Review Article

The Role of Microbiome in Preterm Labor: Recent Advances and Future Challenges

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Abstract

Problem: Our current understanding of preterm labor is limited in the context of dysbiosis of microbiome.

Background: Human microbiome is comprised of trillions of microorganisms, residing on and within our bodies that are associated with the vital function of organs and systems. Few studies have highlighted the role of maternal gut microbiome dysbiosis as a contributing factor for metabolic syndrome and may be associated with risk of preterm labor. A clear understanding of dysbiosis in placenta or vagina with early ripening of cervix for initiation of preterm labor is not fully elucidated.

Aim: To contextualize the alteration in microbial community structure in maternal and fetal organs in association with risk of preterm labor.

Methods: In this review, we summarized the studies published from 2000 to 2016 in the field of microbiome in association with preterm labor.

Findings & Discussion: Evidence suggest that placental microbiome has promising correlation with preterm labor, while findings are equivocal for maternal gut and vaginal microbiome in inducing preterm labor. Similar alteration in fetal intestinal microbiome of meconium is supposed to evoke an inflammatory response that may lead to preterm labor.

Conclusion: To understand the relationship of microbiome and preterm labor, both maternal and fetal microbiome should be studied in parallel to determine the causality. Dysregulation of immune response due to disturbance in normal or commensal flora might be an underlying cause of preterm labor.

Keywords: Microbiome, Preterm labor, Dysbiosis.

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Introduction

Preterm labor is defined as initiation of uterine contractions at any stage before 37 weeks of gestation.[1] Despite advances in antenatal care, the rate of preterm labor is still as high as 18%.[2] Lower middle income countries (LMIC) contribute up to 60% of all premature births.[3] Infant death associated with prematurity outnumbered the combined death toll due to malaria, tuberculosis and AIDS.[4] These infants are at higher risk of developing comorbidities such as respiratory distress syndrome, neonatal sepsis leading to multi system organ failure, intra ventricular hemorrhage, seizures and autism[5] while 40% of these cases succumb to neonatal deaths.[3]

Among various factors contributing to preterm birth, inflammation and release of inflammatory mediators are thought to be the primary trigger for initiation of labor. The infiltration of immune cells into the gestational sac leads to weakening of fetal membranes, placental detachment and cervical ripening which are the hallmark of uterine

contractions.[2] Commensal organisms or normal flora seems to maintain the balance of immune homeostasis in gut as well as in reproductive organs. Therefore, the changes in normal flora were assessed by microbiome research to understand its role in normal pregnancy and associated complications such as preterm birth. With the advent of latest technology such as Next Generation Sequencing (NGS), in-depth analysis of commensal microbe using 16S ribosomal gene can be performed for a number of biological specimens including vaginal fluid, meconium, amniotic fluid and placental tissue. This review summarizes key papers and hand searches of references retrieved from the literature published during 2000 to 2016. Here we discuss the potential role of microbiome in preterm labor. Finally, it takes into account of benefit and relevance of conducting future studies on maternal and fetal microbiome and associated challenges in LMIC countries.

Maternal Microbiome in Pregnancy

The microorganisms that resides on and inside human bodies are known as the microbiota. It is estimated that this microbiota outnumber the human somatic and germ cells by a factor of ten.[6] Together, the genomes of these microbial symbionts is collectively termed as the microbiome. On the other hand, disturbance in the homeostasis between microbiota and the host is termed as “Dysbiosis”. The human microbiome comprises of trillions of commensal microorganisms, residing on and within our bodies.[7] These microbial communities depend on our diet, lifestyle, environment and genetic factors, and therefore play an important role in functioning of various organs and systems. Although, there is 99.9% genetic resemblance among two persons, yet dissimilarity in the microbiome is almost 90%.[8] Therefore, it has potential impact on physiological as well as pathological state of an individual. Numerous studies have already highlighted this linkage between microbial dysbiosis and disorders such as type 2 diabetes, Crohn’s disease and obesity.[9]

Maternal microbiome and fetal wellbeing was first studied by Koen *et al*., who reported the first ever evidence of alteration of gut microbiome during pregnancy; suggesting its role in maintenance of nutritional requirements during the course of pregnancy.[10] Afterwards, site specific microbiota (vagina, gut, saliva and gums, etc.) have also been investigated for involvement in various complications of pregnancy.[11] Here, we discuss the role of maternal, placental and fetal microbiome in the perspective of preterm labor.

