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Remodelling of Mother's Microbiome in Pregnancy: A Review

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Abstract
Pregnancy is a natural process of growth and development and in this period many physiological changes occur including changes in body composition, inflammation, endocrine and metabolic changes. This period of life is driven by a series of immunological, hormonal, and metabolic changes that are essential for the normal fetal development and successful delivery. These various changes have distinct effects on microbiota which is altered accordingly. The microbial changes during pregnancy are likely co-ordinated with the immune, endocrine, and metabolic states. The maternal pregnancy microbiome is important for fetal development and the mother's health. The microbiome of the mother, the placenta and the fetus influence the fetal growth and undoubtedly plays a major role in the adequate development of the newborn infant. The microbiome modulates the inflammatory mechanisms related to physiological and pathological processes that are involved in the perinatal progress through different mechanisms. In this review the known changes in microbial composition in the tenure of pregnancy at a variety of body sites including the gut, vagina, oral cavity and placenta are discussed.

Keywords
Gestation, Gut, Vagina, Placenta, Microbiota

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INTRODUCTION
Pregnancy is a condition that causes physiological impact on musculoskeletal, endocrine, cardiovascular, respiratory, and gastrointestinal system of women. It induces immunological and metabolic changes manifested as immune tolerance that allow implantation and placentation; and insulin resistance to support growth of the fetus [1]. Pregnancy is a diabetogenic state and the adaptations in glucose metabolism allow shunting of glucose to the fetus to promote development, while maintaining adequate maternal nutrition [2]. Insulin-secreting pancreatic β-cells undergo hyperplasia resulting in increased insulin secretion and increased insulin sensitivity in early pregnancy, followed by progressive insulin resistance [3]. With the progression of pregnancy, progesterone, prolactin and estrogen level rises in female body. Additionally, the women is also exposed to placental hormones such as human chorionic gonadotropin, human placental lactogen, placental gonadotropin. Changes in estrogen and progesterone levels during gestation apart from tissue specific modification at endometrium and breast, also influence the structural alterations in the gastrointestinal tract. These include abnormalities in gastric neural activity and smooth muscle function, leading to gastric dysrhythmia or gastroparesis. The alterations are pronounced in women with pre-existing gastrointestinal diseases such as gastroesophageal reflux disease, diabetic gastroparesis, gastric bypass surgery or inflammatory bowel disease [3,4]. At the

onset of pregnancy, the immune system represses itself to recognize and assist implantation of the fetus and restrengthens towards mid and later stages of pregnancy to prevent invasion by pathogens [5]. This 'rewiring of immune system' throughout pregnancy induces a low-grade inflammation at the mucosal surfaces of gut, oral cavity, vagina, and placenta. This leads to structural and compositional changes in the microbiota of these body sites [1,5].

The Healthy Human Microbiome

The human microbiome is a collection of bacteria, archaea, protozoa, fungi and viruses all residing in our body including their genetic material. Sequence-based screening and analytical techniques establish that they play important roles in host metabolism, immunity, endocrinology, and overall health. The microbial compositions vary between people and greatly affected by diet, additional environmental factors and immune status [6] . The diverse gastrointestinal microbiota is predominantly composed of bacteria from three major phyla, namely *Firmicutes*, *Bacteroidetes* and *Actinobacteria* [7]. The symbiotic relationship between the gut microbiota and the host is regulated and stabilized by complex network of interactions that encompass metabolic, immune, and neuroendocrine crosstalk between them. The human vaginal microbiome is dominated by *Lactobacillus* which create an acidic environment that keep safe women from sexually transmitted pathogens and opportunistic infections. Relative abundance of lactobacilli in human vagina is typically >70% whereas in other mammals lactobacilli concentration is rarely more than 1% of the total vaginal microbiota. Furthermore, the loss of *Lactobacillus*-dominance is linked to bacterial vaginosis (BV) which is associated with an overgrowth of anaerobic bacteria [8]. Additionally, the skin, vagina, and oral cavity provide important niches for distinct bacterial communities, which contribute to the immune system by defense against potential pathogens [9].

