

# Placental colonization with periodontal pathogens: the potential missing link



Lori A. Fischer, PhD; Ellen Demerath, PhD; Peter Bittner-Eddy, PhD; Massimo Costalonga, DMD, PhD

Adverse pregnancy outcomes (APOs) including preterm birth (PTB), low birthweight (LBW), and comorbid preterm low birthweight (PLBW) occur in a significant number of women without an apparent etiology, suggesting that undiscovered risk factors for APOs exist. Periodontitis has been proposed as a novel risk factor for APOs.<sup>1</sup>

Disagreement on the association between periodontitis and APOs derives from three major sources. First, there has been heterogeneity in the clinical definition of severity and extent of periodontitis used to distinguish cases vs controls.<sup>2-4</sup> Second, studies fail to control for shared risk factors or confounders between periodontitis and APOs.<sup>2-4</sup> Lastly, most studies did not consider the spread and survival of periodontal pathogens to the placenta as a mechanism that could induce APOs independently of ongoing disease in the oral cavity.<sup>2-4</sup>

## Defining periodontitis

Periodontitis is evaluated as a permanent loss of clinical attachment level

From the Division of Periodontology, Department of Developmental and Surgical Sciences, School of Dentistry (Drs Fischer, Bittner-Eddy, and Costalonga), and the Division of Epidemiology and Community Health, School of Public Health (Dr Demerath), University of Minnesota, Minneapolis MN.

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Corresponding author: Massimo Costalonga, DMD, PhD. [costa002@umn.edu](mailto:costa002@umn.edu)

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Observational studies demonstrate that women with severe periodontitis have a higher risk of adverse pregnancy outcomes like preterm birth and low birthweight. Standard treatment for periodontitis in the form of scaling and root planing during the second trimester failed to reduce the risk of preterm or low birthweight. It is premature to dismiss the association between periodontitis and adverse pregnancy outcomes because one explanation for the failure of scaling and root planing to reduce the risk of adverse pregnancy outcomes is that periodontal pathogens spread to the placental tissue prior to periodontal treatment. In the placenta, orally derived organisms could cause direct tissue damage or mediate a maternal immune response that impairs the growth of the developing fetus. Sequencing studies demonstrate the presence of organisms derived from the oral microbiome in the placenta, but DNA-based sequencing studies should not be the only technique to evaluate the placental microbiome because they may not detect important shifts in the metabolic capability of the microbiome. In humans, polymerase chain reaction and histology have detected periodontal pathogens in placental tissue in association with multiple adverse pregnancy outcomes. We conclude that both placental and oral microbiomes may play a role in periodontitis-associated adverse pregnancy outcomes. However, the measure to determine the association between periodontal pathogens in the placenta and adverse pregnancy outcomes should be the amount and prevalence, not the mere presence of such microorganisms. Placental colonization with periodontal pathogens thus potentially represents the missing link between periodontitis and adverse pregnancy outcomes.

**Key words:** adverse pregnancy outcomes, low birthweight, periodontitis, preterm birth

(CAL).<sup>5-10</sup> CAL measures the distance between an anatomical reference on tooth crown and the bottom of the periodontal pocket. Probing depth (PD) is a measure related to CAL that estimates the depth of the periodontal pocket. The deeper the pocket, the more inflammation is present around a diseased tooth. These 2 parameters are generally measured at 6 independent sites around each tooth.

The strength of association between periodontitis and APOs varies, depending on the severity (amount of CAL and/or PD) and the extent of the disease (number of sites within the mouth with a given level of CAL or PD) used to separate cases from controls.<sup>11</sup> One astute study demonstrated this principle by applying multiple published definitions of periodontitis to the same data set. Six definitions of periodontitis resulted in statistically significant associations with

APOs, whereas 8 did not.<sup>11</sup> Heterogeneity in the periodontitis definition also means that it is difficult to combine data across multiple studies for a meta-analysis.<sup>12,13</sup> This problem may be mitigated if future studies utilize the 2017 consensus classification of periodontal diseases.<sup>10</sup>

Periodontitis in pregnancy should not be confused with pregnancy gingivitis. Pregnancy gingivitis is a common, reversible condition of gingival inflammation associated with high levels of estrogens and blooms of microbial species such as *Prevotella intermedia*.<sup>14-19</sup> In contrast, periodontitis is associated with a fundamental shift in the relative composition of the oral microbiome induced by anaerobic keystone pathogens.<sup>20</sup> In periodontitis the modification of the microbial composition is unrelated to pregnancy status or pregnancy hormones. When

good oral hygiene practices are implemented, pregnancy gingivitis resolves within a few months of birth with no permanent changes in CAL. Pregnancy gingivitis is not considered a risk factor for adverse pregnancy outcomes.<sup>16,17,19</sup>

### Risk factors

The drive to discover whether periodontitis represents a risk factor for APOs stems from the need to develop interventions that reduce the impact of APOs on families, children, and society. Because APOs and periodontitis have many risk factors in common, shared risk factors must be considered in any valid attempt to establish periodontitis as causative of APOs<sup>12,13</sup> (Table 1). Interestingly, many of these risk factors are associated with inflammation or inflammation-induced pathologies. There has been some suggestion that individuals with a more proinflammatory genotype may be more susceptible to inflammation-mediated disorders like periodontitis or chorioamnionitis. For example, polymorphisms of the interleukin (IL)-1 gene are associated with both periodontitis and APOs.<sup>21-29</sup>

