I, *et al*. The evolving microbiome from pregnancy to early infancy: a comprehensive review. *Nutrients* 2020;12:133.

49 Fujiwara N, Tsuruda K, Iwamoto Y, *et al*. Significant increase of oral bacteria in the early pregnancy period in japanese women. *J Investig Clin Dent* 2017;8:e12189.

50 Kumar PS. Sex and the subgingival microbiome: do female sex steroids affect periodontal bacteria? *Periodontology 2000* 2013;61:103–24.

51 Koren O, Goodrich JK, Cullender TC, *et al*. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012;150:470–80.

52 Nuriel-Ohayon M, Neuman H, Ziv O, *et al*. Progesterone increases Bifidobacterium relative abundance during late pregnancy. *Cell Rep* 2019;27:S2211-1247(19)30405-X:730–736.

53 France M, Alizadeh M, Brown S, *et al*. Towards a deeper understanding of the vaginal microbiota. *Nat Microbiol* 2022;7:367–78.

54 Zhang X, Zhai Q, Wang J, *et al*. Variation of the vaginal microbiome during and after pregnancy in Chinese women. *Genomics Proteomics Bioinformatics* 2022;20:S1672-0229(22)00009-2:322–33.:

55 Romero R, Hassan SS, Gajer P, *et al*. The composition and stability of the vaginal. *Microbiome* 2014;2

56 Aagaard K, Riehle K, Ma J, *et al*. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS ONE* 2012;7:e36466.

57 Stout MJ, Zhou Y, Wylie KM, *et al*. Early pregnancy vaginal microbiome trends and preterm birth. *Am J Obstet Gynecol* 2017;217:S0002-9378(17)30641-5:356.:.

58 Stoyancheva G, Marzotto M, Dellaglio F, *et al*. Bacteriocin production and gene sequencing analysis from vaginal lactobacillus strains. *Arch Microbiol* 2014;196:645–53.

59 Smith SB, Ravel J. The vaginal microbiota, host defence and reproductive physiology. *J Physiol* 2017;595:451–63.

60 O'Hanlon DE, Moench TR, Cone RA, *et al*. Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. *PLoS ONE* 2013;8:e80074.

61 Fettweis JM, Serrano MG, Brooks JP, *et al*. The vaginal microbiome and preterm birth. *Nat Med* 2019;25:1012–21.

62 Callahan BJ, DiGiulio DB, Goltsman DSA, *et al*. Replication and refinement of a vaginal microbial signature of preterm birth in two racially distinct cohorts of US women. *Proc Natl Acad Sci U S A* 2017;114:9966–71.

63 Liu ZZ, Sun JH, Wang WJ. Gut microbiota in gastrointestinal diseases during pregnancy. *World J Clin Cases* 2022;10:2976–89.

64 Fuhler GM. The immune system and microbiome in pregnancy. *Best Pract Res Clin Gastroenterol* 2020;44–45:S1521-6918(20)30006-8:101671.:.

65 van Deuren T, Blaak EE, Canfora EE. Butyrate to combat obesity and obesity-associated metabolic disorders: current status and future implications for therapeutic use. *Obes Rev* 2022;23:e13498.

66 Zhang M, Zhou Q, Dorfman RG, *et al*. Butyrate inhibits interleukin-17 and generates tregs to ameliorate colorectal colitis in rats. *BMC Gastroenterol* 2016;16:84:84.:.

67 Barbian ME, Owens JA, Naudin CR, *et al*. Butyrate supplementation to pregnant mice elicits cytoprotection against colonic injury in the offspring. *Pediatr Res* 2022;92:125–34.

68 Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol* 2014;16:1024–33.

69 Bodnar TS, Lee C, Wong A, *et al*. Evidence for long-lasting alterations in the fecal microbiota following prenatal alcohol exposure. *Alcohol Clin Exp Res* 2022;46:542–55.

70 McLean C, Jun S, Kozyrskyj A. Impact of maternal smoking on the infant gut microbiota and its association with child overweight: a scoping review. *World J Pediatr* 2019;15:341–9.

71 Ma J, Prince AL, Bader D, *et al*. High-Fat maternal diet during pregnancy persistently alters the offspring microbiome in a primate model. *Nat Commun* 2014;5:3889.

