Journal of Reproductive Immunology 104–105 (2014) 12–19

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

The microbiome, parturition, and timing of birth: more questions than answers

Amanda L. Prince [a], Kathleen M. Antony [a], Derrick M. Chu [a], Kjersti M. Aagaard [a,b,c,*]

[a] Departments of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Baylor College of Medicine, Houston, TX, USA
[b] Bioinformatics Research Lab, Baylor College of Medicine, Houston, TX, USA
[c] Molecular and Cell Biology, Baylor College of Medicine, Houston, TX, USA

ARTICLE INFO

Article history:
Received 30 January 2014
Received in revised form 15 March 2014
Accepted 17 March 2014

Keywords:
Pregnancy
Microbiome
Preterm birth
Infection
Inflammation
Metagenomics

ABSTRACT

The causes of preterm birth are multifactorial, but its association with infection has been well-established. The predominant paradigm describes an ascending infection from the lower genital tract through the cervix and into the presumably sterile fetal membranes and placenta. Thus, an evaluation of the role of the vaginal microbiome in preterm birth is implicated. However, emerging fields of data described in this review suggest that the placenta might not be sterile, even in the absence of clinical infection. We thus propose an additional mechanism for placental colonization and infection: hematogenous spread. When considered in the context of decades of evidence demonstrating a strong risk of recurrence for preterm birth, studies on parturition are ideal for applying the rapidly expanding field of metagenomics and analytic pipelines. The translational implications toward identification of innovative treatments for the prevention of preterm birth are further discussed. In sum, exciting advances in understanding the role of both host and microbiota in parturition and preterm birth are on the horizon.

1. Introduction

In 2005, the World Health Organization estimated that 12.9 million births worldwide occurred preterm; up to 42% of these resulted in mortality (Beck et al., 2010). Preterm birth is the leading cause of neonatal morbidity and mortality, yet little is understood regarding the underlying etiology (Kilpatrick, 2013). It is traditionally thought that an ascending infection from the vagina causes preterm premature rupture of membranes (PPROM), which initiates preterm labor and ultimately birth. However, more recent studies have shown that bacteria from the oral cavity are most often found in the amniotic fluid of patients with preterm labor (Fig. 1) (Madianos et al., 2013). Additionally, studies are now demonstrating that bacteria are naturally found in placental tissue, and our own lab has shown that the placenta harbors its own unique microbiome (Aagaard et al., in press). Here we will discuss how the microbiome changes during pregnancy and how these changes may influence preterm birth (Table 1). Further research in this area will lead to a greater understanding of the etiologies of preterm birth and may result in innovative treatments to prevent preterm birth.

2. The microbiome during pregnancy

During a normal pregnancy, the gravidae undergoes a spectrum of anatomical, physiological, and biochemical

* Corresponding author at: Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX 77030, USA. Tel.: +1 801 585 2550; fax: +1 801 585 2594.
E-mail addresses: aagaardt@bcm.edu, kjersti131@prodigy.net (K.M. Aagaard).