Because complications associated with APOs are a leading cause of infant mortality in the United States, identifying novel risk factors for APOs are important.<sup>30,31</sup> Some racial and ethnic groups are especially susceptible to APOs and subsequent infant mortality (Table 2).<sup>32</sup> Notably, increased maternal age was associated with both increased prevalence of periodontitis and increased infant mortality rate.<sup>31,33</sup>

APOs can also lead to life-long consequences. For example, 52% of infants born at 24–28 weeks have neurodevelopment impairment as compared with 5% of those born at 32–36 weeks.<sup>34</sup> Additionally, young adults who were born with very low birthweight (<1500 g) had lower bone mineral density than term-birth, same-age peers.<sup>35</sup>

In summary, if severe periodontitis represents a potential novel risk factor for APOs, then treating periodontitis has the potential to be a low-cost public intervention to reduce the impact of APOs on society.

### Periodontitis as a risk factor for APOs

One meta-analysis of 15 observational studies found an increased risk of PTB (odds ratio [OR], 2.73, 95% confidence interval [CI], 2.06–3.6) and LBW (OR, 1.5, 95% CI 1.26–1.79) in women with periodontitis.<sup>36</sup> Other systematic reviews of case-control and cohort studies support an association between periodontitis and APOs.<sup>13,37</sup> Again, these studies must be considered with caution because of heterogeneity in periodontitis definitions.<sup>11</sup> The observational evidence associating periodontitis with APOs leads to the hypothesis that treating periodontitis through standard periodontal therapy could result in a significant reduction in the devastating impact of PLBW on individuals and families.

### Scaling and root-planing trials

Several randomized, controlled treatment trials using scaling and root planing (SRP) tested whether a periodontal therapeutic intervention could reduce the risk of APOs in women with periodontal disease. Studies of high quality, low risk of bias<sup>38</sup> in multiple meta-analysis reports agreed that SRP does not reduce the risk of PTB or the risk of LBW.<sup>39-42</sup> These findings prompted researchers to question the mechanism behind the association between periodontitis and increased risk of APOs found in observational studies.<sup>12,13 36,37</sup>

### Why did SRP therapy fail?

SRP changes the composition of the periodontal microbiota by mechanically disrupting the biofilm within the periodontal pocket. SRP is strictly a local intervention because it does not prevent hematogenous spread of periodontal pathogens. Because gingival inflammation increases in periodontitis, the likelihood of transient bacteremia also increases.<sup>43-45</sup> It is therefore reasonable to speculate that periodontal pathogens seeding the placental tissue prior to second-trimester SRP treatment may impair the developing fetus.<sup>4</sup>

Infection and inflammation associated with overgrowth of urinary tract and vaginal microorganisms have been

well characterized as a risk factor for PLBW. Interestingly, both bacterial vaginosis and periodontitis involve a change in the microbiome that favors overgrowth of anaerobic species normally found in low numbers in their respective human ecological niches. This observation leads to the possibility that anaerobic species originating from the oral cavity and now found in the placenta could be involved in APOs, paralleling the observation that overgrowth of anaerobic species from or in the vagina is involved in APOs.<sup>46,47</sup>

### Anaerobic species of APOs and periodontitis

Bacterial vaginosis is an independent risk factor for PLBW.<sup>32,48,49</sup> Bacterial vaginosis results from an overgrowth of organisms such as the anaerobes *Gardnerella vaginalis* and *Mycoplasma hominis* in the vaginal canal. These low-frequency organisms grow to replace the normal *Lactobacillus*-dominated vaginal microbiome.<sup>32,46</sup>

Anaerobic periodontal pathogens have also been detected in the vaginal microbiome in individuals with bacterial vaginosis.<sup>50,51</sup> Interestingly, black women were more likely to have vaginal microbiomes dominated by anaerobes, while white women were more likely to have a vaginal flora dominated by *Lactobacillus* species.<sup>52</sup> This suggests the possibility that the increased rate of PLBW in black women (Table 2) is associated with the increased proportions of vaginal anaerobes. The increased prevalence of vaginal anaerobes may also influence the higher rate of bacterial vaginosis in black vs white women (51% vs 23%, respectively).<sup>53</sup>

*Porphyromonas gingivalis* and *Fusobacterium nucleatum* are two anaerobic species commonly associated with periodontitis that have been detected in the vaginal or placental microbiome in association with APOs.<sup>50,51,54-56</sup> The amount of *P gingivalis* is higher in the oral cavity of pregnant women who subsequently experienced PTB as compared with women with term births.<sup>47</sup> Both *P gingivalis* and *F nucleatum* are also found in the oral cavity of

periodontally healthy individuals, albeit in significantly lower numbers.<sup>55,56</sup>

Perhaps because anaerobic species from the vagina can lead to inflammation associated with bacterial vaginosis, anaerobic species from the oral cavity can lead to inflammation-mediated placental destruction and APOs. The anaerobic periodontal pathogens *F nucleatum* and *P gingivalis* are two prime candidates that could potentially induce placental inflammation and cause subsequent placental damage.