Pregnancy Induced Changes in Vaginal Microbiota

The vaginal mucosal ecosystem is comprised of a stratified squamous non-keratinized epithelium overlaid by a mucosal layer continuously lubricated by cervicovaginal fluid (CVF). The vaginal microbiota is unique as it undergoes remarkable compositional changes throughout a women's lifespan from birth to puberty and menopause. The organization of the vaginal microbiota is exclusively dynamic which corresponds to the hormonal fluctuations throughout the woman's reproductive life, and during pregnancy. During the menstrual cycle, stability of microbial communities is higher between 14 and 21 days of menstrual cycle i.e at the time when estrogen concentrations are high [10]. This has been attributed to the effect of estrogens on the maturation of the vaginal epithelium causing deposition of glycogen on the upper layer of the epithelium [10,11]. Glycogen is a carbon source metabolized to lactic acid by *Lactobacillus* spp., causing a low vaginal pH [10,12].

In most of the women of reproductive age a good number of protective lactic acid-producing *Lactobacillus* species dominates the healthy vaginal microbiota. The primary colonizing bacteria of a healthy individual are of the genus *Lactobacillus* (90–95%), the most common being *L. crispatus, L. iners, and L. gasseri* [13]. These bacteria protect against vaginal dysbiosis and inhibit opportunistic infections through the direct and indirect protective effects of *Lactobacillus* products, such as lactic acid and bacteriocin among others. Lactic acid decreases vaginal pH and thus restricts a broad range of infections which directly affects host immune functions by inhibiting pro-inflammatory responses and help to release mediators from vaginal epithelial cells to stimulate antiviral response [12,13]. In addition, *Lactobacillus*-derived bacteriocins (ribosomally-synthesized bacterial antimicrobial peptides) may inhibit pathogen growth [14].

During normal pregnancy, the composition of the vaginal microbiota changes as a function of gestational age, with a rise in the relative abundance for *Lactobacillus* spp., such as *L. crispatus*, *L. jensenii*, *L. gasserii*, *L. vaginalis*, and a decrease in anaerobe or strict anaerobe microbial species. The composition of the vaginal microbiome associated with pregnancy may have functional (that is, metabolic, immune) implications for the host. Indeed, low risk pregnant women have more stable vaginal flora throughout the pregnancy than non-pregnant women [13, 14]. Normal changes in the vaginal flora during pregnancy are transitions to another *Lactobacillus* community, and this stability would protect against ascending infections through the genital tract.

Lactobacilluscrispatus and *Lactobacillusjensenii* num ber rises as part of the vaginal microbiomial stability associated with gestation. These species metabolise glycogen hydrolysates to produce lactic acid to propagate their growth and replication. This is facilitated by high levels of estrogen that stimulates glycogen accumulation in vaginal epithelial cells and epithelial-derived α-amylase that depolymerizes the α-glucan molecules [15,16]. Other than lactic acid, the lactobacilli produce hydrogen peroxide and bacteriocins that inhibit the growth of bacterial vaginosis-associated pathogens [15,16] thereby

preventing infection associated spontaneous preterm birth. Interestingly, the dominant *Lactobacillus* species in pregnancy varies according to ethnic group; while *L. jensenii* is predominantly observed in women of Asian and Caucasian ethnicity, *L. gasseri* is absent in samples from Black women [17].

The vaginal microbiota at late gestation is indistinguishable to that of a non-pregnant (non-menstruating and bacterial vaginosis- negative) state characterized by a decrease in α-diversity (species richness – within individual diversity or amount of different species detected in the vaginal sample), and a corresponding rise in *Lactobacillus* spp [18]. This resemblance is believed to trigger the physiological changes that are associated with parturition and of course this is not without the support of rising estrogen, increased vaginal epithelial glycogen accumulation, and low pH [15,18]. The rise of disease-causing pathogens in the vagina is analogous to complications of pregnancy, with an increased risk of preterm birth and spontaneous abortion [15]. A meta-analysis reported significant diversity dissimilarities in vaginal microbiomes in the first trimester, between women with term and preterm outcomes which indicates a prospective diagnostic significance of microbiome-related biomarkers [19].