http://dx.doi.org/10.1016/j.jri.2014.03.006
0165-0378/

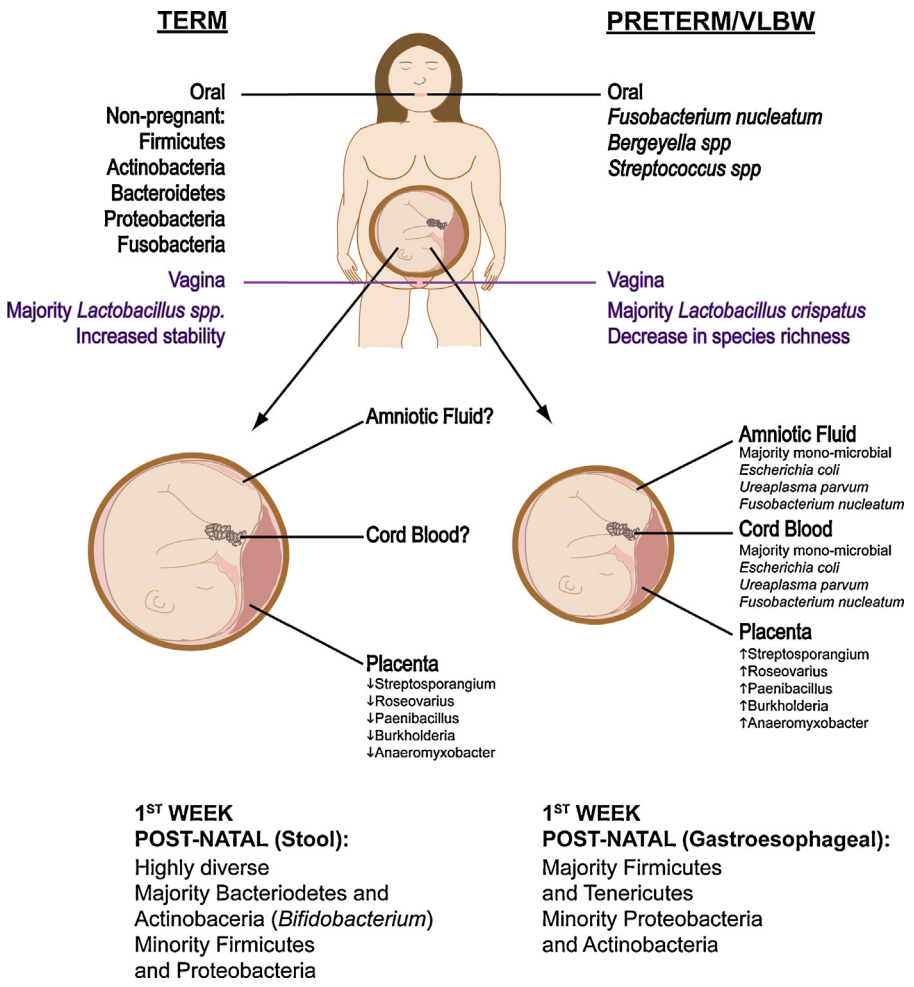

Fig. 1. The fetal and neonatal microbiome does not replicate the vaginal microbiome in pregnancy, but more closely resembles the oral and placental microbiome. The oral microbiome has been previously characterized in the non-gravid population by the Human Microbiome Project Consortium (2012). Oral bacteria associations with preterm birth have been described in a very limited number of studies, and primarily with targeted 16S-based methodologies (Bearfield, 2002; Douvier et al., 1999; Fardini et al., 2010; Han et al., 2006, 2004). However, recent studies have characterized the vaginal microbiome during pregnancy, and demonstrated that the species richness decreases during pregnancy with a corresponding dominance in *Lactobacillus* spp. (Aagaard et al., 2012; Romero et al., 2014). While one recent study reported an association between the vaginal microbiome richness and diversity and preterm birth (Hyman et al., 2014), others suggest that the vaginal microbiome normally varies across the gestational age spectrum (Aagaard et al., 2012; Romero et al., 2014). What is striking is the lack of shared taxonomy in the microbiome profiles of term or VLBW or preterm infant microbiomes in the first week of life with the maternal vaginal microbiome. So what might colonize the infant at birth? We recently described the placental microbiome, including comparisons between term and preterm subjects, and found differences in the overall beta diversity with distinct clustering by virtue of preterm birth (Stout et al., 2013; Aagaard et al., in press). Of interest, we similarly observed that the placental microbiome (in aggregate) most closely resembles the oral microbiome (Aagaard et al., in press). While the examination of the cord blood and amniotic fluid microbiome is still needed in term neonates, recent studies in preterm neonates have revealed a prevalence of bacteria normally residing in the maternal oral cavity (Wang et al., 2013). Within the first week of life, the development of the microbiome has been studied in term and preterm neonates (Jost et al., 2012; Milisavljevic et al., 2013; Palmer et al., 2007). While Firmicutes has been described to be a major phylum colonizing neonates, these are not predominantly *Lactobacillus*. At a coarse taxonomic level, these observations collectively lead one to speculate that it is neither ascending bacteria from the vaginal tract (nor contact with bacteria from the vaginal tract during delivery) that are the sole or even majority colonizers of the infant microbiome. Alternatively, it appears that the placenta and maternal oral flora are most closely mirrored in the infant during the first week of life.

changes. These functional alterations result from the influences of hormonal and physical fluctuations, and they affect every organ of the body. These are accompanied by concomitant changes in the microbiome, at least in the vagina and gut, which are the only sites that have been specifically examined in pregnancy to date (Aagaard et al., 2012; Koren et al., 2012; Romero et al., 2014).

During pregnancy, hormonal changes result in increased thickness of the vaginal mucosa, hypertrophy of the smooth muscle cells, and relaxation of the connective tissues. Recently, we cataloged the "normal" microbiota signature during pregnancy in a cross-sectional study sampling women at a variety of gestational ages (Aagaard et al., 2012). Using 454 pyrosequencing technology, we deep sequenced the V3–V5 region of 16S rRNA from samples obtained from the vaginal introitus, midvagina, and posterior fornix. Interestingly, we found that the vaginal microbial community differed by gestational age and

Table 1
Microbiome studies of non-gravid and gravid populations.

Authors	Site	Technique(s)	Study Design	Findings
Non-gravid				
NIH HMP Consortium (Human and Project, 2012)	Skin, nares, oral, vagina	Sequencing	Longitudinal	Characterized healthy reference population
Ravel et al. (2011)	Mid-vagina (self-collected)	Sequencing	Cross-sectional	Characterized healthy, non-gravid vaginal microbiome
Gravid				
Aagaard et al. (2012)	Vaginal introitus, posterior fornix, and midvagina	Sequencing	Cross-sectional	Characterized healthy, gravid vaginal microbiome
Romero et al. (2014)	Posterior fornix	Sequencing	Longitudinal	Characterized healthy, gravid vaginal microbiome throughout pregnancy
Koren et al. (2012)	Stool	Sequencing	Longitudinal	Characterized 1st and 3rd trimester stool
Douvier et al. (1999)	Amniotic fluid, placenta, maternal endocervix	Bacterial culture	Case study	Placenta colonized with oral bacteria, *Capnocytophaga sputigena*
Han et al. (2006)	Amniotic fluid	PCR	Cross-sectional	Amniotic fluid was colonized with oral bacteria of the *Bergeyella spp*
Bearfield (2002)	Amniotic fluid, vagina, and dental plaque	Bacterial culture and PCR	Cross-sectional	*Streptococcus* spp or *F. nucleatum* mostly found in the oral cavity of gravidae testing positive in the amniotic fluid.
Stout et al. (2013)	Placenta	Histopathology	Cross-sectional	Bacteria were found in the basal plate of the placenta
Han et al. (2004)	Placenta, liver, spleen, amniotic fluid, fetus	Bacterial culture	Murine study	*F. nucleatum* isolated from preterm births colonized placenta of gravid mice
Fardini et al. (2010)	Placenta	Sequencing	Murine study	Oral bacteria colonize murine placenta
Aagaard et al. (in press)	Placenta	Sequencing	Population-based, cross-sectional	The placenta harbors a unique microbiome profile, most akin to the oral microbiome and varies by virtue of preterm birth and a remote history of antenatal infection
Neonatal				
Palmer et al. (2007)	Stool	Sequencing, microarray, PCR	Longitudinal	Characterized healthy neonatal microbiome
Jost et al. (2012)	Stool	Sequencing	Longitudinal	Characterized healthy neonatal microbiome
Wang et al. (2013)	Cord blood, amniotic fluid	Bacterial culture and Sequencing	Cross-sectional	Neonates with necrotizing colitis had predominantly one species of bacteria dominating
Milisavljevic et al. (2013)	Gastro-esophageal	Sequencing	Longitudinal	Characterized the microbiome in VLBW infants