### Relevance of Fusobacteria and Porphyromonas species

The periodontal pathogen *F nucleatum* has been frequently detected in association with APOs.<sup>57-61</sup> Experiments using a mouse model have demonstrated biological plausibility for spread of *F nucleatum* to the murine placenta<sup>43</sup> and subsequent murine PTB or stillbirth.<sup>62,63</sup> The adhesion complexes FadA<sup>64</sup> and Fap2<sup>65</sup> may be critical to *Fusobacterium* invasion of placental tissue. Higher vaginal IL-8 concentrations have been found in women with *Fusobacterium* infections of the amniotic fluid, suggesting *Fusobacterium* may also elicit an immune response that can be detrimental to fetal development.<sup>66</sup>

In comparison, *P gingivalis*, a low-abundance keystone pathogen, is capable of altering nutrients in the local environment in a way that shifts the composition of the microbial community.<sup>20</sup> The microbiome is essential to the pathogenesis of *P gingivalis*-mediated periodontitis because germ-free mice monocolonized with *P gingivalis* do not develop alveolar bone loss.<sup>67</sup>

*P gingivalis* may similarly be capable of shifting the placental microbiome toward a more virulent profile. PLBW could be directly induced through bacterial products or through disruption of the normal placental microbiome composition, resulting in damage to placental structures and nutrient transfer (Figure, A).

Alternatively, the more virulent placental microbiome may attract the attention of the immune system. When the balance of microbiome, trophoblast, and immune cells at implantation site is disturbed, the resulting maternal

**TABLE 1**

### Risk factors for APOs and periodontitis in the United States

| Risk factor                                  | APOs (reference)        | Periodontitis (reference)                 |
|--|-------------------------|---|
| Age greater than 34 years                    | ✓ <sup>120</sup>        | ✓ <sup>121, 122</sup>                     |
| Age less than 17 years                       | ✓ <sup>120</sup>        |   |
| Low socioeconomic status                     | ✓ <sup>120</sup>        | ✓ <sup>121, 122</sup>                     |
| Recreational drug abuse                      | ✓ <sup>120</sup>        | ✓ <sup>123</sup>                          |
| Alcohol use                                  | ✓ <sup>120</sup>        | ✓ <sup>124</sup>                          |
| Smoking or other tobacco use                 | ✓ <sup>120</sup>        | ✓ <sup>121, 122, 124</sup>                |
| Hypertension/cardiovascular disease          | ✓ <sup>120</sup>        | ✓ <sup>121</sup>                          |
| Diabetes                                     | ✓ <sup>120</sup>        | ✓ <sup>121, 122, 124</sup>                |
| Obesity/metabolic syndrome                   | ✓ <sup>120</sup>        | ✓ <sup>121, 122, 124</sup>                |
| Osteoporosis                                 |                         | ✓ <sup>124</sup>                          |
| Deficiencies in dietary calcium or vitamin D |                         | ✓ <sup>124</sup>                          |
| Stress                                       | ✓ <sup>120</sup>        | ✓ <sup>121, 124</sup>                     |
| Inadequate prenatal care                     | ✓ <sup>120</sup>        |   |
| Multiple pregnancies                         | ✓ <sup>120</sup>        |   |
| Previous incidence of APOs                   | ✓ <sup>120</sup>        |   |
| Racial/ethnic group                          | ✓ <sup>120</sup>        | ✓ <sup>121, 122</sup>                     |
| Anaerobic bacterial infections               | ✓ <sup>32, 48, 49</sup> | ✓ <sup>55, 56, 92, 93, 121, 125-129</sup> |
| Polymorphisms of IL-1 gene                   | ✓ <sup>21-24</sup>      | ✓ <sup>25-29</sup>                        |

APO, adverse pregnancy outcome; IL, interleukin.

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inflammatory condition has the potential to result in indirect placental collateral damage and APOs (Figure, B and C).<sup>1,68</sup> For example, subcutaneous inoculation of BALB/c mice with *P gingivalis* leads to fetal growth restriction in correlation with an increase in CD4<sup>+</sup> T-helper cells expressing interferon gamma.<sup>69,70</sup>

*P gingivalis* is also detrimental to fetal development in hamsters and rats<sup>71-73</sup> but not to rabbits, despite the ability to translocate to the placenta.<sup>74,75</sup> Systemic interferon gamma is hypothesized to be detrimental for pregnancy outcomes in multiple species, although not all studies are in agreement.<sup>76-85</sup>

As described earlier, many of the shared risk factors between periodontitis and APOs involve an increase in local or systemic inflammation. Indeed, labor itself involves the upregulation of inflammatory mediators in a positive feedback mechanism. It is possible therefore that preterm labor may be induced by too

much inflammation too early in pregnancy. Inflammation induced by periodontal pathogens or the influence of periodontal pathogens on the normal placental microbiome is consequently central to the hypothesized connection between periodontitis and APOs.

### Detection of periodontal pathogens in PLBW placentas

#### Polymerase chain reaction

Placental tissue samples taken from women with PLBW infants yielded a higher prevalence of microorganisms than controls.<sup>86</sup> More specifically, *F nucleatum* was detected in 94% of placentas from mothers with periodontitis and PLBW as compared with 36.4% of full-term placentas from mothers without periodontitis.