72 Chu DM, Antony KM, Ma J, *et al*. The early infant gut microbiome varies in association with a maternal high-fat diet. *Genome Med* 2016;8:77.

73 Bhagavata Srinivasan SP, Raipuria M, Bahari H, *et al*. n.d. Impacts of diet and exercise on maternal gut microbiota are transferred to offspring. *Front Endocrinol* 9

74 Lundgren SN, Madan JC, Emond JA, *et al*. Maternal diet during pregnancy is related with the infant stool microbiome in a delivery mode-dependent manner. *Microbiome* 2018;6:109.

75 Dierikx TH, Visser DH, Benninga MA, *et al*. The influence of prenatal and intrapartum antibiotics on intestinal microbiota colonisation in infants: a systematic review. *Journal of Infection* 2020;81:190–204.

76 Mirzaei S, Oroomiei N, Nakhaee N. The first 1000 days of life and the risk of future drug consumption. *Int J High Risk Behav Addict* 2022;11

77 Krisanits B, Randise JF, Burton CE, *et al*. Pubertal mammary development as a " susceptibility window " for breast cancer disparity. *Adv Cancer Res* 2020;146:S0065-230X(20)30004-X:57–82.:

78 Gohir W, Ratcliffe EM, Sloboda DM. Of the bugs that shape us: maternal obesity, the gut microbiome, and long-term disease risk. *Pediatr Res* 2015;77:196–204.

79 Romieu I, Torrent M, Garcia-Esteban R, *et al*. Maternal fish intake during pregnancy and atopy and asthma in infancy. *Clin Exp Allergy* 2007;37:518–25.

80 Mennitti LV, Oliveira JL, Morais CA, *et al*. Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. *J Nutr Biochem* 2015;26:S0955-2863(14)00203-4:99–111.:

81 Li D, Wu M. Pattern recognition receptors in health and diseases. *Signal Transduct Target Ther* 2021;6:291.

82 Robertson SA, Hutchinson MR, Rice KC, *et al*. Targeting Toll-like receptor-4 to tackle preterm birth and fetal inflammatory injury. *Clin Transl Immunology* 2020;9:e1121

83 Murphy EA, Velazquez KT, Herbert KM. Influence of high-fat diet on gut microbiota: a driving force for chronic disease risk. *Curr Opin Clin Nutr Metab Care* 2015;18:515–20.

84 Moreira APB, Texeira TFS, Ferreira AB, *et al*. Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. *Br J Nutr* 2012;108:801–9.

85 Cani PD, Bibiloni R, Knauf C, *et al*. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008;57:1470–81.

86 de Vos WM, Tilg H, Van Hul M, *et al*. Gut microbiome and health: mechanistic insights. *Gut* 2022;71:1020–32.

87 Edwards SM, Cunningham SA, Dunlop AL, *et al*. The maternal gut microbiome during pregnancy. *MCN Am J Matern Child Nurs* 2017;42:310–7.

88 Camilleri M. Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut* 2019;68:1516–26.

89 Xie R, Sun Y, Wu J, *et al*. Maternal high fat diet alters gut microbiota of offspring and exacerbates DSS-induced colitis in adulthood. *Front Immunol* 2018;9:2608.

90 Mirpuri J. Evidence for maternal diet-mediated effects on the offspring microbiome and immunity: implications for public health initiatives. *Pediatr Res* 2021;89:301–6.

91 Wang J, Zheng J, Shi W, *et al*. Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. *Gut* 2018;67:1614–25.

92 Kuperman AA, Koren O. Antibiotic use during pregnancy: how bad is it? *BMC Med* 2016;14:91.

93 Wong WSW, Sabu P, Deopujari V, *et al*. Prenatal and Peripartum exposure to antibiotics and cesarean section delivery are associated with differences in diversity and composition of the infant meconium microbiome. *Microorganisms* 2020;8:179

94 Li J, Zhao F, Wang Y, *et al*. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* 2017;5:14.

95 Yu Y, Zhao F. Microbiota-Gut-Brain axis in autism spectrum disorder. *J Genet Genomics* 2021;48:S1673-8527(21)00206-X:755–62.:

96 Hampe CS, Roth CL. Probiotic strains and mechanistic insights for the treatment of type 2 diabetes. *Endocrine* 2017;58:207–27.

