

# Microbiome in Parturition and Preterm Birth

Indira U. Mysorekar, PhD<sup>1,2</sup> Bin Cao, PhD<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynecology

<sup>2</sup>Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri

**Address for correspondence** Indira U. Mysorekar, PhD, Department of Obstetrics and Gynecology, Department of Pathology and Immunology, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110 (e-mail: indira@wustl.edu).

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## Abstract

Preterm parturition is a one of the most significant global maternal-child health problem. In recent years, there has been an explosion in reports on a role for microbiomes (i.e., a microbial biomass) on a plethora of physiologic and pathologic human conditions. This review aims to describe our current understanding of the microbiome and its impact on parturition, with particular emphasis on preterm birth. We will focus on the roles of vaginal and oral mucosal microbiomes in premature parturition and describe the state-of-the-art methodologies used in microbiome studies. Next, we will present new studies on a potential microbiome in the placenta and how it may affect pregnancy outcomes. Finally, we will propose that host genetic factors can perturb the normal “pregnancy microbiome” and trigger adverse pregnancy outcomes.

## Keywords

- ▶ pregnancy
- ▶ microbiome
- ▶ preterm birth
- ▶ parturition
- ▶ bacteria

## Preterm Birth and Associated Morbidity and Mortality

Preterm birth (PTB), defined as a live birth between 20 and 37 weeks of gestation, is a significant public health concern, affecting 12.7% of all births in the United States. PTB is a leading cause of perinatal morbidity and mortality in both developed and developing countries<sup>1,2</sup>; 27% of neonatal deaths are primarily attributable to PTB, and an additional 36% of neonatal deaths secondary to opportunistic infections (e.g., sepsis, pneumonia) are attributable to prematurity. Of those infants that survive, PTB is an independent risk factor for motor (cerebral palsy) and sensory (retinopathy and neuropathy) deficits, learning disabilities, and respiratory disorders (bronchopulmonary dysplasia). In fact, nearly 50% of preterm babies suffer from long-term neurologic sequelae.<sup>3</sup> In addition to the global PTB, rate of prematurity is the astounding financial and social burden on the affected families. PTB-associated costs have been estimated in 2005 to exceed \$26 billion.<sup>4</sup>

PTB-associated morbidity and mortality is tightly linked to the gestational age of the fetus. Therefore, PTBs are classified into extremely preterm (more than 20 weeks but fewer than 28 weeks of gestation), very preterm (28 weeks through 32

weeks), and moderate-to-late preterm (more than 32 weeks but less than 37 weeks). Over the past 25 years, the rate of PTB in the United States has increased by 36%, and most of the increase has been in moderate-to-late PTBs. Increasing maternal age, rising rates of multiple gestations due to the use of assisted reproductive technologies, and a concomitant increase in indicated PTBs are major contributing factors driving this increase.<sup>5</sup>

PTB causes are generally grouped into the following categories: 30% are associated with preterm premature rupture of membranes, 45 to 50% are idiopathic, and 15 to 20% are medically indicated.<sup>6</sup> However, the underlying etiologies (e.g., genetic factors, socioeconomic factors, or environmental factors) and mechanisms which may trigger various PTB subtypes remain largely unknown. Furthermore, to date, clinical interventions to reduce PTB have focused largely on specific risk factors, rather than on molecular-cellular etiology, and have arguably had less than optimal impact on elucidating PTB mechanisms.<sup>7</sup>

Because PTB is currently the most significant problem in maternal–fetal health, it is of the greatest importance to determine its causes, which will allow the field to develop novel methods of prevention and treatment. This review will focus on microbial (bacterial) etiologies for PTB.