The same clonotype of *F nucleatum* was detected in the placenta of a stillborn infant and in the mouth of its mother, who experienced excessive gingival

TABLE 2

## Prevalence of APOs, periodontitis, and infant mortality rate by race

| Racial/ethnic group | Prevalence of periodontitis, <sup>a</sup><br>1999–2004 <sup>33</sup> | Infant mortality<br>rate, <sup>b</sup> 2005 <sup>31</sup> | Percent of live<br>births, 2013 <sup>31</sup> |       |
|---------------------|--|---|---|-------|
| Non-Hispanic white  | 2.0%   | 5.06  | PTB   | 10.2% |
|                     |  |   | LBW <2500 g                                   | 7.0%  |
|                     |  |   | LBW <1500 g                                   | 1.13% |
| Non-Hispanic black  | 2.2%   | 13.63   | PTB   | 16.3% |
|                     |  |   | LBW <2500 g                                   | 13.2% |
|                     |  |   | LBW <1500 g                                   | 2.96% |
| Mexican American    | 9.3%   | 5.62  | PTB   | 10.8% |
|                     |  |   | LBW <2500 g                                   | 6.6%  |
|                     |  |   | LBW <1500 g                                   | 1.15% |

APO, adverse pregnancy outcome; CAL, clinical attachment level; LBW, low birthweight; NHANES, National Health and Nutrition Examination Survey; PD, probing depth; PTB, preterm birth.

<sup>a</sup> A patient having periodontitis was defined by having 2 teeth with each having at least 1 periodontal site with both  $\geq 3$  mm of CAL and  $\geq 4$  mm PD measuring 2 of 4 possible quadrants.<sup>33</sup> A follow-up study suggested NHANES examinations using recordings in 2 of 4 quadrants may have underestimated the prevalence of periodontal disease by 50% or more.<sup>130</sup> <sup>b</sup> Number of deaths per 1000 live births.

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bleeding during pregnancy.<sup>63</sup> *Parvimonas micra*, a known periodontal pathogen, was detected only in placentas of mothers with PLBW and was not detected in placentas of full-term mothers without periodontitis.<sup>86</sup> Interestingly, *Actinomyces israelii*, a microorganism found in healthy mouths, was detected more frequently in mothers without periodontitis with full-term, normal-weight births.

Preeclampsia is an APO that has been associated with pathological inflammation.<sup>87-89</sup> Among the genera of microorganisms detected in placentas from preeclampsia patient were *Bacillus*, *Variovorax*, *Prevotella*, *Porphyromonas*, the proposed periodontal pathogen *Dialister*,<sup>56</sup> and *Lactobacillus*.<sup>90</sup>

In another study, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*, *F nucleatum*, and *P intermedia* were detected only in placental samples from the preeclampsia patient group.<sup>91</sup> *P gingivalis*, *F nucleatum*, *Tannerella forsythensis*, and *Treponema denticola* were found in significantly higher numbers in the preeclampsia patients than in controls.<sup>91</sup> *A actinomycetemcomitans* and *T denticola* are also strongly associated with periodontitis,<sup>56,92,93</sup> and *T denticola* in vaginal swabs has also been associated

with PTB.<sup>47</sup> Increased amounts of *P intermedia* have been implicated in the pathogenesis of pregnancy gingivitis.<sup>14-19</sup> These studies thus demonstrate that a few select periodontal pathogens can be detected in placentas associated with APOs via polymerase chain reaction (PCR)-based methods.

Because the placenta receives most of the 10% of uterine blood flow with each stroke volume, there is the possibility that the placenta filters out microbes present in the blood like the spleen. However, oral pathogens were not detected in the maternal blood in any subjects with preeclampsia or controls.<sup>90</sup> This suggests that oral microorganisms can enter the blood stream infrequently or at below detectable levels after manipulation of the gingiva with brushing or even after chewing food.<sup>43-45</sup> Ultimately, these bacteria may be able to colonize and grow to detectable levels only upon reaching the microbial niche of the placenta.