97 de Brito Alves JL, de Oliveira Y, Carvalho NNC, *et al*. Gut microbiota and probiotic intervention as a promising therapeutic for pregnant women with cardiometabolic disorders: present and future directions. *Pharmacological Research* 2019;145:104252.

98 Abbasi A, Aghebati-Maleki A, Yousefi M, *et al*. Probiotic intervention as a potential therapeutic for managing gestational disorders and improving pregnancy outcomes. *Journal of Reproductive Immunology* 2021;143:103244.

99 Rinne M, Kalliomäki M, Salminen S, *et al*. Probiotic intervention in the first months of life: short-term effects on gastrointestinal symptoms and long-term effects on gut microbiota. *J Pediatr Gastroenterol Nutr* 2006;43:200–5.

100 Gomez Arango LF, Barrett HL, Callaway LK, *et al*. Probiotics and pregnancy. *Curr Diab Rep* 2015;15:567.

101 Ilmonen J, Isolauri E, Poussa T, *et al*. Impact of dietary counselling and probiotic intervention on maternal anthropometric measurements during and after pregnancy: a randomized placebo-controlled trial. *Clin Nutr* 2011;30:156–64.

102 Luoto R, Laitinen K, Nermes M, *et al*. Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. *Br J Nutr* 2010;103:1792–9.

103 Luoto R, Kalliomäki M, Laitinen K, *et al*. Initial dietary and microbiological environments deviate in normal-weight compared to overweight children at 10 years of age. *J Pediatr Gastroenterol Nutr* 2011;52:90–5.

104 Luoto R, Kalliomäki M, Laitinen K, *et al*. The impact of perinatal probiotic intervention on the development of overweight and obesity: follow-up study from birth to 10 years. *Int J Obes (Lond)* 2010;34:1531–7.

105 Obuchowska A, Gorczyca K, Standyło A, *et al*. Effects of probiotic supplementation during pregnancy on the future maternal risk of metabolic syndrome. *Int J Mol Sci* 2022;23:8253

106 Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, *et al*. Probiotic mechanisms of action. *Ann Nutr Metab* 2012;61:160–74.

107 Sanders ME, Benson A, Lebeer S, *et al*. Shared mechanisms among probiotic taxa: implications for general probiotic claims. *Current Opinion in Biotechnology* 2018;49:207–16.

108 Simon E, Călinoiu LF, Mitrea L, *et al*. Probiotics, prebiotics, and synbiotics: implications and beneficial effects against irritable bowel syndrome. *Nutrients* 2021;13:2112.

109 Poudel R, Jumpponen A, Schlatter DC, *et al*. Microbiome networks: a systems framework for identifying candidate microbial assemblages for disease management. *Phytopathology®* 2016;106:1083–96.

110 Greenbaum S, Greenbaum G, Moran-Gilad J, *et al*. Ecological dynamics of the vaginal microbiome in relation to health and disease. *Am J Obstet Gynecol* 2019;220:S0002-9378(18)32114-8:324–35.::

111 Li Z, Zhang B, Wang N, *et al*. A novel peptide protects against diet-induced obesity by suppressing appetite and modulating the gut microbiota. *Gut* 2022;gutjnl-2022-328035

112 Xiao L, Zhang F, Zhao F. Large-Scale microbiome data integration enables robust biomarker identification. *Nat Comput Sci* 2022;2:307–16.

113 Kinshella M-LW, Omar S, Scherbinsky K, *et al*. Effects of maternal nutritional supplements and dietary interventions on placental complications: an umbrella review, meta-analysis and evidence map. *Nutrients* 2021;13:472.

114 Zhao Z, Ji X, Zhang T, *et al*. Washed microbiota transplantation improves the fertility of patients with inflammatory bowel disease. *Chin Med J (Engl)* 2022;135:1489–91.

115 Gresham E, Bisquera A, Byles JE, *et al*. Effects of dietary interventions on pregnancy outcomes: a systematic review and meta-analysis. *Matern Child Nutr* 2016;12:5–23.

116 He R, Li P, Wang J, *et al*. The interplay of gut microbiota between donors and recipients determines the efficacy of fecal microbiota transplantation. *Gut Microbes* 2022;14:2100197

117 Wei S, Jespersen ML, Baunwall SMD, *et al*. Cross-generational bacterial strain transfer to an infant after fecal microbiota transplantation to a pregnant patient: a case report. *Microbiome* 2022;10:193.