PCR, polymer chain reaction; VLBW, very low birth weight.

proximity to the cervix (Aagaard et al., 2012). Furthermore, the microbial community structure resembled a non-pregnant state in late gestation, and we saw a decrease in alpha diversity, or within-sample diversity, with a corresponding increase in *Lactobacillus* species in gravid patients compared with nonpregnant subjects (Aagaard et al., 2012). Recently, Romero et al. took these studies further by examining the vaginal microbiome longitudinally during pregnancy at the posterior fornix (Romero et al., 2014). While the vaginal microbiome of gravid women could still be classified into distinct community state types, as previously described in nonpregnant women (Ravel et al., 2011), the vaginal microbiome became more stable and less diverse throughout pregnancy, as we previously described (Aagaard et al., 2012; Romero et al., 2014).

One such species that was discriminately and specifically enriched in our study was *Lactobacillus johnsonii*. This species encodes enzymes and transporters that are essential for the release of bile salt hydrolase and is primarily found in the upper gastrointestinal tract (Pridmore et al., 2004). *L. johnsonii* also produces Lactacin F, which limits other lactobacillus and *Enterococcus* species in the gastrointestinal tract (Abee et al., 1994). Thus, the increase in *L. johnsonii* may be important for the inoculation of neonates in order to promote the digestion of breast milk postpartum.

While these alterations in the microbiome may serve to inoculate the neonatal gut, they may also contribute to pregnancy maintenance. In addition to the aforementioned enrichment in *L. johnsonii*, there was enrichment of *L. jensenii* and *L. crispatus*. *L. jensenii* anaerobically metabolizes glycogen, and the increased estrogen levels in pregnancy lead to increased glycogen, which, through *L. jensenii* metabolism, contributes to the acidic vaginal environment. This acidic environment likely suppresses the growth of bacteria associated with bacterial vaginosis, which may be a natural mechanism for preventing infections associated with preterm birth (O'Hanlon et al., 2011). The finding that the vaginal microbiome becomes more similar to the nonpregnant microbiome in late pregnancy may also support the microbiome's role in pregnancy maintenance, as a return to a nonpregnant state may herald changes associated with parturition.

With regard to the enteric microbiome, alpha diversity is decreased as the pregnancy progresses from the first to third trimester; however, beta diversity increases during this time (Koren et al., 2012; Romero et al., 2014). Further, as gravidae progress through their pregnancy, the ratio of *Firmicutes* and *Bacteroidetes* changes similar

to that seen between lean and obese subjects in various studies (Koren et al., 2012; Turnbaugh et al., 2009, 2006). In fact, when the enteric microbiome from gravidae in their first or third trimester is transplanted into germ-free mice, mice inoculated with microbiota from third-trimester patients are more prone to an increase in adipose tissue and the inflammatory cytokines, IL-1β, IL-5, IL-6, and GM-CSF (Koren et al., 2012). These data indicate that as pregnancy progresses, the body undergoes changes to increase the capacity for energy-harvesting microbes.

3. The microbiome, parturition, and preterm birth

As we begin to understand changes in the microbiome that occur during pregnancy, a natural question to ponder is the role of the microbiome in the regulation of the timing of parturition and notably preterm birth. While the dogma currently states that preterm birth is initiated by an ascending infection from the vaginal cavity to the placenta, recent studies have challenged this idea (Gonçalves et al., 2002; Madianos et al., 2013). In fact, the colonization of the placenta with bacteria may not be exclusive to patients with evidence of infection, such as preterm labor. For example, Stout et al. recently demonstrated that bacteria could be found in the basal plate of term and preterm placentas following spontaneous deliveries (Stout et al., 2013). Additionally, our own lab has found that the placenta harbors a unique microbiome, and that this microbiome differs in term and preterm subjects (Fig. 1) (Aagaard et al., in press). Supporting our observations, a bacterium found in the amniotic fluid of preterm patients was most likely to be found in the oral cavity as well, rather than the vaginal cavity (Bearfield, 2002; Douvier et al., 1999; Han et al., 2006) and both term and preterm neonates appear to be colonized early with bacteria normally found in the oral cavity (Fig. 1) (Human Microbiome Project Consortium, 2012; Jost et al., 2012; Milisavljevic et al., 2013; Palmer et al., 2007). When *Fusobacterium nucleatum* is isolated from preterm patients and injected intravenously into pregnant mice, the placenta becomes selectively colonized with bacteria while other organs, such as the liver or spleen, do not (Han et al., 2004). This study also documented that while *F. nucleatum* infection did not result in preterm birth in mice, it resulted in stillbirth (Han et al., 2004). This is akin to the "failed escape hypothesis" of stillbirth, which postulates that stillbirth occurs if a fetus fails to initiate a fetal inflammatory response with subsequent preterm birth (Blackwell et al., 2003; Romero et al., 1998).