#### Immunohistochemistry and immunofluorescence

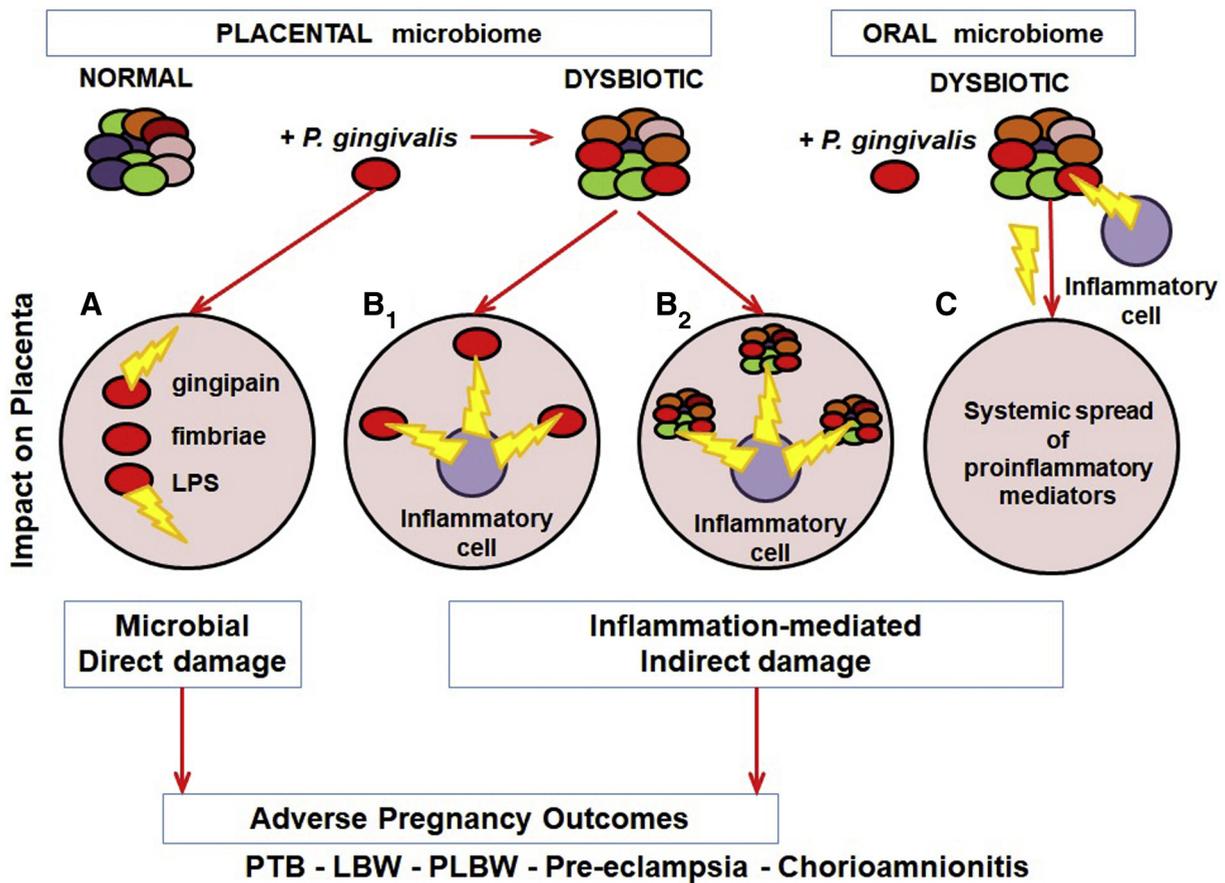
As compared with PCR, immunohistochemistry and immunofluorescence have the advantage of being able to definitively detect whether periodontal pathogens in the placenta are transient visitors within the maternal blood

vessels or whether true placental tissue invasion has occurred.

Placental sections from chorioamnionitis-affected placentas associated with PTB showed 30% more intense immunostaining for *P gingivalis* antigens than normal placental controls.<sup>94</sup> A second study reported that *P gingivalis* antigens in the umbilical cord were associated with preeclampsia. In this study, *P gingivalis* in the placenta was associated with PTB and delivery by caesarean delivery but not with chorioamnionitis or preeclampsia.<sup>95</sup> These findings collectively suggest that well-established periodontal pathogens can be detected within the cells and tissue of placentas associated with inflammation-mediated APOs and not merely within the placental blood vessels.

Ultimately though, we must interpret the PCR, immunohistochemical, and immunofluorescent data with caution because the mere presence of oral *P gingivalis* does not indicate presence or risk of developing periodontitis.<sup>44</sup> Rather, periodontitis is associated with an increase in *P gingivalis* abundance in the oral microbiome. Perhaps it is a similar situation in the placenta, in which an increase in abundance rather than simple presence of a periodontal pathogen determines the development of APOs.<sup>96</sup>

**FIGURE**  
Model connecting periodontitis to APOs



Damage to placental structures could result from direct action of *P. gingivalis* virulence factors (A), proinflammatory cells responding to *P. gingivalis* (B<sub>1</sub>), proinflammatory cells responding to the altered placental microbiome (B<sub>2</sub>), systemic spread of proinflammatory mediators responding to a dysbiotic oral microbiome (C), or damage to the placental structure may then lead to various APOs.

APO, adverse pregnancy outcome.

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### The placental microbiome

Given that an oral microbiome is necessary for *P. gingivalis* to induce inflammation that drives the pathology of periodontitis, a local microbiome would likely be necessary for *P. gingivalis* to induce APOs. The existence of a placental microbiome is a recent, and still controversial, discovery.<sup>57-59,97-100</sup> One side of the controversy ascertains that a unique, low-abundance, placental microbiome exists. In contrast, some believe the detection of bacterial species in placental samples represents environmental contamination and not true placental colonization.<sup>57,101-110</sup>

On the “pro-placental microbiome” side, placentas obtained from elective caesarian sections harbor distinct microbial populations in the amniotic fluid, placenta, and infant meconium (first feces). These three populations shared enough features to suggest that placental and amniotic fluid species transferred at the fetomaternal interface are the first colonizers of the infant gut.<sup>97</sup> Among the low abundance microbes present in amniotic fluid or placenta, Proteobacteria and Enterobacteriaceae were the most represented phyla. The most predominant genera of the Proteobacteria phyla were

*Enterobacter*, *Escheria*, and *Shigella*. Another study verified that Proteobacteria was the most abundant placental phyla, while also reporting that the microbial composition of placentas differed between women with and without gestational diabetes mellitus.<sup>98</sup> Additionally, more than half of placentas delivered between the 24<sup>th</sup> week of gestation and one-third of those delivered between of the 27<sup>th</sup> week harbored one or more microbial species.<sup>99</sup> In this study, the number of microorganisms recovered declined with increasing gestational age among placentas recovered after labor in the

absence of preeclampsia, whether the infant was delivered vaginally or by caesarean section.