118 Yu J, Liu X, Li Y, *et al*. Maternal exposure to farming environment protects offspring against allergic diseases by modulating the neonatal TLR-tregs-th axis. *Clin Transl Allergy* 2018;8:34.

119 D'Argenio V. The prenatal microbiome: a new player for human health. *High-Throughput* 2018;7:38.

120 Jenmalm MC. The mother-offspring dyad: microbial transmission, immune interactions and allergy development. *J Intern Med* 2017;282:484–95.

121 Simister NE. Placental transport of immunoglobulin G. *Vaccine* 2003;21:3365–9.

122 Clements T, Rice TF, Vamvakas G, *et al*. Update on transplacental transfer of igg subclasses: impact of maternal and fetal factors. *Front Immunol* 2020;11:1920.

123 Hall JM, Lingenfelter P, Adams SL, *et al*. Detection of maternal cells in human umbilical cord blood using fluorescence in situ hybridization. *Blood* 1995;86:2829–32.

124 Kanaan SB, Gammill HS, Harrington WE, *et al*. Maternal microchimerism is prevalent in cord blood in memory T cells and other cell subsets, and persists post-transplant. *Oncoimmunology* 2017;6:e1311436

125 Mold JE, Michaëlsson J, Burt TD, *et al*. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. *Science* 2008;322:1562–5.

126 Gao Y, Nanan R, Macia L, *et al*. The maternal gut microbiome during pregnancy and offspring allergy and asthma. *Journal of Allergy and Clinical Immunology* 2021;148:669–78.

127 Gomez de Agüero M, Ganal-Vonarburg SC, Fuhrer T, *et al*. The maternal microbiota drives early postnatal innate immune development. *Science* 2016;351:1296–302.

128 Megli CJ, Coyne CB. Infections at the maternal–fetal interface: an overview of pathogenesis and defence. *Nat Rev Microbiol* 2022;20:67–82.

129 Yang J, Hou L, Wang J, *et al*. Unfavourable intrauterine environment contributes to abnormal gut microbiome and metabolome in twins. *Gut* 2022;71:2451–62.

130 Walter J, Hornef MW. A philosophical perspective on the prenatal in utero microbiome debate. *Microbiome* 2021;9:5.

131 de Goffau MC, Charnock-Jones DS, Smith GCS, *et al*. Batch effects account for the main findings of an in utero human intestinal bacterial colonization study. *Microbiome* 2021;9:6.

132 Rackaityte E, Halkias J, Fukui EM, *et al*. Viable bacterial colonization is highly limited in the human intestine in utero. *Nat Med* 2020;26:599–607.

133 Mishra A, Lai GC, Yao LJ, *et al*. Microbial exposure during early human development primes fetal immune cells. *Cell* 2021;184:S0092-8674(21)00574-2:3394–3409.

134 Kennedy KM, Bellissimo CJ, Breznik JA, *et al*. Over-celling fetal microbial exposure. *Cell* 2021;184:S0092-8674(21)01281-2:5839–41.:

135 Wang J, Li Z, Ma X, *et al*. Translocation of vaginal microbiota is involved in impairment and protection of uterine health. *Nat Commun* 2021;12:4191.

136 Olaniyi KS, Moodley J, Mahabeer Y, *et al*. Placental microbial colonization and its association with pre-eclampsia. *Front Cell Infect Microbiol* 2020;10:413.

137 Gomez-Arango LF, Barrett HL, McIntyre HD, *et al*. Contributions of the maternal oral and gut microbiome to placental microbial colonization in overweight and obese pregnant women. *Sci Rep* 2017;7:2860.

138 Pelzer E, Gomez-Arango LF, Barrett HL, *et al*. Review: maternal health and the placental microbiome. *Placenta* 2017;54:S0143-4004(16)30649-X:30–7.:

139 Zheng J, Xiao X, Zhang Q, *et al*. The placental microbiome varies in association with low birth weight in full-term neonates. *Nutrients* 2015;7:6924–37.

140 Romero R, Miranda J, Kusanovic JP, *et al*. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. *J Perinat Med* 2015;43:19–36.

141 Aagaard K, Ma J, Antony KM, *et al*. The placenta harbors a unique microbiome. *Sci Transl Med* 2014;6:237ra65

142 Lannon SMR, Adams Waldorf KM, Fiedler T, *et al*. Parallel detection of lactobacillus and bacterial vaginosis-associated bacterial DNA in the chorioamnion and vagina of pregnant women at term. *J Matern Fetal Neonatal Med* 2019;32:2702–10.