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## Infection and Preterm Birth Are Strongly Linked

Infection is one of the most consistently identified risk factors for PTB. Intrauterine infection contributes to at least 25% of PTBs. Predisposing factors for infection-related PTB are diverse, including subclinical intrauterine infections,<sup>8</sup> intra-amniotic infections,<sup>9,10</sup> and pyelonephritis.<sup>11</sup> Eleven to 80% of PTBs can be accompanied by intra-amniotic bacterial colonization.<sup>12,13</sup> Further, experimental evidence for an unequivocal association between infection and PTB includes data that antibiotic treatment of ascending intrauterine infections can prevent PTB in experimental models of chorioamnionitis, treatment of asymptomatic bacteriuria prevents PTB, and systemic administration of microbial products to pregnant animals results in spontaneous preterm labor and PTB.<sup>14,15</sup> PTB-causing infections have been postulated to begin in the reproductive or genitourinary tract, ascend upward toward the uterus, and possibly cross the placental barrier. Potential sites of infection in proximity to the fetus include the amniotic fluid, placenta, fetal membranes, and umbilical cord.<sup>16</sup>

Because the risk of PTB recurrence can reach as high as 48%,<sup>17</sup> identification of the etiologic infectious agents (both culturable and nonculturable organisms, known and unknown) is essential to better stratify recurrence estimates, provide targeted and efficacious interventions, and reduce the risk of PTB in subsequent pregnancies. The development of effective treatments will require identifying the relevant sites of infection, the pathogens involved, and the mechanisms by which pathogens induce pathological change during pregnancy.

## Infectious Organisms and Etiologies Associated with Preterm Birth

A wide variety of bacterial species have been identified in PTB-associated infections; the most prevalent being *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Bacteroides* spp., *Gardnerella vaginalis*, and *Fusobacterium nucleatum*.<sup>16,18</sup> We refer the reader to a recent review<sup>19</sup> for a detailed description of the bacterial species that have been associated with PTB.<sup>20,21</sup> It has been suggested that the majority of these bacterial species exhibit typically low virulent properties. However, once inside the uterine environment, they are capable of stimulating production and release of proinflammatory cytokines, prostaglandins, and metalloproteases together with myeloid cell influx. These events can trigger cervical ripening and shortening, membrane weakening and rupture, uterine contractions, and PTB.<sup>16</sup> In addition, the presence of organisms in the amniotic cavity may elicit a fetal inflammatory response, which predisposes preterm neonates to short- and long-term consequences, such as neonatal sepsis, bronchopulmonary dysplasia, and cerebral palsy.

## Relevance of the Human Microbiome in Pregnancy

Although microbes have historically been viewed only as pathogens, many microorganisms live in a symbiotic rela-

tionship with the host and protect the host from harmful pathogens.<sup>22-24</sup> In fact, the microbial genome exceeds the human genome by a 100-fold, and adult human cells are outnumbered 10:1 by microbial cells.<sup>25</sup> The human genome interacts with, and has evolved alongside, the genomes of 10 to 100 trillion bacterial cells.<sup>26</sup> To understand this coevolution and begin to understand how bacterial cells we play host to, govern human health and disease, it is essential to understand the bacterial genomic diversity and composition at various mucosal sites in the human body. Accordingly, the National Institutes of Health launched the 5-year Human Microbiome Project (HMP) in 2007 with the ambitious goal of understanding human-microbe and microbe-microbe interactions in healthy adults.<sup>27</sup> These efforts are producing comprehensive genomic characterization of the healthy adult human microbiota, which can be used as a comparison for diseases states. With the advent of next-generation sequencing and whole genome shotgun sequencing, the field of metagenomics has been evolving rapidly. Traditional sequencing methods, such as Sanger sequencing, only allowed analysis of a few samples at a time, and enabled sampling of only the most predominant species present. In the past decade, however, next-generation sequencing techniques have made it possible to obtain a comprehensive analysis of the species present within a given body site by enabling the simultaneous reading of tens to even hundreds of thousands of sequences. It is now possible to obtain a detailed catalog of the bacterial species present in a given human sample. Furthermore, metagenomics has facilitated the sequencing and identification of nonculturable species thus providing a wealth of information into microbial etiologies for several disease conditions. Finally, given the increasing sequencing capacity at diminished cost, our ability to acquire and analyze complex metagenomic data will undoubtedly continue to increase.