An independent study isolated bacteria from the oral cavity of a variety of individuals and subsequently used this mixture to infect gravid mice. Similar results were seen in this study where the placenta was colonized by the oral bacteria (Fardini et al., 2010). Arce et al. took these types of studies one step further and inoculated mice by feeding them chow containing either the gram-negative bacteria *Campylobacter rectus* alone or a combination of *C. rectus* and *Porphyromonas gingivalis*. They found that infected mice had decreased fecundity and increased resorption rates (Arce et al., 2009). Additionally, infected mice that were impregnated had inflammation in the placenta, along with an increase in the expression of Toll-like receptor-4 (TLR-4), which recognizes lipopolysaccharide (LPS) on gram-negative bacteria (Arce et al., 2009). Taken together, these studies demonstrate that hematogenous infections have the ability to colonize and infect the placenta, which provides support to the notion that preterm birth might be caused by infections not originating in the vaginal cavity.

Despite the recent explosion of metagenomic studies involving the microbiome, there are relatively few studies using metagenomics to examine the role of the microbiome in preterm birth. Most recently, Hyman et al. examined the posterior fornix of term and preterm patients using 16S sequencing. When examining 37 Caucasian gravidae, with only seven patients resulting in a preterm birth, Hyman et al. found that there was a decrease in the alpha diversity of the overall microflora of preterm patients (Hyman et al., 2014). Interestingly, while *Lactobacillus* has been found to be the dominating genus of the vaginal cavity, there were no differences in this genus between term and preterm patients, and there appeared to be no bacterium in particular dominating the posterior fornix in preterm patients (Hyman et al., 2014). For example, in one preterm patient *Bifidobacterium* dominated the vaginal cavity while in another the vaginal cavity was dominated by *Ureaplasma* (Hyman et al., 2014). These results are interesting because when infants with early-onset necrotizing colitis (EONS) are examined by 16S sequencing, Wang et al. determined that the majority of patients had a mono-microbial infection (Wang et al., 2013). Notably, the study by Hyman et al. neglected to examine the oral cavity of term and preterm patients, which the recent studies above implicate as playing an important role in preterm birth.

4. Preterm birth and inflammation

Given the early and emerging evidence pertaining to the potential role of oral and hematogenous infections (of oral microbes) in preterm birth, one wonders why the placenta might harbor largely commensal microbes. This may be due to the unique immune environment of the placenta during pregnancy which ultimately prevents rejection of the fetus. While increased levels of IL-6 have been found in the amniotic fluid of preterm gravidae, the immunosuppressive/immunomodulatory cytokine, IL-10, has also been found (Greig et al., 1995; Han et al., 2009). Further, T cells are found at the maternal–fetal interface, and specialized NK cells have also been characterized in the placenta (Christiansen, 2013; Zenclussen, 2013). The effect of these changes may not only prevent rejection of the fetus, but may alter the composition of the mother's microbiome in order to promote energy harvest required for pregnancy as well as to inoculate the neonate with the proper microbiome required for normal digestion and development.

However, these changes in the gravid immune system may functionally alter susceptibility of the placenta to hematogenous colonization, which in turn may trigger an inflammatory response (Fig. 2). While the microbial species themselves may not be pathogenic, the relatively altered community structure (*e.g.*, dysbiosis) would thereby render the risk of localized inflammation. For example, infusion of LPS into placental explants *in vitro* has been shown to trigger production of pro-inflammatory cytokines, such