143 Amarasekara R, Jayasekara RW, Senanayake H, *et al*. Microbiome of the placenta in pre-eclampsia supports the role of bacteria in the multifactorial cause of pre-eclampsia. *J Obstet Gynaecol Res* 2015;41:662–9.

144 Jiménez E, Marín ML, Martín R, *et al*. Is meconium from healthy newborns actually sterile? *Res Microbiol* 2008;159:187–93.

145 Mshvildadze M, Neu J, Shuster J, *et al*. Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. *J Pediatr* 2010;156:20–5.

146 Perez-Muñoz ME, Arrieta M-C, Ramer-Tait AE, *et al*. A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. *Microbiome* 2017;5:48:48.:

147 Lauder AP, Roche AM, Sherrill-Mix S, *et al*. Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. *Microbiome* 2016;4:29:29.:.

148 de Goffau MC, Lager S, Sovio U, *et al*. Human placenta has no microbiome but can contain potential pathogens. *Nature* 2019;572:329–34.

149 Lim ES, Rodriguez C, Holtz LR. Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community. *Microbiome* 2018;6:87:87.:.

150 Olomu IN, Pena-Cortes LC, Long RA, *et al*. Elimination of "kitome" and "splashome" contamination results in lack of detection of a unique placental microbiome. *BMC Microbiol* 2020;20:157:157.:.

151 Theis KR, Romero R, Winters AD, *et al*. Does the human placenta delivered at term have a microbiota? results of cultivation, quantitative real-time PCR, 16S rrna gene sequencing, and metagenomics. *Am J Obstet Gynecol* 2019;220:S0002-9378(18)30900-1:267.:.

152 Theis KR, Romero R, Winters AD, *et al*. Lack of evidence for microbiota in the placental and fetal tissues of rhesus macaques. *MSphere* 2020;5:e00210-20.

153 Leon LJ, Doyle R, Diez-Benavente E, *et al*. Enrichment of clinically relevant organisms in spontaneous preterm-delivered placentas and reagent contamination across all clinical groups in a large pregnancy cohort in the United Kingdom. *Appl Environ Microbiol* 2018;84

154 Sterpu I, Fransson E, Hugerth LW, *et al*. No evidence for a placental microbiome in human pregnancies at term. *American Journal of Obstetrics and Gynecology* 2021;224:296.

155 Lager S, de Goffau MC, Sovio U, *et al*. Detecting eukaryotic microbiota with single-cell sensitivity in human tissue. *Microbiome* 2018;6:151.

156 Kuperman AA, Zimmerman A, Hamadia S, *et al*. Deep microbial analysis of multiple placentas shows no evidence for a placental microbiome. *BJOG* 2020;127:159–69.

157 Kennedy KM, Gerlach MJ, Adam T, *et al*. Fetal meconium does not have a detectable microbiota before birth. *Nat Microbiol* 2021;6:865–73.

158 Stout MJ, Conlon B, Landeau M, *et al*. Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations. *American Journal of Obstetrics and Gynecology* 2013;208:226.

159 Antony KM, Ma J, Mitchell KB, *et al*. The preterm placental microbiome varies in association with excess maternal gestational weight gain. *American Journal of Obstetrics and Gynecology* 2015;212:653.

160 Parnell LA, Briggs CM, Cao B, *et al*. Microbial communities in placentas from term normal pregnancy exhibit spatially variable profiles. *Sci Rep* 2017;7:11200.

161 Liu C-J, Liang X, Niu Z-Y, *et al*. Is the delivery mode a critical factor for the microbial communities in the meconium? *EBioMedicine* 2019;49:S2352-3964(19)30715-7:354–63.:.

162 Tuominen H, Collado MC, Rautava J, *et al*. Composition and maternal origin of the neonatal oral cavity microbiota. *J Oral Microbiol* 2019;11:1663084.

163 Seferovic MD, Pace RM, Carroll M, *et al*. Visualization of microbes by 16S in situ hybridization in term and preterm placentas without intraamniotic infection. *American Journal of Obstetrics and Gynecology* 2019;221:146.