On the other side, contamination of placental samples from environmental microbial sources has been a contentious issue when it comes to characterizing the placental microbiome.<sup>57,101-108</sup> Some publications have even suggested that all or most sequencing reads detected from “placental microbiomes” actually represent environmental and reagent contamination.<sup>109,110</sup> However, these studies utilized V1-V2 16s bacterial primers, which are biased towards detecting environmental contaminants.<sup>107</sup>

To illustrate this issue, one study that detected numerous species associated with the vaginal microflora in the placenta, including the *Lactobacillus* genera, *Mycoplasma hominis*, and *Gardnerella vaginalis*, did not discard fetal membranes.<sup>60</sup> As fetal membranes are the placental tissues most likely to be contaminated by exposure to maternal body surfaces, we cannot determine with certainty whether these organisms represented true placental colonization or maternal microbiome contamination. Another study that detected *Mycoplasma* spp. and *Ureaplasma* spp. in placentas of spontaneous PTB infants found the same taxonomic reads in reagent and environmental control samples.<sup>110</sup> Lastly, detection of the *Lactobacillus* genera has been strongly associated with vaginal but not caesarean section placentas, suggesting vaginal microbiome contamination of the placenta.

Therefore, studies that included fetal membranes or used maternal sections of the placenta such as the maternal basal plate are not considered here in this review.<sup>60,111,112</sup> Instead, this review focuses on studies utilizing only placentas from sterile cesarean births or studies that removed the placental membranes, as these are the most likely to be contaminated with bacteria from maternal, amniotic fluid, or environmental surfaces (Supplementary Table S1). Additionally, these studies sampled areas of the placenta with a low chance of maternal microbial or blood

contamination such as the placental parenchyma, or chorionic and intervillous spaces. Finally, given that the placentas were harvested and processed in a random fashion, any environmental or reagent contamination would be the same in both, term placentas and placentas associated with APOs. Thus, if we focus only on those species that vary in abundance we can remove environmental contaminant noise.

### Placental Microbiome and APOs

At the phylum level, the normal placental microbiome was found to be more closely related to human non-pregnant oral microbiomes than to the vaginal, skin, or gut microbiomes (Bray-Curtis dissimilarity <0.3).<sup>57,59</sup> However, a limitation of this data set is that the oral samples were not collected from the same pregnant mothers but from a cohort of non-pregnant subjects of the Human Microbiome Project. Thus, the periodontal pathogens in the PLBW placentas could not be directly associated with maternal periodontal disease. The placental microbiome detected within this data set was mostly composed of *Firmicutes*, *Tenericutes*, *Proteobacteria*, *Bacteroidetes*, and *Fusobacteria* phyla.<sup>57,59</sup> A separate study utilizing a different patient population confirmed within the same mother that placental microbial communities are more similar to the ones found in the oral microbiome than the gut microbiome, especially at higher levels of taxonomy.<sup>107</sup> This study utilized matched maternal oral, gut, and placental samples. Additionally, this study verified that the placental microbiome has a distinct metabolic profile. Lastly, in humans the taxa of the placental microbiome varied by gestational age between week 24 to 41 (Adonis [PERMANOVA],  $p = 0.001$ ).<sup>57</sup> This suggests that the placental microbiome also changes over time.

Chorioamnionitis-associated placentas of preterm infants universally had less diverse microbiomes.<sup>58</sup> Such decreased species richness may be important since similar decreased diversity in the gut has been associated with inflammatory phenotypes and infection.<sup>113-116</sup> Sequencing studies also

revealed that the placental microbiome changes associated with PTB are not the same as the changes associated with LBW, suggesting a unique microbial community shift for each condition (Supplementary Table S2).

### Microbiome metabolic pathways altered in APOs

In APOs, differences in bacterial metabolic pathways have been detected between term and preterm placental samples.<sup>57</sup> Changes in the metabolic pathways of the placental microbiome may partially explain inflammation of the placenta in subjects with chorioamnionitis.<sup>58</sup> In periodontitis, disease-associated microbial communities have highly conserved metabolic and virulence factor gene expression profiles, even though microbial species within the disease-associated microbiome were not conserved from patient to patient. Therefore, changes in the metabolic capabilities of an altered oral and an altered placental microbiome may be the drivers of both periodontitis and certain APOs. It may be more important to know which metabolic pathways are altered in dysbiotic placental microbiomes than the individual species represented.

For example, in both chronic periodontitis subgingival microbiome and the preterm placental microbiome, methane metabolism was increased.<sup>57,117</sup> However, some pathway alterations differ between APOs and periodontitis. Butyrate or butanoate metabolism is increased in aggressive periodontitis but decreased in preterm placentas from women with excess gestational weight gain as well as being lower in term placentas without chorioamnionitis as compared with those with chorioamnionitis.<sup>58,59,118</sup>

Benzoate degradation was also decreased in term placentas without chorioamnionitis as compared with those with the condition, whereas it was increased in the subgingival plaque of chronic periodontitis patients.<sup>58,119</sup> To date, we have very few studies with limited numbers of patients analyzing the metabolic capabilities of placental or

oral microbiomes. It is therefore premature to draw definitive conclusions of specific metabolic pathways influencing APOs.