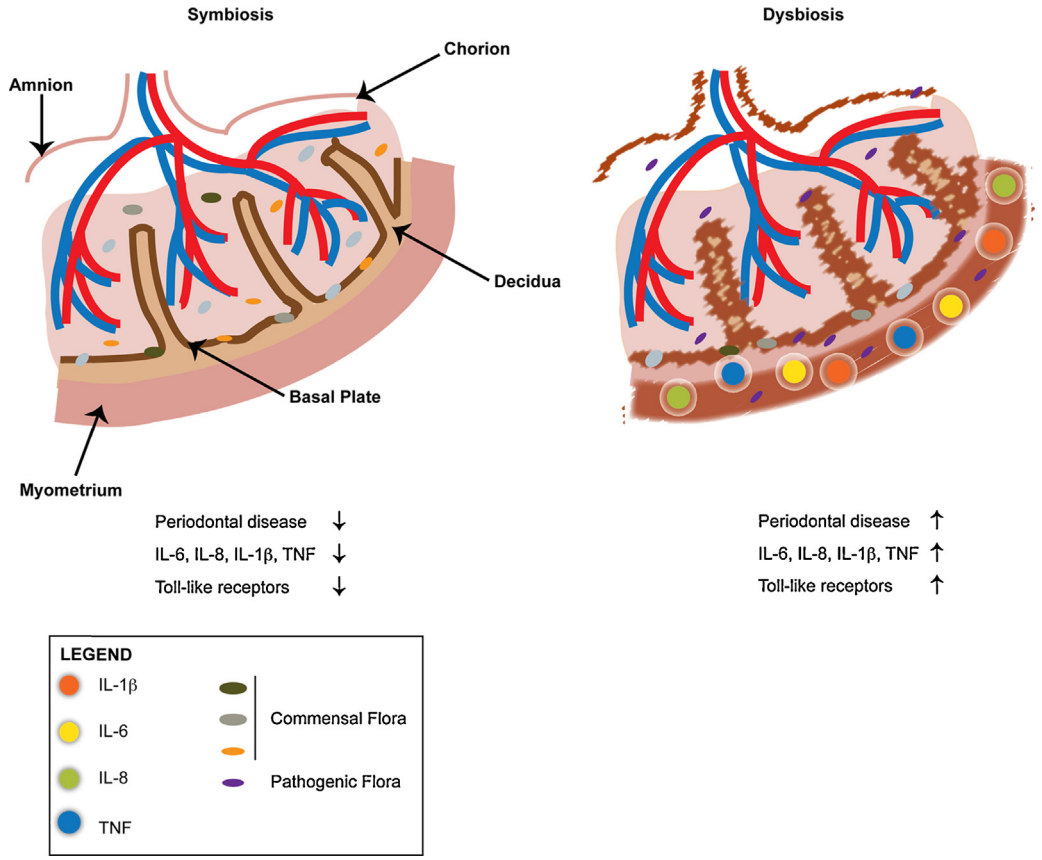

Fig. 2. Dysbiosis of the placental microbiome and risk of preterm birth. Recent studies have suggested that the placenta harbors a low-abundance community of microbes (Stout et al., 2013; Aagaard et al., in press). On the left, we schematically depict a term labor placenta harboring commensal flora with few associations with periodontal disease, inflammation, or innate immune receptors. On the right, we depict the alternative state of dysbiotic flora, which would be associated with an induction or propagation of inflammation. The resulting imbalance favoring perpetuation of inflammation would lead to a weakening of MMP and other structural proteins, resulting in preterm premature rupture of membranes (PPROM) and induction of preterm labor.

as IL-1β, IL-6, and IL-8 (Fortunato et al., 1996; Holcberg et al., 2008). Furthermore, when IL-1β is infused intra-amniotically into pregnant rhesus macaques, amniotic levels of TNF and prostaglandins are raised and uterine contractions are induced (Sadowsky et al., 2003). These findings imply a pro-inflammatory etiology, but it is not clear whether this can be attributed to a single taxa or a dysbiotic placental microbiome community profile.

These changes in the gravid immune system within the placental microenvironment must strike a delicate balance between the prevention of fetal rejection and susceptibility to dysbiosis and ensuing inflammation. The observation that preterm birth tends to recur in individual mothers suggests that human host genetic variation may disrupt this balance and render the placenta more susceptible to infection in the face of a microbial challenge. In support of this hypothesis, significant efforts have been made to associate preterm birth with small nucleotide polymorphisms (SNPs) within key cytokines altered in the gravid immune system. For example, in a study of 62 Caucasian women who delivered preterm, mothers, with a homozygous C to T polymorphism within the promoter region of IL-1β at position −31 (IL-1β −31C > T), were found to have a higher rate of preterm birth (odds ratio = 6.36) (Hollegaard et al., 2008). The strength of this SNP association appears to be dependent on both the fetal and maternal genotypes (Yılmaz et al., 2012). However, most polymorphisms of IL-1β do not increase total serum levels, but the TT genotype at position −31 increases local monocyte IL-1β secretion in response to LPS (Chen et al., 2006; Cullup et al., 2004). Additionally, similar studies have examined polymorphisms of the IL-6 promoter. As mentioned previously, IL-6 is a pro-inflammatory cytokine increased in the amniotic fluid of preterm mothers (Han et al., 2009). Individuals homozygous for a G to C polymorphism at position −174 within the IL-6 promoter (IL-6 −175G > C) have significantly decreased plasma levels of IL-6 (Fishman et al., 1998). In concordance with this result, Simhan et al. found that the CC genotype at this locus was associated with fewer preterm births (Simhan, 2003). In further stratifications, Macones et al. discovered that women with a polymorphism in the promoter region of TNFα (−308) and diagnosed with bacterial vaginosis had a higher rate of preterm birth (adjusted odds ratio = 6.0) (Macones et al., 2004). Altogether, these results suggest a host gene–microbiome interaction that influences a mother's risk of preterm birth. Hematogenous microbial colonization of the placenta (albeit in low abundance) may

cause local inflammation that induces premature labor. However, certain polymorphisms of key cytokines may heighten the sensitivity of the gravid immune system to bacterial stimuli, leading to an inappropriate immune response to an otherwise normal placental microbiome.

5. Timing of placental colonization and inflammation

Another intriguing idea is that bacterial infiltration into placental tissue and the resulting inflammation may occur in term labors as a method of inducing labor. Recently, Lappas examined the expression of cytokines and transcription factors in the myometrium of gravidae undergoing an elective or laboring cesarean. This group found that the forkhead box O1 (FOXO1) was upregulated in patients undergoing a labored cesarean (Lappas, 2013). Further, when FOXO1 expression was diminished in myometrial cell lines using small interfering RNAs (siRNAs), this group demonstrated that IL-6 and IL-8 expression was diminished in the absence of FOXO1 upon stimulation with IL-1β (Lappas, 2013). This study, combined with the finding that bacteria were found in the basal plate of the placenta of term and preterm gravidae (Stout et al., 2013), indicates that microbes may permeate the placenta to induce labor. However, questions pondering why this process occurs early in preterm patients and how to prevent the early onset of labor still remain. The evolving knowledge base of human genomics, the microbiome, and microbial metabolic pathways will hopefully elucidate answers to these questions. Further work in these areas is desperately needed in order to answer these questions definitively.