164 Leoni C, Ceci O, Manzari C, *et al*. Human endometrial microbiota at term of normal pregnancies. *Genes (Basel)* 2019;10:971.

165 Zhu L, Luo F, Hu W, *et al*. Bacterial communities in the womb during healthy pregnancy. *Front Microbiol* 2018;9:2163:2163.:.

166 Stinson LF, Boyce MC, Payne MS, *et al*. The not-so-sterile womb: evidence that the human fetus is exposed to bacteria prior to birth. *Front Microbiol* 2019;10:1124.

167 Younge N, McCann JR, Ballard J, *et al*. Fetal exposure to the maternal microbiota in humans and mice. *JCI Insight* 2019;4

168 Walker AW. A lot on your plate? well-to-well contamination as an additional confounder in microbiome sequence analyses. *MSystems* 2019;4:e00362-19.

169 Eisenhofer R, Minich JJ, Marotz C, *et al*. Contamination in low microbial biomass microbiome studies: issues and recommendations. *Trends Microbiol* 2019;27:105–17.

170 Dominguez-Bello MG, Costello EK, Contreras M, *et al*. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010;107:11971–5.

171 Rutayisire E, Huang K, Liu Y, *et al*. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterol* 2016;16:86:86.:.

172 Chu DM, Ma J, Prince AL, *et al*. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat Med* 2017;23:314–26.

173 Reyman M, van Houten MA, van Baarle D, *et al*. Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life. *Nat Commun* 2019;10:4997:4997.:.

174 Shao Y, Forster SC, Tsaliki E, *et al*. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature* 2019;574:117–21.

175 Ferretti P, Pasolli E, Tett A, *et al*. Mother-To-Infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host & Microbe* 2018;24:133–145.

176 He Q, Kwok L-Y, Xi X, *et al*. The meconium microbiota shares more features with the amniotic fluid microbiota than the maternal fecal and vaginal microbiota. *Gut Microbes* 2020;12:1794266.

177 Granger CL, Embleton ND, Palmer JM, *et al*. Maternal breastmilk, infant gut microbiome and the impact on preterm infant health. *Acta Paediatr* 2021;110:450–7.

178 Nuzzi G, Di Cicco ME, Peroni DG. Breastfeeding and allergic diseases: what's new? *Children (Basel)* 2021;8:330.

179 McWilliam V, Venter C, Greenhawt M, *et al*. A pragmatic approach to infant feeding for food allergy prevention. *Pediatr Allergy Immunol* 2022;33:e13849

180 Donald K, Petersen C, Turvey SE, *et al*. Secretory iga: linking microbes, maternal health, and infant health through human milk. *Cell Host Microbe* 2022;30:650–9.

181 Gołębiewski M, Łoś-Rycharska E, Sikora M, *et al*. Mother's milk microbiome shaping fecal and skin microbiota in infants with food allergy and atopic dermatitis: a pilot analysis. *Nutrients* 2021;13:3600

182 Gopalakrishna KP, Hand TW. Influence of maternal milk on the neonatal intestinal microbiome. *Nutrients* 2020;12:823

183 Huus KE, Petersen C, Finlay BB. Diversity and dynamism of iga-microbiota interactions. *Nat Rev Immunol* 2021;21:514–25.

184 Boutin RCT, Sbihi H, Dsouza M, *et al*. Mining the infant gut microbiota for therapeutic targets against atopic disease. *Allergy* 2020;75:2065–8.

185 Orndorff PE, Devapali A, Palestrant S, *et al*. Immunoglobulin-mediated agglutination of and biofilm formation by Escherichia coli K-12 require the type 1 pilus fiber. *Infect Immun* 2004;72:1929–38.

186 Dieterich CM, Felice JP, O'Sullivan E, *et al*. Breastfeeding and health outcomes for the mother-infant dyad. *Pediatr Clin North Am* 2013;60:S0031-3955(12)00157-5:31–48.:.

187 Mennini M, Arasi S, Fiocchi AG. Allergy prevention through breastfeeding. *Curr Opin Allergy Clin Immunol* 2021;21:216–21.

188 Garcia-Larsen V, Ierodiakonou D, Jarrold K, *et al*. Diet during pregnancy and infancy and risk of allergic or autoimmune disease: A systematic review and meta-analysis. *PLoS Med* 2018;15:e1002507.