In summary, the microbiome data and preliminary metabolic data indicate that it may not be the presence of a specific periodontal pathogen in the placenta that leads to APOs. Instead, multiple keystone periodontal pathogens may be able to alter the nutrient environment in a way that leads to a disruption of the normal microbial metabolic balance and creates a more proinflammatory environment.

### Discussion and conclusion

A causal relationship between periodontitis and APOs was prematurely dismissed by some authors because SRP improved periodontal outcomes but did not reduce the risk of APOs.<sup>39-42</sup> This conclusion was predicated on the concept that oral pathogens persisting in the periodontal pocket are the sole contributors to periodontitis-associated APOs.

The evidence we presented here introduces the possibility that the placental microbiome is similar to the oral microbiome and may be amendable to colonization by oral pathogens via hematogenous spread. Clearly standard scaling and root planing treatment would not be effective at eliminating oral pathogens that have seeded the placental tissues prior to periodontal treatment.

Because periodontal pathogens are measured in the placental tissue at delivery, it is unclear exactly when the placenta is seeded. However, the failure of second-trimester therapeutic interventions to prevent APOs suggests that pathogens colonize the placenta within the first trimester. Preventing periodontal pathogens from reaching the placenta by treating periodontitis prior to pregnancy may be safer and more effective than trying to prevent APOs by treating ongoing severe periodontitis during pregnancy. Thus, while standard periodontal therapy at the second trimester is safe during pregnancy and does not increase the risk of PLBW, it should not be recommended to pregnant women with severe

periodontitis as a means of preventing PLBW.<sup>1,4</sup>

As previously discussed, the mere presence of *P gingivalis* and *F nucleatum* does not necessarily imply periodontal pathology.<sup>55,96</sup> Rather, increased frequency to levels greater than those found in the healthy state is necessary for progression to periodontitis. It is reasonable to assume that these periodontal bacteria would act similarly in a placental environment. Therefore, a key issue raised by this review is that the measure to determine the association between periodontal pathogens in the placenta and APOs should be amount and prevalence, not mere presence of such microorganisms.

There are multiple things that can be done to make future studies of the association between placental-periodontal pathogens and APOs more rigorous. First, studies need more rigorous contamination controls, in the form of collecting maternal blood, vaginal, environmental, and reagent microbiome samples simultaneously or within a short time frame of collecting the placental samples. Studies should also define a null hypothesis, specifying what results would be expected if the isolated microorganisms represent contamination and not true placental colonization.<sup>99</sup> Lastly, while it is difficult to distinguish contamination from true colonization at the genus or family level, it is possible to discern contamination when working at the strain level. Shotgun metagenomics, strain culture, and/or strain-directed sequencing may be utilized to make this distinction between contaminants and colonizers.

If future studies intend to prove that the placental microbiome resembles the maternal oral microbiome, maternal oral microbiome samples should also be taken within a short time frame of collecting the placental samples. Importantly, DNA-based sequencing studies should not be the sole technique used to evaluate the placental microbiome. DNA-based studies may miss important shifts in the metabolic capability of the altered microbiome that can be assessed only by transcriptomics or proteomics. Once potential pathogens of interest are

identified, immunohistochemical or immunofluorescent methods should be utilized to verify whether the species detected were transients contained within maternal blood or true placental tissue residents. This is especially important if the same species can be detected in both placental tissue and maternal blood samples.

Lastly, studies should utilize the new 2017 World Workshop consensus definitions of periodontitis to allow for greater clarity within the data set and enable combination of data sets across multiple patient populations for future meta-analysis.<sup>10</sup> It may really be that only women with severe forms of periodontitis are at risk of PLBW or would benefit the most from therapeutic intervention. ■

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**SUPPLEMENTAL TABLE 1****Sample collection method and adverse pregnancy outcomes**

| <b>Study</b>                | <b>Tissue collection</b>   | <b>Comparison</b>  |
|-----------------------------|--|--|
| Antony et al <sup>59</sup>  | Discard placental membranes and maternal decidua; collection 4 cm from cord insertion site                 | Term gestation, no excess gestational weight gain vs PTB with excess gestational weight gain   |
| Aagaard et al <sup>57</sup> | Discard placental membranes and maternal decidua; collection 4 cm from cord insertion site                 | Placental microbiome associated with term birthweight compared with PTB placentas and nonpregnant oral microbiome reported within Human Microbiome Project |
| Prince et al <sup>58</sup>  | Swabs from fetal chorion and/or villous placental membranes adjacent to fetal side, avoiding maternal side | Term birth placenta with and without chorioamnionitis vs PTB placentas with and without chorioamnionitis   |
| Zheng et al <sup>61</sup>   | Discard placental membranes and maternal decidua; collection 4 cm from cord insertion site                 | Full-term infants; normal vs LBW   |

LBW, low birthweight; PTB, preterm birth.

Fischer. Placental colonization with periodontal pathogens: the potential missing link. *Am J Obstet Gynecol* 2019.