It has been observed that the relationship between the vaginal microbiota and obstetric complications is population dependent. Women of European ancestry are more likely to develop a *Lactobacillus*-dominated microbiome, whereas African American women are more likely to exhibit a diverse microbiotic profile [20].

The gestation-associated changes in the vaginal microbiota also gives prospective postnatal benefits to the offspring. *Lactobacillus johnsonii* is primarily found in the upper gut of newborn where it sustains its survival and persistence by catalysing the hydrolysis (releasing of taurine and glycine) and uptake of bile through its bile salt hydrolase and transporters [21]. It inhibits the growth and survival of other *lactobacilli* and *enterococci* in the gut through the production of bacteriocin-Lactacin F. These properties aid postnatal digestion of breast milk by neonates inoculated with vaginal *L. johnsonii* at birth [18].

Placental Microbiota

Placenta is a highly complex and fascinating organ. During the course of a pregnancy, it acts as the lungs, gut, kidneys, and liver of the fetus. The placenta also has major endocrine actions that modulate maternal physiology and metabolism and provides a safe and protective milieu in which the fetus can develop. The placenta is a potential barrier against infections and plays an important role in modulation of pregnancy and immunity. Dysfunctions of placenta are linked with complications such as preeclampsia, intrauterine growth restriction and stillbirth [22]. The composition of the placental microbiome is distinct from that of the vagina and has been reported to resemble the oral microbiome. Compared to the gut microbiome, the placental microbiome exhibits limited microbial diversity during pregnancy. A unique placental microbiome niche, composed of nonpathogenic commensal microbiota from the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla have been reported using whole-genome shotgun sequencing of samples from 320 subjects [23]. Several findings using both culture and metagenomic techniques have suggested the presence of a low biomass microbial community in the healthy placenta [23]. The abundance of different species of *Lactobacillus*, *Propionibacterium*, and members of the *Enterobacteriaceae* family have been detected by DNA-based studies in placental tissue of pregnant women at term but it is under debate [24]. Several species of the oral microbiome were detected in the placenta, including *Prevotella tannerae* (gingival crevices) and nonpathogenic *Neisseria* species (mucosal surfaces) [24].

In addition, other authors have confirmed a distinct microbiota in both the placenta and amniotic fluid of healthy women at the time of elective C-section which is characterized by low richness, low diversity, and the predominance of *Proteobacteria* [24]. Similarly, other studies have found microbes in amniotic fluid and umbilical cord blood in healthy asymptomatic women, as well as in those with pregnancy complications [25]. During characterizing of the placental microbial community, it was observed that the major phylum was *Proteobacteria*, and while comparing to all other organs, the microbial composition was most similar to the oral microbiota, including species such as *Prevotella tannerae* and *Neisseria* [25]. The similarity between the oral and placental microbiota suggests that bacteria may pass from the oral cavity to the placenta which possibly explains many observations of women with periodontal disease having increased risk of pregnancy complications [24]. Some of those oral bacteria, such as *Fusobacterium nucleatum*, may be transmitted hematogenously during placentation by binding to the vascular endothelium, and modifying its permeability and the translation of other common commensals, such as *Escherichia coli* [26]. In other study no evidence for existence of placental microbiome was reported [27].