6. Conclusions

Preterm birth is one of the leading causes of neonatal mortality in the United States, yet its definitive attributable etiology remains to be clarified. Its known association with genitourinary infections and periodontal disease has prompted investigations into the microbiome of these sites. Until recently, the paradigm was that the majority of intrauterine infections originated in the lower genital tract with microbiota ascending into an otherwise sterile environment resulting in infection of the placenta, fetal membranes (chorioamnionitis), umbilical cord (funisitis), and the fetus (sepsis) (Gonçalves et al., 2002). However, mounting evidence from human and animal models points to an alternative hematogenous source of infection, namely from the oral cavity (Madianos et al., 2013). The discovery of associations between the oral and placental microbiome, particularly in cases of periodontal disease and preterm birth, would have a particularly high impact, and the current innovations in metagenomic research serve as the perfect arena in which to apply microbiome principles to research and prevention of preterm birth. Utilizing current and developing state-of-the-science technology and analysis tools, science is at the point in which the role of the microbiome in preterm birth can, and should, be prospectively investigated. Only this will lead to the understanding necessary to propose causative etiologies and diagnostics of preterm birth. Once understood, innovative treatments, including simple dietary changes, may be developed to tackle this pressing perinatal epidemic.

Whether we are obstetrician gynecologists, reproductive biologists, or physician scientists, we are called upon to challenge long-standing assumptions that have failed to lead to major breakthroughs in peripartum care for women and their children. Beyond the introduction of progesterone-based prophylaxis and cerclage for the prevention of recurrent preterm birth, we have yet to make a meaningful dent in the rate of preterm births. Both nationally and globally our rates of prematurity have only incrementally fluctuated over the past decades. Concomitant with our emerging understanding that our microbiome largely comprises microbiota that are more friend than foe is an appreciation that longitudinal studies with deep phenotypic and clinical metadata are going to be instrumental in discerning association from causation. As more whole genome shotgun data are combined with metatranscriptomics analysis (thereby telling us not only who is present, but what they are actually doing), we will begin to acquire necessary snapshots of biological flow and disease progression over time. However, these more complex datasets will necessitate tools and personnel with the capacity to convert big data to applicable knowledge. We anticipate that future studies that are capable of making a significant and lasting impact will be those that marry big data metagenomic science with inherent clinical knowledge from larger population-based cohorts with longitudinal samples. These studies will ultimately enable inferred causation analysis, which is critical to deciphering the role of the microbiome in modulating the risk of preterm birth, and the impact of known and novel interventions on this process. Such approaches will yield not only potentially novel biomarkers, but the capacity for knowledge-based interventions.

Conflict of interest

None.

References

Aagaard, K., Riehle, K., Ma, J., Segata, N., Mistretta, T.-A., Coarfa, C., Raza, S., Rosenbaum, S., Van den Veyver, I., Milosavljevic, A., Gevers, D., Huttenhower, C., Petrosino, J., Versalovic, J., 2012. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PLoS ONE 7, e36466, http://dx.doi.org/10.1371/journal.pone.0036466.

Aagaard, K., Ma, J., Antony, K.M., Anders, A., Ganu, R., Petrosino, J., Versalovic, J., 2014. The placenta harbors a unique microbiome. Sci. Transl. Med. (in press).

Abee, T., Klaenhammer, T.R., Letellier, L., 1994. Kinetic studies of the action of lactacin F, a bacteriocin produced by *Lactobacillus johnsonii* that forms poration complexes in the cytoplasmic membrane. Appl. Environ. Microbiol. 60, 1006–1013.

Arce, R.M., Barros, S.P., Wacker, B., Peters, B., Moss, K., Offenbacher, S., 2009. Increased TLR4 expression in murine placentas after oral infection with periodontal pathogens. Placenta 30, 156–162, http://dx.doi.org/10.1016/j.placenta.2008.11.017.

Bearfield, C., 2002. Possible association between amniotic fluid micro-organism infection and microflora in the mouth. Brit. J. Obst. Gynaecol. 109, 527–533.

Beck, S., Wojdyla, D., Say, L., Betran, A.P., Merialdi, M., Requejo, J.H., Rubens, C., Menon, R., Van Look, P.F.A., 2010. The worldwide

incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull. World Health Organ. 88, 31–38, http://dx.doi.org/10.2471/BLT.08.062554.

Blackwell, S., Romero, R., Chaiworapongsa, T., Kim, Y.M., Bujold, E., Espinoza, J., Camacho, N., Hassan, S., Yoon, B.H., Refuerzo, J.S., 2003. Maternal and fetal inflammatory responses in unexplained fetal death. J. Matern. Fetal. Neonatal Med. 14, 151–157, http://dx.doi.org/10.1080/jmf.14.3.151.157.

Chen, H., Wilkins, L.M., Aziz, N., Cannings, C., Wyllie, D.H., Bingle, C., Rogus, J., Beck, J.D., Offenbacher, S., Cork, M.J., Rafie-Kolpin, M., Hsieh, C.-M., Kornman, K.S., Duff, G.W., 2006. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Hum. Mol. Genet. 15, 519–529, http://dx.doi.org/10.1093/hmg/ddi469.

Christiansen, O.B., 2013. Reproductive immunology. Mol. Immunol. 55, 8–15, http://dx.doi.org/10.1016/j.molimm.2012.08.025.