Maternal Gut microbiome and preterm labor

The gut microbiome influence digestion, absorption and metabolism during gut homeostasis. Alteration in gut microbial community known as “gut dysfunction” is also associated with obesity and type 2 diabetes.[12] Impact of gut microbiome on brain functioning generated a new concept of gut-brain axis. Both animal and human studies showed the influence of gut microbiome on neurocognitive functions.[13]

The study in Danish population reported a low diversity of gut microbiota in obese people compared to non-obese.[14] This suggests that absence of bacterial diversity is associated with adiposity, insulin resistance, dyslipidemia and progressive weight gain[14]. Similar to this, patients with insulin resistance had elevated branched chain amino acids, produced by a particular gut resident bacteria which was missing in healthy controls.[15]

Unfavorable consequences on fetal well-being and a high incidence of preterm labor in mothers with metabolic syndrome was also reported.[16] Dysbiosis of gut microbiota may serve as the underlying cause of preterm labor, however, there is no proof to support the hypothesis of variation of gut microbiome in term or preterm labor except an evidence of maternal weight gain across the pregnancy with associated changes in gut microbiome.[17] Abundance of Bacteroidetes in maternal gut correlated with fetuses born with higher birth weight as well as with the risk of preterm labor. This point towards the use of probiotics in facilitation of normal pregnancy while preventing preterm labor. In future, it would be worth investigating the role of gut microbiome in malnourished pregnant women as it is well established that it changes rapidly with alteration in diet.[18]

Vaginal Microbiome and Preterm labor

Ascending infections from the vagina is one of the causes of preterm labor. A higher load of Ureaplasma

parvum was found in vaginal samples in preterm labor[19]. Vaginal microbiome of healthy women is enriched with Lactobacillus species and is considered as part of good/healthy microbiome. In-depth study of vaginal microbiome across the gestation was first observed in a longitudinal study, where microbiota of non-pregnant and pregnant females was compared during antenatal period. A high diversity in microbial community was reported in pregnant females[20] which showed a dynamic nature of microbiome during pregnancy. Since Lactobacilli are considered as the part of healthy pregnancy, it was also thought that elevated estrogen levels are associated with proliferation of Lactobacilli and maintenance of healthy pregnancy.[4] Contrary to this, vaginal environment lacking Lactobacillus species have failed to correlate with preterm birth. However, relationship existed in preterm labor where mothers had a history of at least one preterm birth.[21] Lactobacilli have a controversial role in protection and prevention of complications of pregnancy, and further evidence is required to develop a consensus for its use as probiotics.

Table I summarizes the work published on vaginal microbiome and preterm pregnancy.

Table I Summary of findings of vaginal microbiome with term and preterm pregnancy.

Author	Study design	Technique	Key finding
Romero. et al. [20] (2013)	Longitudinal case-control study	16S rDNA	Lactobacillus is predominant specie among microbial communities during normal pregnancy.
Romero. et al. [22] (2014)	Longitudinal case-control study	16S rRNA	No significant difference in vaginal communities was observed in preterm vs. term deliveries.
Marina R. S. etal.[23] (2014)	Longitudinal study	16S rDNA and sequencing	Normal pregnancy is characterized by a microbiome that has low diversity and high stability with predominance of lactobacillus specie. Dynamics of microbiome is highly influenced by ethnic differences and different risk of complications associated with pregnancy.
Hyman R. et al[21] (2013)	Prospective longitudinal study	16S rDNA and sequencing	Ethnicity and sampling sites are important determinants of the vaginal microbiome that plays a significant role in preterm birth. No variation in vaginal microbiome reported between trimesters.
Cox, C. et al[19] (2016)	Case control study	Histopathology, PCR	Detection of Ureaplasma Parvum (a vaginal commensal) from the placental tissue, is significantly associated with acute chorioamnionitis in women presenting with preterm labor.