Endometrial Microbiota

After sequencing and analysis of specific regions of bacterial ribosomal RNA, a resident endometrial microbiota has been defined [28]. Early studies on the endometrial microbiota reported the dominance of *Lactobacillus* species. Moreno *et al report* an association between low levels of *Lactobacillus* species (<90% *Lactobacillus* with >10% other bacteria) in the endometrial microbiota and poor pregnancy outcomes regarding implantation success and ongoing and term pregnancy rates [29]. A further study describes an endometrial microbiota mainly dominated by *Bacteroides* [1].

Pregnancy Induced Changes in Intestinal Microbiota

The normal gut microbiota imparts specific function in host nutrient and drug metabolism, maintenance of structural integrity of the gut mucosal barrier, immunomodulation, and protection against pathogens. The microbiota is distributed throughout the gut varying according to region and determined by pH, nutrient, and oxygen availability. Overall, the gut microbiome influence energy utilization, hormonal, immunologic and metabolic health of the host. In general, *Firmicutes* and *Bacteroides*, which constitute >95% of the bacterial population, dominate the physiologic gut microbiota in the non-pregnant state. Other bacteria phyla/divisions present in small proportions include Proteobacteria, Fusobacteria, Actinobacteria and *Verruco microbia* [30].

These microorganisms ferment non-digestible dietary fibres to produce metabolites (short chain fatty acids, SCFAs) which are accountable for maintaining intestinal barrier integrity and prevent leakage of bacteria lipopolysaccharide (LPS) into systemic circulation [30]. The SCFA butyrate, propionate or acetate are the key drivers of T-cell subset proliferation and activity [31,32].

The microbial diversity in the gut at the initial stage of pregnancy appears to be similar to that of non-pregnant women [1]. With the progression of pregnancy, the abundance of gut bacteria associated with inflammatory states increases in nearly 70% of women. The greatest change in the gut microbiota occurs in the ratio of certain key bacteria (*Firmicutes: Bacteroidetes*), mimicking the higher levels of *Firmicutes* seen in obesity [33]. Levels of proinflammatory cytokines (including IFN-y, IL-2, IL-6 and TNF-α) also rise in serum, adipose and placental tissue later In pregnancy and also in the mucosal surfaces throughout the gastrointestinal tract which reflect a low-grade inflammatory state [34]. In the context of a normal pregnancy, these metabolic and immunologic changes improve energy storage in maternal tissue which is required for fetal growth and lactation [34, 35].

From the first to the third trimester, the gut microbiota composition changes dramatically. These changes are characterized by increased abundance of members of the *Actinobacteria* and *Proteobacteria* phyla, as well as a reduction in individual richness (alpha diversity) [36]. In addition, levels of *Faecalibacterium*, a butyrate-producing bacterium with anti-inflammatory activities, which is depleted in metabolic syndrome patients are significantly decreased in the third trimester of pregnancy [1]. Between-subject diversity (beta diversity), is increased in the thsird trimester, coupled with weight gain, insulin insensitivity, and higher levels of fecal cytokines, reflecting inflammation [36]. The third trimester microbiota has been proved to cause increased weight gain, insulin resistance and significant higher inflammatory response when transferred to germ – free mice [36] . With the advancement of pregnancy, hormonal and immunological changes increase nutrient and energy harvest from the gut. These changes are induced by increased estrogen and progesterone that inhibit gastrointestinal contractility and prolong transit providing a suitable environment (substrates) for energy-harvesting microbes. The consequent dysbiosis triggers a low-grade inflammatory state propagated by pro-inflammatory chemocytokines leading to insulin resistance and hyperglycemia.

Intestinal microbiota has also been suggested to play a role in host weight gain in pregnancy through increased absorption of glucose and fatty acids, increased fasting induced adipocyte factor (FIAF) secretion, induction of catabolic pathways and stimulation of immune system [37]. Also, overweight pregnant women have significant higher levels of gut *Bacteroides* and *Staphylococcus* than pregnant women having normal body weight [37]. The gut microbiota during pregnancy is a critical determinant of offspring health which regulate the development of atopy and autoimmune phenotypes in the offspring [34]. The commensal microbiota has a role in regulating host immunity to pathogens and autoimmune responses. Indeed, the microbiota is a source of metabolites and peptide ligands for T cell recognition, known as pathogen-associated molecular patterns (PAMPs), which are recognized by immune receptors. Microbiota-derived metabolites and PAMPs can affect target organs and activate the autoimmune cascade [35].