Cullup, H., Middleton, P.G., Duggan, G., Conn, J.S., Dickinson, a.M., 2004. Environmental factors and not genotype influence the plasma level of interleukin-1 receptor antagonist in normal individuals. Clin. Exp. Immunol. 137, 351–358, http://dx.doi.org/10.1111/j.1365-2249.2004.02531.x.

Douvier, S., Neuwirth, C., Filipuzzi, L., Kisterman, J.P., 1999. Chorioamnionitis with intact membranes caused by *Capnocytophaga sputigena*. Eur. J. Obstet. Gynecol. Reprod. Biol. 83, 109–112.

Fardini, Y., Chung, P., Dumm, R., Joshi, N., Han, Y.W., 2010. Transmission of diverse oral bacteria to murine placenta: evidence for the oral microbiome as a potential source of intrauterine infection. Infect. Immun. 78, 1789–1796, http://dx.doi.org/10.1128/IAI.01395-09.

Fishman, D., Faulds, G., Jeffery, R., Mohamed-Ali, V., Yudkin, J.S., Humphries, S., Woo, P., 1998. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J. Clin. Invest. 102, 1369–1376, http://dx.doi.org/10.1172/JCI2629.

Fortunato, S.J., Menon, R.P., Swan, K.F., Menon, R., 1996. Inflammatory cytokine (interleukins 1, 6 and 8 and tumor necrosis factor-alpha) release from cultured human fetal membranes in response to endotoxic lipopolysaccharide mirrors amniotic fluid concentrations. Am. J. Obstet. Gynecol. 174, 1855–1861 (discussion 1861–1862).

Gonçalves, L.F., Chaiworapongsa, T., Romero, R., 2002. Intrauterine infection and prematurity. Ment. Retard. Dev. Disabil. Res. Rev. 8, 3–13, http://dx.doi.org/10.1002/mrdd.10008.

Greig, P.C., Herbert, W.N., Robinette, B.L., Teot, L.a., 1995. Amniotic fluid interleukin-10 concentrations increase through pregnancy and are elevated in patients with preterm labor associated with intrauterine infection. Am. J. Obstet. Gynecol. 173, 1223–1227.

Han, Y., Redline, R., Li, M., Yin, L., 2004. *Fusobacterium nucleatum* induces premature and term stillbirths in pregnant mice: implication of oral bacteria in preterm birth. Infect. Immun. 72, 2272–2279, http://dx.doi.org/10.1128/IAI.72.4.2272.

Han, Y., Ikegami, A., Bissada, N., 2006. Transmission of an uncultivated Bergeyella strain from the oral cavity to amniotic fluid in a case of preterm birth. J. Clin. Microbiol. 44, 1475–1483, http://dx.doi.org/10.1128/JCM.44.4.1475-1483.2006.

Han, Y.W., Shen, T., Chung, P., Buhimschi, I.A., Buhimschi, C.S., 2009. Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth. J. Clin. Microbiol. 47, 38–47, http://dx.doi.org/10.1128/JCM.01206-08.

Holcberg, G., Amash, A., Sapir, O., Sheiner, E., Levy, S., Myatt, L., Huleihel, M., 2008. Perfusion with lipopolysaccharide differently affects the secretion of interleukin-1 beta and interleukin-1 receptor antagonist by term and preterm human placentae. Placenta 29, 593–601, http://dx.doi.org/10.1016/j.placenta.2008.03.008.

Hollegaard, M.V., Grove, J., Thorsen, P., Wang, X., Mandrup, S., Christiansen, M., Norgaard-Pedersen, B., Wojdemann, K.R., Tabor, A., Attermann, J., Hougaard, D.M., 2008. Polymorphisms in the tumor necrosis factor alpha and interleukin 1-beta promoters with possible gene regulatory functions increase the risk of preterm birth. Acta Obstet. Gynecol. Scand. 87, 1285–1290, http://dx.doi.org/10.1080/00016340802468340.

Human Microbiome Project Consortium, 2012. Structure, function and diversity of the healthy human microbiome. Nature 486, 207–214, http://dx.doi.org/10.1038/nature11234.

Hyman, R.W., Fukushima, M., Jiang, H., Fung, E., Rand, L., Johnson, B., Vo, K.C., Caughey, A.B., Hilton, J.F., Davis, R.W., Giudice, L.C., 2014. Diversity of the vaginal microbiome correlates with preterm birth. Reprod. Sci. 21, 32–40, http://dx.doi.org/10.1177/1933719113488838.

Jost, T., Lacroix, C., Braegger, C.P., Chassard, C., 2012. New insights in gut microbiota establishment in healthy breast fed neonates. PLoS ONE 7, e44595, http://dx.doi.org/10.1371/journal.pone.0044595.

Kilpatrick, S.J., 2013. Prediction and Prevention of Preterm Birth: What Have We Learned Since 2001? [WWW Document]. http://contemporaryobgyn.modernmedicine.com/sites/default/files/images/Logos/coglogo.jpg, http://contemporaryobgyn.modernmedicine.com/contemporary-obgyn/news/modernmedicine/modern-medicine-feature-articles/prediction-and-prevention-pr

Koren, O., Goodrich, J.K., Cullender, T.C., Spor, A., Laitinen, K., Bäckhed, H.K., Gonzalez, A., Werner, J.J., Angenent, L.T., Knight, R., Bäckhed, F., Isolauri, E., Salminen, S., Ley, R.E., 2012. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 150, 470–480, http://dx.doi.org/10.1016/j.cell.2012.07.008.