With the ever-increasing quantity of genomic data, analytic methods are also rapidly evolving (for a thorough description of the methods, we refer the reader to an excellent recent review<sup>28</sup>). For example, it is now possible to generate a microbiome gene catalog. Functional genomic information regarding presence and abundance of microbial pathways can be determined with relative ease. Meta-RNA sequencing can be used to map gene expression data to determine bacterial gene expression profiles relative to the total gene pool of the human microbiome. Finally, on the host side, host genomic sequences can also be analyzed to obtain information on genomic variants, including single nucleotide polymorphism (SNPs) and copy number variations. This could, for example, allow investigations into how host genomic variants associated with PTB may affect microbiome composition.

## Defining the Microbiome of Preterm Birth

### Vaginal Microbiome, Bacterial Vaginosis, and Preterm Birth

There are currently no published next-generation sequencing-based analyses of a microbiome associated with PTB. In the past decade, however, there have been several reports

describing utero-invasive bacteria using polymerase chain reaction (PCR) or culture-based approaches. For example, *Ureaplasma* cultures are positively associated with PTB.<sup>29</sup> Culture-based analyses of chorioamniotic membranes most commonly detected *Ureaplasma* and *G. vaginalis*, whereas *Mycoplasma* were most commonly detected in amniotic fluids, using culture or PCR methods.<sup>30,31</sup> Additional bacterial species associated with amniotic fluids include *Leptotrichia*, *Sneathia*, and *Bergeyella*.<sup>30,32</sup>

Recent studies have also started to flesh out the complexity and diversity of the vaginal microbiome in the nonpregnant female and in pregnant women through the course of pregnancy.<sup>28</sup> The healthy vagina is predominantly colonized by lactobacilli, which can prevent colonization by other species and are thought to maintain a healthy, stable low pH environment through the production of lactic acid and hydrogen peroxide.<sup>33</sup> The vaginal microbiome of reproductive-age women reveals tremendous variance in the dominance of *Lactobacillus* species. For example, sequencing of vaginal swabs from 200 randomly selected subjects revealed that more than half exhibited significant diversity in the composition of their vaginal microbiomes, considerable variability in vaginal pH, and that differences in pH and bacterial composition were associated with ethnicity.<sup>34</sup> Prominent changes in the vaginal flora that result in pH variation are associated with increased risk of PTB.<sup>35</sup> Furthermore, Hyman and colleagues recently showed that the overall diversity of the vaginal microbiome correlated positively with PTB with race and ethnicity and sampling sites being key variables.

Culture-based methods have also identified bacterial vaginosis (BV, a condition in which the normal vaginal flora of lactobacilli are replaced by other low- and high-grade pathogens)-associated species including *G. vaginalis*, *Prevotella bivia*, and *Peptostreptococcus*.<sup>30,36</sup> BV has been implicated as a risk factor for PTB and associated morbidities.<sup>37-41</sup> The racial disparity in rates of PTB is mirrored in rates of BV: 11% of Caucasian women deliver preterm and 10 to 20% of Caucasians have BV, 18% of African American women deliver preterm and 30 to 50% of African American women have BV.<sup>21</sup> Thus, BV doubles the relative risk for PTB in the Caucasian population, but this disorder more than triples the risk in African American women.<sup>42</sup> In fact, BV may account for as much as 30% of the racial gap in rates of PTB.<sup>43</sup> During BV, the numbers of lactobacilli drop and the numbers of anaerobes increase dramatically, resulting in a tremendous increase in bacterial burden and species diversity. Studies have analyzed the roles of *Atopobium vaginae*, *Mycoplasma hominis*, and *G. vaginalis* among other species to establish an association between bacterial colonization and preterm labor, but as of yet, there has been no unequivocally clear role determined for any single species in PTB.<sup>44,45</sup>

HMPs dedicated to the vaginal microbiome are now revealing the full spectrum of species that are associated with BV.<sup>46</sup> Understanding the balance between vaginal colonization with lactobacilli and BV-associated organisms will enable more targeted therapeutic interventions.