## SUPPLEMENTAL TABLE 2

## Changes in placental microbiome in association with APOs as compared with normal for gestational weight term births

| Components of the microbiome             | LBW <sup>61</sup>       | PTB <sup>57,58,a,b</sup> PTB with excess gestational weight gain <sup>59,c</sup> |
|--|-------------------------|--|
| Phylum                                   |                         |  |
| <i>Actinobacteria</i>                    | ↑                       | Specific order reported <sup>a</sup><br>Phylum reported <sup>c</sup>             |
| <i>Bacteroidetes</i>                     | Specific genus reported | NR   |
| <i>Cyanobacteria</i>                     | ↓                       | Specific genus reported <sup>a</sup><br>Phylum reported <sup>c</sup>             |
| <i>Firmicutes</i>                        | ↓                       | Specific species reported <sup>a</sup><br>Phylum reported <sup>c</sup>           |
| <i>Fusobacteria</i>                      | ↑                       | Specific genus reported <sup>a</sup>   |
| <i>Proteobacteria</i>                    | ↑                       | Specific class or genus reported <sup>a,c</sup>                                  |
| Class                                    |                         |  |
| Phylum Proteobacteria                    |                         |  |
| Class Alphaproteobacteria                | NR                      | ↑ <sup>a</sup>   |
| Order                                    |                         |  |
| Phylum Actinobacteria                    |                         |  |
| Order Actinomycetales                    | NR                      | ↑  |
| Genus                                    |                         |  |
| Phylum Bacteroidetes                     |                         |  |
| Genus <i>Shingobacterium</i>             | ↑                       | NR   |
| Phylum Cyanobacteria                     |                         |  |
| Genus <i>Cyanobacteria_unclassified</i>  | ↓                       | NR   |
| Genus <i>Cyanobacteria_norank</i>        | ↓                       | NR   |
| Phylum Firmicutes                        |                         |  |
| Genus <i>Paenibacillus</i>               | NR                      | ↑  |
| Genus <i>Lactococcus</i>                 | ↑                       | NR   |
| Genus <i>Bacillus</i>                    | ↑                       | NR   |
| Genus <i>Megasphaera</i>                 | ↑                       | NR   |
| Genus <i>Faecalibacterium</i>            | ↑                       | NR   |
| Genus <i>Jeotgalicoccus</i>              | ↑                       | NR   |
| Genus <i>Pediococcus</i>                 | ↑                       | NR   |
| Genus <i>Lactobacillus</i>               | ↓                       | Specific species reported  |
| Genus <i>Enterococcus</i>                | ↓                       | NR   |
| Genus <i>Clostridium_sensu_stricto_1</i> | ↓                       | NR   |
| Genus <i>Ruminococcus</i>                | ↓                       | NR   |

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(continued)

## SUPPLEMENTAL TABLE 2

## Changes in placental microbiome in association with APOs as compared with normal for gestational weight term births (continued)

| Components of the microbiome              | LBW <sup>61</sup> | PTB <sup>57,58,a,b</sup> PTB with excess gestational weight gain <sup>59,c</sup> |
|---|-------------------|--|
| Phylum Fusobacteria                       |                   |  |
| Genus <i>Sneathia</i>                     | ↑                 | NR   |
| Genus <i>Fusobacterium</i>                | NR                | ↑ <sup>d</sup>   |
| Phylum Proteobacteria                     |                   |  |
| Genus <i>Lawsonia</i>                     | ↓                 | NR   |
| Phylum Proteobacteria                     |                   |  |
| Genus <i>Beijerinckia</i>                 |                   | Direction not indicated <sup>c</sup>   |
| Genus <i>Burkholderia</i>                 | NR                | ↑ <sup>a,c</sup>   |
| Genus <i>Vibrio</i>                       |                   | ↑ <sup>c</sup>   |
| Phylogeny unknown                         |                   |  |
| DSSF69_norank                             | ↑                 | NR   |
| Species                                   |                   |  |
| Phylum Firmicutes                         |                   |  |
| Genus <i>Lactobacillus</i>                |                   |  |
| Species <i>Lactobacillus crispatus</i>    | NR                | ↓  |
| Species <i>Lactobacillus acidophilus</i>  | NR                | ↓  |
| Genus <i>Streptococcus</i>                |                   |  |
| Species <i>Streptococcus thermophiles</i> | NR                | ↑  |
| Species <i>Streptococcus agalactiae</i>   | NR                | ↑ <sup>d</sup>   |
| Genus <i>Ureaplasma</i>                   |                   |  |
| Species <i>Ureaplasma urealyticum</i>     | NR                | ↑ <sup>d</sup>   |
| Species <i>Ureaplasma parvum</i>          | NR                | ↑ <sup>d</sup>   |
| Phylum Fusobacteria                       |                   |  |
| Genus <i>Fusobacterium</i>                |                   |  |
| Species <i>Fusobacterium nucleatum</i>    | NR                | ↑ <sup>d</sup>   |

APO, adverse pregnancy outcome; LBW, low birthweight; NR, not reported; PTB, preterm birth.

<sup>a</sup> Preterm birth outcomes in study<sup>57</sup>; <sup>b</sup> Preterm birth outcomes in study<sup>58</sup>; <sup>c</sup> Preterm birth with excess gestational weight gain in study<sup>59</sup>; <sup>d</sup> With concurrent chorioamnionitis.

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