Placental microbiome:

Placenta has a complex role during pregnancy. A number of physiological functions such as homeostasis, protected transportation across membranes and protection against infectious agents are carried out by it. Advances in the last decade has shifted the paradigm of our prior understanding of sterile environment of placenta to numerous colonies of microorganisms residing within this organ.[24] With the successful completion of Human Microbiome Project, the role of placental microbiome has revealed in various physiological as well as pathological conditions during pregnancy.[7]

In the absence of any intrauterine infection or inflammation, microbial colonies have still been isolated from placental tissue probably as part of commensal organisms.[25] These communities are thought to be involved in the uptake of nutrients and maintenance of homeostasis within the organ.[10] For example, it has been hypothesized that inflammatory environment is essential for basic events in the first trimester such as trophoblastic invasion and implantation of the blastocyst. This mechanism of inflammation is regulated by Toll like receptors (TLR) present on placental cells which recognize bacteria, viruses, fungi and parasites. These TLR are the mediators of an “educated” immune response that pedals various physiological immune reactions during pregnancy such as initiation of parturition. Presence of microbial communities in the microenvironment of a placental tissue may regulate the maternal immune response that is essential to maintain hormone with the fetus.[26] Maternal-fetal tolerance, being a key

feature of healthy pregnancy proposes an important role of commensal population that maintains quiescence in maternal as well as in fetal tissue.[27] The roles of physiological and pathological inflammation have been hypothetically associated with microbiome observed in preterm and term labor in the figure- 1.

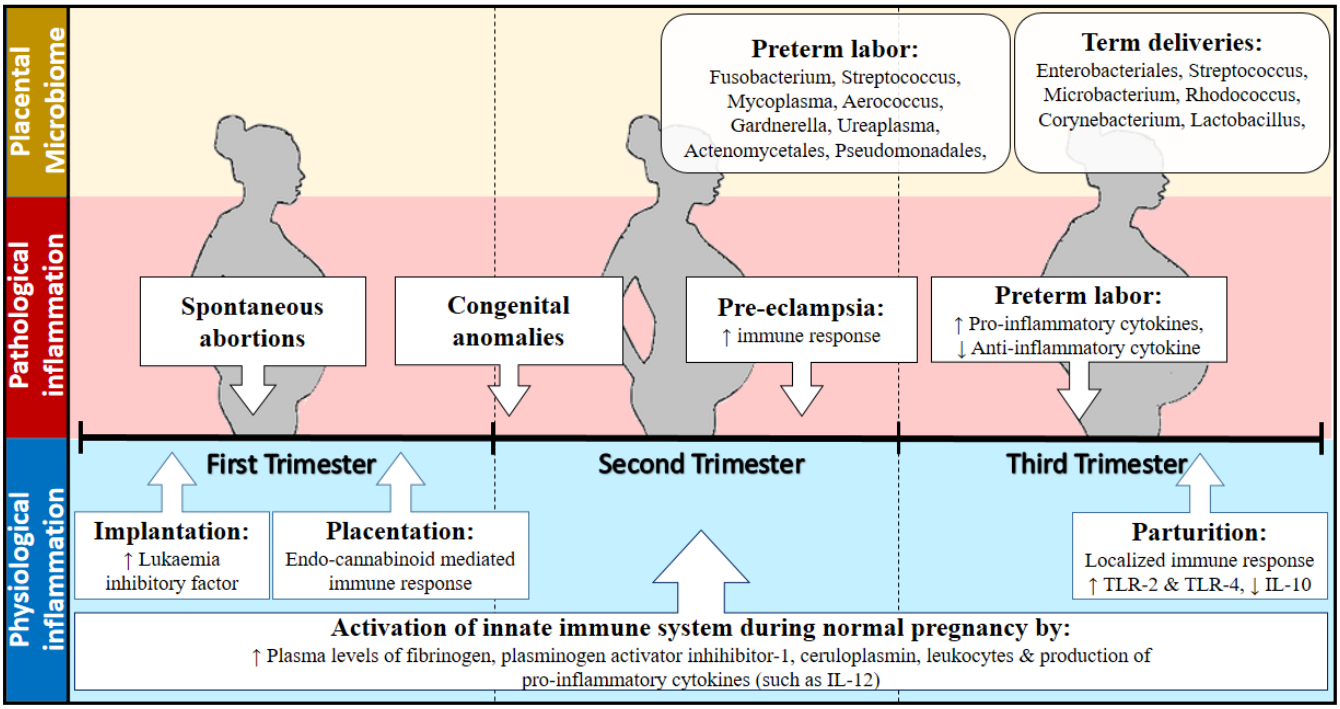

Figure 1: Describes the length of pregnancy, hypothetically divided into three phases: Role of physiological inflammation and pathological inflammation in association with placental microbiome in term and preterm labor.