## Oral Microbiome and Preterm Birth

Intrauterine infections are thought to predominantly originate from pathogens in the vaginal tract that ascend into the sterile uterus. However, improved genome sequencing efforts have determined that in fact many utero-invasive bacteria do not belong to the vaginal microflora. Instead, several studies have elucidated that intrauterine infections may be caused by bacterial species that are components of the commensal oral flora, such as *F. nucleatum* and *Bergeyella*, *Eikenella*, and *Capnocytophaga* spp.<sup>18,32,47</sup> Oral flora comprises more than 700 microbial taxa<sup>48,49</sup> and thus may serve as a potential reservoir for infection. These findings suggest a hematogenous route of pathogenic transmission.<sup>50</sup>

The link between oral flora, periodontal disease, and PTB gained further attention after Offenbacher et al observed a possible association between periodontal disease and PTBs in a case-control study.<sup>51</sup> Periodontal diseases are among the most common chronic infections, affecting up to 50% of humans. During periodontal infection, oral bacterial titers increase dramatically, accompanied by inflammation and bleeding in the gingival tissue. These conditions lead to increased bacteremia, which enhances the opportunities for hematogenous transmission. Consistent with this idea, periodontal pathogens have been detected in placentas of women with preeclampsia<sup>52</sup> and in the amniotic fluid of pregnant women with preterm labor.<sup>51,53,54</sup> These findings have led to the proposal that periodontitis may represent a distant source of systemic inflammation in the mother. Alternatively, periodontitis may affect the placenta directly through the hematogenous translocation of periodontal pathogens. However, associative studies have produced different results in different population groups, and no conclusive evidence has yet demonstrated that preventive periodontal care can reduce the risk of PTB.<sup>55</sup>

One important question is to determine the proportion of the oral microbiome that is capable of oral-uterus transmission. Given that the majority of the bacteria in the oral cavity are uncultivated, it is very likely that the involvement of oral bacteria in intrauterine infection has been significantly underestimated.<sup>32</sup> As a result, clinical evidence linking oral bacteria to PTB is incomplete. State-of-the-art next-generation sequencing and metagenomic analyses are warranted to provide comprehensive knowledge of the diversity of oral bacterial involved in PTB.

## Intracellular Microbes and Placenta: Is There a Placental Microbiome?

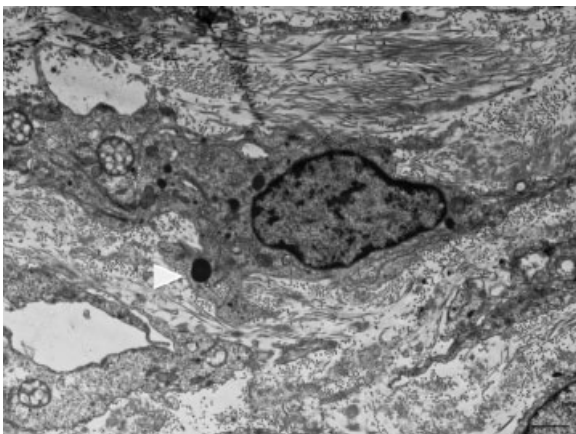
Among the important contributors to PTB are microorganisms (both pathogenic and perturbed microbiome communities) that we have already discussed and genetic variants that modify and mediate host susceptibility to infection. The role of maternal genomic variation in mediating susceptibility to spontaneous PTB has been extensively studied,<sup>56-60</sup> but studies imputing susceptibility loci and microbiome profiles to PTB are needed. These are likely essential steps in elucidating underlying mechanisms of spontaneous PTB.