189 Lin B, Dai R, Lu L, *et al*. Breastfeeding and atopic dermatitis risk: A systematic review and meta-analysis of prospective cohort studies. *Dermatology* 2020;236:345–60.

190 Davis EC, Wang M, Donovan SM. The role of early life nutrition in the establishment of gastrointestinal microbial composition and function. *Gut Microbes* 2017;8:143–71.

191 Jost T, Lacroix C, Braegger CP, *et al*. New insights in gut microbiota establishment in healthy breast fed neonates. *PLoS One* 2012;7:e44595

192 Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. *Front Microbiol* 2014;5:494:494.:.

193 Tamburini S, Shen N, Wu HC, *et al*. The microbiome in early life: implications for health outcomes. *Nat Med* 2016;22:713–22.

194 Stewart CJ, Ajami NJ, O'Brien JL, *et al*. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* 2018;562:583–8.

195 Moore RE, Townsend SD. Temporal development of the infant gut microbiome. *Open Biol* 2019;9:190128:190128.:.

196 Wiciński M, Sawicka E, Gębalski J, *et al*. Human milk oligosaccharides: health benefits, potential applications in infant formulas, and pharmacology. *Nutrients* 2020;12:266

197 Beghetti I, Biagi E, Martini S, *et al*. Human milk's hidden gift: implications of the milk microbiome for preterm infants' health. *Nutrients* 2019;11:2944

198 Lackey KA, Williams JE, Meehan CL, *et al*. What's normal? microbiomes in human milk and infant feces are related to each other but vary geographically: the INSPIRE study. *Front Nutr* 2019;6:45:45.:.

199 Togo A, Dufour J-C, Lagier J-C, *et al*. Repertoire of human breast and milk microbiota: a systematic review. *Future Microbiol* 2019;14:623–41.

200 Dwyer LR, Scharschmidt TC. Early life host-microbe interactions in skin. *Cell Host Microbe* 2022;30:S1931-3128(22)00102-0:684–95.:.

201 Xiao L, Wang J, Zheng J, *et al*. Deterministic transition of enterotypes shapes the infant gut microbiome at an early age. *Genome Biol* 2021;22:243:243.:.

202 Dzidic M, Mira A, Artacho A, *et al*. Allergy development is associated with consumption of breastmilk with a reduced microbial richness in the first month of life. *Pediatr Allergy Immunol* 2020;31:250–7.

203 Wang S, Wei Y, Liu L, *et al*. Association between breastmilk microbiota and food allergy in infants. *Front Cell Infect Microbiol* 2021;11:770913:770913.:.

204 Fehr K, Moossavi S, Sbihi H, *et al*. Breastmilk feeding practices are associated with the co-occurrence of bacteria in mothers' milk and the infant gut: the child cohort study. *Cell Host Microbe* 2020;28:S1931-3128(20)30350-4:285–297.

205 Moossavi S, Sepehri S, Robertson B, *et al*. Composition and variation of the human milk microbiota are influenced by maternal and early-life factors. *Cell Host Microbe* 2019;25:S1931-3128(19)30049-6:324–335.

206 Baumann-Dudenhoeffer AM, D'Souza AW, Tarr PI, *et al*. Infant diet and maternal gestational weight gain predict early metabolic maturation of gut microbiomes. *Nat Med* 2018;24:1822–9.

207 Davis KL, Marks SN. Enterovirus infections in early childhood and the risk of atopic disease-a nested case-control study. *Pediatrics* 2014;134 Suppl 3:S135–6.

208 Valles-Colomer M, Bacigalupe R, Vieira-Silva S, *et al*. Variation and transmission of the human gut microbiota across multiple familial generations. *Nat Microbiol* 2022;7:87–96.

209 Rosenberg E, Zilber-Rosenberg I. Reconstitution and transmission of gut microbiomes and their genes between generations. *Microorganisms* 2021;10:70

210 Tochitani S. Vertical transmission of gut microbiota: points of action of environmental factors influencing brain development. *Neurosci Res* 2021;168:S0168-0102(20)30490-9:83–94.:

211 the CHILD Study Investigators, Tun HM, Konya T, *et al*. Exposure to household furry pets influences the gut microbiota of infants at 3–4 months following various birth scenarios. *Microbiome* 2017;5:40.