In the context of preterm labor, there was an obvious difference in the microbiome found in very preterm placental tissue as compared to labor induced after 37 weeks, suggesting correlation with the gestational age.[28] In addition, role of microbiome is also evident in assisted reproduction where experimental use of Lactobacillus Crispatus showed an enhanced rate of implantation and live births, at the same time decreasing the rate of uterine infection[29]. Mechanistic studies on microbiome and TLR will advance our understanding in the development of probiotics to be used in pregnancy for reducing the incidence of recurrent preterm labor.

Antibiotics therapies (co administration of metronidazole and azithromycin) have failed to reduce the risk of preterm labor[30], therefore it implies that disturbance of commensal microorganism leads to more harm than any good. Table II presents a summary of studies conducted on correlation of placental microbiome with term and preterm labor as well as other conditions associated with pregnancy. As preterm labor is now being referred as a polymicrobial disease[1], wider investigations covering viruses and fungi might further improve our understanding of healthy placental tissue that is essential to develop interventions for placental insufficiencies.

Table II: Summary of microbiome studies conducted on placental tissue

Author	Study design	Technique	Key finding
Jones, H.E., et al[31] (2009)	Longitudinal case-control study	16S rDNA and real-time assay	A greater diversity in bacterial communities in fetal membranes was observed in preterm. Tissue histology confirmed the presence of bacteria in chorioamnionitis and maternal immune paresis.
Aagaard, K., et al[24] (2014)	Population bases cohort study	16S rDNA and short-gun metagenomic studies	Placental microbiome has similarity with oral microbiome of mothers which also correlated with an outcome of their birth.

Doyle, R.M et al[25] (2014)	Case-control study	16S rDNA pyrosequencing	Term pregnancy has a constant microbial community structure irrespective of mode of delivery. Preterm labor is strongly associated with a particular group of organisms such as *Mycoplasma hominis, Aerococcus christensenii, Gardnerella vaginalis and Fusobacterium nucleatum*
Stout, M.J., et al[32] (2013)	Cross-sectional study	Histopathological evaluation	Maternal basal plate of placenta is a possible source of intrauterine colonization that may have a role in maternal–fetal interface.
Zheng, J., et al[33] (2015)	Longitudinal case-control study	16S ribosomal DNAamplicon	Significant variation in the composition of placenta microbiota was observed between the low and normal birth weight neonates. Lactobacillus is a hallmark species associated with birth weight and term delivery.

Fetal Microbiome and Preterm labor

Beside maternal infections, fetal subclinical inflammation is also related to preterm labor.[34] Fetal inflammatory response syndrome (FIRS) is commonly accompanied with chronic chorioamnionitis that leads to initiation of preterm uterine contractions. Stimulation of T cells and release of a specific T cell chemokine initiates an inflammatory response.[35] Such immune activation was also showed by Kim et al. in babies born with FIRS without any maternal immune activation[36]. Therefore, fetal immune activation can also lead to disruption of a normal pregnancy.

Meconium was considered to be a sterile until first week of birth. However, in 1936 Synder was the first one to suggest in-utero colonization of *Lactobacillus* in fetal gut even in a nine minutes old baby. Later, proof of placental transfer of microbial flora in the intestine of pups was established by oral administration of *Enterococcus faecium* in pregnant mice.[37] Nevertheless, the actual programming of microbial colonization of the fetus is not fully elucidated and our current understanding on the role of placental symbiosis versus dysbiosis is very limited.