Lappas, M., 2013. Forkhead box O1 (FOXO1) in pregnant human myometrial cells: a role as a pro-inflammatory mediator in human parturition. J. Reprod. Immunol. 99, 24–32, http://dx.doi.org/10.1016/j.jri.2013.04.005.

Macones, G.A., Parry, S., Elkousy, M., Clothier, B., Ural, S.H., Strauss, J.F., 2004. A polymorphism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. Am. J. Obstet. Gynecol. 190, 1504–1508, http://dx.doi.org/10.1016/j.ajog.2004.01.001 (discussion 3A).

Madianos, P.N., Bobetsis, Y.a, Offenbacher, S., 2013. Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms. J. Periodontol. 84, S170–S180, http://dx.doi.org/10.1902/jop.2013.1340015.

Milisavljevic, V., Garg, M., Vuletic, I., Miller, J.F., Kim, L., Cunningham, T.D., Schröder, I., 2013. Prospective assessment of the gastroesophageal microbiome in VLBW neonates. BMC Pediatr. 13, 49, http://dx.doi.org/10.1186/1471-2431-13-49.

O'Hanlon, D.E., Moench, T.R., Cone, R.A., 2011. In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. BMC Infect. Dis. 11, 200, http://dx.doi.org/10.1186/1471-2334-11-200.

Palmer, C., Bik, E.M., DiGiulio, D.B., Relman, D.A., Brown, P.O., 2007. Development of the human infant intestinal microbiota. PLoS Biol. 5, e177, http://dx.doi.org/10.1371/journal.pbio.0050177.

Pridmore, R.D., Berger, B., Desiere, F., Vilanova, D., Barretto, C., Pittet, A.-C., Zwahlen, M.-C., Rouvet, M., Altermann, E., Barrangou, R., Mollet, B., Mercenier, A., Klaenhammer, T., Arigoni, F., Schell, M.A., 2004. The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonii* NCC 533. Proc. Natl. Acad. Sci. U. S. A. 101, 2512–2517.

Ravel, J., Gajer, P., Abdo, Z., Schneider, G.M., Koenig, S.S.K., McCulle, S.L., Karlebach, S., Gorle, R., Russell, J., Tacket, C.O., Brotman, R.M., Davis, C.C., Ault, K., Peralta, L., Forney, L.J., 2011. Vaginal microbiome of reproductive-age women. Proc. Natl. Acad. Sci. U. S. A. 108 Suppl, 4680–4687, http://dx.doi.org/10.1073/pnas.1002611107.

Romero, R., Gomez, R., Ghezzi, F., Yoon, B.H., Mazor, M., Edwin, S.S., Berry, S.M., 1998. A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. Am. J. Obstet. Gynecol. 179, 186–193.

Romero, R., Hassan, S.S., Gajer, P., Tarca, A.L., Fadrosh, D.W., Nikita, L., Galuppi, M., Lamont, R.F., Chaemsaithong, P., Miranda, J., Chaiworapongsa, T., Ravel, J., 2014. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome 2, 4, http://dx.doi.org/10.1186/2049-2618-2-4.

Sadowsky, D.W., Novy, M.J., Witkin, S.S., Gravett, M.G., 2003. Dexamethasone or interleukin-10 blocks interleukin-1β-induced uterine contractions in pregnant rhesus monkeys. Am. J. Obstet. Gynecol. 188, 252–263, http://dx.doi.org/10.1067/mob.2003.70.

Simhan, H., 2003. Interleukin-6 promoter −174 polymorphism and spontaneous preterm birth. Am. J. Obstet. Gynecol. 189, 915–918, http://dx.doi.org/10.1067/S0002-9378(03)00843-3.

Stout, M.J., Conlon, B., Landeau, M., Lee, I., Bower, C., Zhao, Q., Roehl, K.A., Nelson, D.M., Macones, G.A., Mysorekar, I.U., 2013. Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations. Am. J. Obstet. Gynecol. 208, 226.e1–226.e7, http://dx.doi.org/10.1016/j.ajog.2013.01.018.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., Gordon, J.I., 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444, 1027–1031, http://dx.doi.org/10.1038/nature05414.

Turnbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., Egholm, M., Henrissat, B., Heath, A.C., Knight, R., Gordon, J.I., 2009. A core gut microbiome in obese and lean twins. Nature 457, 480–484, http://dx.doi.org/10.1038/nature07540.

Wang, X., Buhimschi, C.S., Temoin, S., Bhandari, V., Han, Y.W., Buhimschi, I.A., 2013. Comparative microbial analysis of paired amniotic

fluid and cord blood from pregnancies complicated by preterm birth and early-onset neonatal sepsis. PLoS ONE 8, e56131, http://dx.doi.org/10.1371/journal.pone.0056131.
Yılmaz, Y., Verdi, H., Taneri, A., Yazıcı, A.C., Ecevit, A.N., Karakaş, N.M., Tarcan, A., Haberal, A., Ozbek, N., Atac, F.B., 2012. Maternal–fetal proinflammatory cytokine gene polymorphism and preterm birth. DNA Cell Biol. 31, 92–97, http://dx.doi.org/10.1089/dna.2010.1169.
Zenclussen, A.C., 2013. Adaptive immune responses during pregnancy. Am. J. Reprod. Immunol. 69, 291–303, http://dx.doi.org/10.1111/aji.12097.