Mammalian pregnancy is a state of immune tolerance maintained by multiple mechanisms. One major mechanism

is via the major histocompatibility complex molecule human leukocyte antigen-G (HLA-G), which is expressed on extravillous trophoblast (EVT) cells, and modulates maternal natural killer cells. HLA proteins identify cells as “self” or “nonself” to the host immune system. Dysregulation of antigen recognition of the HLA system occurs in autoimmune disease, transplant organ rejection, or tumor growth,<sup>61</sup> and polymorphisms in HLA-G have been linked to recurrent spontaneous abortion.<sup>62</sup> Studies have found that amniotic fluid levels of HLA-G are significantly higher in pregnancies complicated by toxoplasmosis infection, with the highest levels found in pregnancies with transplacental transmission<sup>63</sup> and correlated significantly with intra-amniotic infection.<sup>14</sup> Levels of soluble HLA-G in the amniotic fluid have been shown to be elevated in patients with spontaneous preterm labor.<sup>64</sup> Furthermore, recent studies have found increased maternal serum HLA class I antibodies to be associated with spontaneous PTB and fetal inflammatory responses, suggesting a component of maternal–antifetal rejection in the pathogenesis of PTB.<sup>65,66</sup> Maternal–fetal gene–environment interactions in terms of HLA-G expression may thus jointly contribute to pregnancy tolerance and PTB.

Recently, we showed that intracellular bacteria were present in the basal plate, the peripheral region of the placenta on the maternal side in contact with the uterine wall, of 27% of all placentas ( $n = 195$ ) and 54% ( $p < 0.05$ ) of placentas from very PTBs.<sup>67</sup> We found that these intracellular bacteria were present in the HLA-G positive EVTs (–Fig. 1), which are found exclusively in the basal plate. EVTs are the fetally derived and invade into the maternal basal plate, thus coming into contact with maternal stromal and immune cells.<sup>68,69</sup>

The presence of intracellular bacteria in a quarter of placental basal plates is noteworthy in terms of potential pathologic effect of basal plate colonization as a contributor to PTB. However, the bacterial type, functions, and host responses to the bacteria within cells in the basal plate are unknown, and may collectively determine the outcome



**Figure 1** Transmission electron micrograph of bacteria (white arrowhead points to the electron dense single bacillus) within an extravillous trophoblast embedded within the basal plate from a term C-sectioned placenta from a woman with no clinical diagnosis of infection. Image =  $\times 3,000$  magnification. Bar =  $2\ \mu\text{m}$ .

from the presence of intracellular bacteria. The presence of bacteria in normal term deliveries, as well as the absence of bacteria in many cases of extreme prematurity, suggests that the phenotype of PTB is mediated not just by the presence or absence of bacteria, but likely by more complex relationships between the bacterial types, host tissue, and host responses. It is possible, for example, that certain microbes in the basal plate are truly commensal and exert no pathologic effect, whereas others microbes may activate the inflammatory cascade predisposing to PTB. The location of bacterial colonization in an immune privileged cell type and the association of HLA-G with PTB may well be a pivot balancing fetal tolerance with maternal immunity. Future investigations regarding the specific type and function of these microbes, and whether they constitute a placental microbiome are required to fully elucidate the normal or pathogenic relationship between host and microbes in the basal plate and indeed other regions of the placenta.

## Conclusions

Several decades of research indicate that infection confers risk for PTB, and this risk is mediated by human host genetic susceptibility. Evidence from both human and animal models suggests that infectious etiologies for PTB could originate in the lower genitourinary tract, but also that hematogenous transmission is an alternative route of infection. Furthermore, there is new evidence that microbes can reside in the placenta. The developing technological advances and analytical tools will provide unprecedented insights into both host and microbial effects on placental function and risk of PTB. To understand the deviation from normal pregnancy, studies must catalog the establishment of the “pregnancy microbiome” at different sites and throughout gestation. Such studies should focus on understanding the evolution of the pregnancy microbiome and the responsible maternal, environmental, and fetal factors. With current deep-sequencing technology, a well-characterized prospective study comparing pregnancy microbiomes in preterm and normal delivery would improve our understanding of microorganisms associated with PTB and possibly guide development of preventive and therapeutic interventions.

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