Using non-culture based techniques, meconium was studied with a focus in microbiome in both term and preterm babies.[38] A correlation of meconium microbiota and the gestational age of neonates at 23 to 41 week were also established in this study. Amniotic fluid and meconium also showed similarity in microbiome, suggesting cross over between fetal intestinal microbiome and amniotic fluid which probably leads to preterm labor. [39]

Mode of delivery is very important in acquisition of microbiome in newborns.[40] It was observed that children delivered by C section had dissimilar microbiome compared to those born by vaginal birth. Longitudinal studies in children delivered by C sections or vaginal birth are essential to assess the risk and outcome of child's health, because of dissimilarity in microbiome encountered during C section. Therefore, both maternal and fetal microbiome is important in understanding the sequel of preterm birth.

Future of research on Microbiome in developing countries

The current figures of preterm labor in LMIC have not changed drastically for the last several years compared to the developing countries. Being multifactorial, the mechanics involved in initiation of uterine contraction may be partially influenced by microbiome if not entirely dependent on dynamics of microbial flora.[41]

Discovery of microbiome has added a new vision to the patho-physiologic understanding of numerous conditions. For example, maternal diabetes and hypertension increases the risk of non-communicable diseases in the subsequent generations and it is also associated with higher risk of preterm births.[42] The use of probiotics to correct the dysbiosis of microbiome in preterm birth requires rigorous analyses and trials to assess its beneficial role. So far, the results are equivocal, as shown in multiple studies. Promising results were achieved with probiotics treatment in bacterial vaginosis[43], reducing the risk of spontaneous preterm delivery. Contrary to these findings, meta-analysis of randomized clinical

trials demonstrated no effect of use of probiotics (*Lactobacillus* and *Bifidobacterium)* on the gestational age.[44] The future of microbiome research demands the use of engineered bacterial strains that may provide an alternative regimen to combat against ever increasing antibiotics resistance.[44]

Sample collection for microbiome analysis is the foremost challenge for the researchers of LMICs. Both labs and field sites are not apt with collection and storages of these samples containing commensal bacteria. For microbial analysis, samples should be rapidly transferred from field site to the main lab for the long and short term storage. This is followed by outsourcing this analysis from overseas lab of DNA Sequencing. Sequencing the DNA of the microbial community determines the order of the "bases" that make up their DNA molecule. This sequence helps the scientists to match the genetic information that is carried in a particular DNA segment with the banked sequencing data in order to identify the bacterial species.

Most of the current microbiome studies have been using specialized dry shippers for the collection and snap freezing of samples in liquid nitrogen within few minutes of samples collection.[45] Gut flora or bacterial community are mainly facultative or strict anaerobes and therefore cannot survive in the presence of oxygen. This makes the transportation of good quality samples is a huge challenge for the researchers. As alternate to the culture techniques, bacterial 16S ribosomal sequencing using NGS for classifying bacteria at phylum and species level is much reliable and robust analysis but requires technological assistance and trained staff.

Bacterial ribosomal genes are highly conserved region and remarkably similar in distantly related organism, therefore this region is preferably used for the classification of taxonomy and phylogeny of different bacterial species.[46] The V4 region of 16S rRNA is highly variable with spacer sequence of conserved region. Compared to the V5 and V6 region, the V4 is preferentially used for sequencing, as it captures prokaryotes and archae. The methodology employs the extraction of DNA in pulverized fecal samples. DNA is further purified (QIAquick column), and quantified by Qubit and then subjected to PCR using primers directed at variable region 4 (V4) of bacterial 16S rRNA genes present in the samples. Bacterial V4-16S rRNA datasets are generated by multiplex sequencing of amplicons prepared from fecal/vaginal DNA samples using the Illumina MiSeq platform. The resulting reads are binned into 97% identity of Operational Taxonomic Units (OTUs). Using an open access software, Qiime and Ribosomal Data base Project (RDP) (https://rdp.cme.msu.edu), the abundance of OTU membership in a single sample is determined by R package software (https://www.r-project.org).

16S ribosomal quantification is a semi quantitative method describing bacterial specie present in the complex biological sample. It gives relative abundance of a particular microbe, and useful for the identification of non-culturable bacteria with lower abundance. It has comparatively lower sensitivity than metagenomics and also restricted to bacteria in the short length of V4 region unless otherwise revealed in the full-length V4 region.

The microbiome research is emerging in the context of preterm birth, further studies in our region are critical to understand the role of microbiome in preterm birth. Such studies will be helpful to devise therapeutic interventions as translational medicine of microbiome.

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