212 PASTURE study group, Depner M, Taft DH, *et al*. Maturation of the gut microbiome during the first year of life contributes to the protective farm effect on childhood asthma. *Nat Med* 2020;26:1766–75.

213 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259–60.

214 Okada H, Kuhn C, Feillet H, *et al*. The"ygiene hypothesis"for autoimmune and allergic diseases: an update. *Clin Exp Immunol* 2010;160:1–9.

215 Rook GAW, Martinelli R, Brunet LR. Innate immune responses to mycobacteria and the downregulation of atopic responses. *Current Opinion in Allergy and Clinical Immunology* 2003;3:337–42.

216 Rook GAW, Lowry CA, Raison CL. Microbial"ld friends" immunoregulation and stress resilience. *Evol Med Public Health* 2013;2013:46–64.

217 Derrien M, Alvarez A-S, de Vos WM. The gut microbiota in the first decade of life. *Trends in Microbiology* 2019;27:997–1010.

218 Robertson RC, Manges AR, Finlay BB, *et al*. The human microbiome and child growth – first 1000 days and beyond. *Trends in Microbiology* 2019;27:131–47.

219 Dogra S, Chung C, Wang D, *et al*. Nurturing the early life gut microbiome and immune maturation for long term health. *Microorganisms* 2021;9:2110.

220 Olin A, Henckel E, Chen Y, *et al*. Stereotypic immune system development in newborn children. *Cell* 2018;174:S0092-8674(18)30848-1:1277–92.:.

221 Torow N, Marsland BJ, Hornef MW, *et al*. Neonatal mucosal immunology. *Mucosal Immunology* 2017;10:5–17.

222 Wopereis H, Oozeer R, Knipping K, *et al*. The first thousand days - intestinal microbiology of early life: establishing a symbiosis. *Pediatr Allergy Immunol* 2014;25:428–38.

223 Sassin AM, Johnson GJ, Goulding AN, *et al*. Crucial nuances in understanding (mis) associations between the neonatal microbiome and cesarean delivery. *Trends in Molecular Medicine* 2022;28:806–22.

224 Wampach L, Heintz-Buschart A, Fritz JV, *et al*. Birth mode is associated with earliest strain-conferred gut microbiome functions and immunostimulatory potential. *Nat Commun* 2018;9:5091.

225 Jakobsson HE, Abrahamsson TR, Jenmalm MC, *et al*. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. *Gut* 2014;63:559–66.

226 Kalbermatter C, Fernandez Trigo N, Christensen S, *et al*. Maternal microbiota, early life colonization and breast milk drive immune development in the newborn. *Front Immunol* 2021;12:683022:683022.:

227 Stokholm J, Thorsen J, Blaser MJ, *et al*. Delivery mode and gut microbial changes correlate with an increased risk of childhood asthma. *Sci Transl Med* 2020;12

228 Hansen CHF, Andersen LSF, Krych L, *et al*. Mode of delivery shapes gut colonization pattern and modulates regulatory immunity in mice. *J Immunol* 2014;193:1213–22.

229 Zachariassen LF, Hansen AK, Krych L, *et al*. Cesarean section increases sensitivity to oxazolone-induced colitis in C57BL/6 mice. *Mucosal Immunol* 2019;12:1348–57.

230 Roswall J, Olsson LM, Kovatcheva-Datchary P, *et al*. Developmental trajectory of the healthy human gut microbiota during the first 5 years of life. *Cell Host Microbe* 2021;29:S1931-3128(21)00100-1:765–776.

231 Bisgaard H, Li N, Bonnelykke K, *et al*. Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *J Allergy Clin Immunol* 2011;128:646–52.

232 Abrahamsson TR, Jakobsson HE, Andersson AF, *et al*. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* 2012;129:434–40,

233 Azad MB, Konya T, Guttman DS, *et al*. Infant gut microbiota and food sensitization: associations in the first year of life. *Clin Exp Allergy* 2015;45:632–43.

234 Al Nabhani Z, Dulauroy S, Marques R, *et al*. A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. *Immunity* 2019;50:S1074-7613(19)30081-0:1276–1288.

235 Al Nabhani Z, Eberl G. Imprinting of the immune system by the microbiota early in life. *Mucosal Immunol* 2020;13:183–9.

236 Bergström A, Skov TH, Bahl MI, *et al*. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. *Appl Environ Microbiol* 2014;80:2889–900.