Pregnancy induced changes in oral microbiota

The oral cavity including the teeth, gingival sulcus, tongue, cheeks, tonsils, hard and soft palates

represent a natural niche for up to 700 different species of Streptococci, *Lactobacilli, Staphylococci, Corynebacteria,* etc [38]. The main change occurring in the oral microbiota during pregnancy is a rise in viable microbial load and dramatic variation throughout pregnancy. Several studies have examined the differences in the levels of oral microorganisms in pregnant and non-pregnant women [39,40] In their study, Fujiwara *et al*. compared the oral microbiota composition in pregnant and non-pregnant women and observed that the number of microorganisms identified in pregnant women's saliva samples was significantly higher than non-pregnant women [40].

They showed that, Porphyromonas *gingivalis*, Aggregati bacter *actino mycetemcomitans*, *Streptococci, Staphylococci*, and *Candida species* were significantly higher in the pregnant women especially during the first and second trimester while both groups carried the same percentages of *Prevotella intermedia*; *Fusobacterium nucleatum* and *Streptococci* species [38,40].. Furthermore, Candida levels were significantly higher during middle and late pregnancy compared to non-pregnant women, further demonstrating a higher prevalence of periodontal pathogens in pregnancy [39].

Microbiome in Adverse Pregnancy Outcomes

The fetal and mother microbiome in relation to adverse outcomes of pregnancy such as low birth weight has been studied. In a study by Ardissone *et al* compared the meconium microbiome in newborn before and after 33 weeks of gestation and concluded that *Enterococcus* and *Enterobacter* negatively correlated with gestational age and *Lactobacillus* and *Phortorhabdus* concentration was much higher in newborns who were born before completing 33 weeks of gestation [41]. Overweight preganant women who harbour a high amount of energy harvesting microbes usually supply high amount of energy to the foetus which leads to a high birth weight and increased risk of complications to both the mother and newborn [42]. The gut bacterial load increases throughout pregnancy irrespective of maternal body weight. Specifically, *Bacteroides. fragilis* and *Staphylococcus* have been shown to increase with weight gain as pregnancy advances in normal weight women [42,43]. Also, placental tissue of preterm delivered mothers has reported to contain high concentration of *Burkholderia*, *Actinomycetales* and *Alphaproteobacteria* and preterm mothers with severe chorioamnionitis had higher abundance of *Ureaplasma parvum*, *Fusobacterium nucleatum* and *Streptococcus agalactiae* [44]. Mothers with a history of antepartum urinary infection have shown the presence of *Streptococcus* and *Acinetobacter in* their placental sample [45]. Periodontal diseases such as gingivitis and periodontitis are chronic oral infections characterized by local and systemic inflammatory responses and have been associated with adverse outcomes of pregnancy like premature birth, preeclamsia and miscarriage [46,47]. Periodontitis affects about 40 % of pregnant women and is associated with a decrease gestational age [48].

CONCLUSION

The endocrine and physical changes that accompany pregnancy trigger an array of anatomical, physiological, and biochemical alterations that affect every organ of the body. The microbiome of different body sites including the vagina and gut are in a continuous crosstalk [15] and exhibit immunological and metabolic changes associated with the progression of normal, healthy gestation. Early microbial colonization of fetus suggests that from the very beginning of development there may be reciprocal interactions between developing host and that maternal microbial components which also helps to build up infant immunity. So, larger and prospective studies are needed to characterize the evolution of the microbiota during different conditions and its influence on healthy and pathological pregnancies, on onset of labor, and on other physiological and metabolic determinants of mother and offspring in perinatal period.

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