



Oral Microbiome and Preterm Birth: Correlation or Coincidence? A Narrative Review

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Abstract

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AIM: Physiological changes that occur during pregnancy involve, as a natural consequence, also modifications of oral microbiome. However, the addition with microbial imbalance due to pre-existing periodontal infection might impair a pathological alteration in the phylogenetic community structure and composition in the oral cavity, exacerbating an inflammatory status, and becoming a potential risk factor for preterm birth. From the empirical findings about the relationship between periodontal pathogens and systemic diseases, a clear interest focused on the potential impact of some periodontal pathogens on the preterm birth risk has emerged. In this close emerging link, the potential interdependence existing between dysbiosis of oral microbiome and changes in maternal-fetal barrier in premature rupture of membranes was explored.

MATERIALS AND METHODS: In accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, a Medline search was performed for studies focusing on oral microbiome and its association with pre-term birth, and completed by additional hand searching. Two reviewers independently selected studies and extracted data. The search was restricted to only reports written in English.

RESULTS: The electronic search produced 66 items. Six duplicates were found. Among the collected studies, 56 were discarded because they met the exclusion criteria. The articles and reports in our review showed a connection between preterm birth and altered oral microbiome, suggesting a potential key role of *Fusobacterium nucleatum*, a notable periodontal pathogen involved in several pathological periodontal conditions, in increasing the risk of premature birth.

CONCLUSIONS: Since *F. nucleatum* is frequently associated with preterm birth, it is coherent to hypothesize a potential role for the oral microbiota for preterm birth risk. Further studies should be carried out to determine the changes of the oral microflora in pregnancy and to provide comprehensive knowledge of the diversity of oral bacteria involved in preterm birth.

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Introduction

Pregnancy is characterized by significant physiological changes, involving the immune and endocrinological system. The accompanying modifications concern also the oral cavity and the oral microbiome, resulting in increased susceptibility to periodontal inflammatory conditions [1], [2], [3], [4], [5]. Microcirculatory alterations induced by estrogen, progesterone, and chorionic gonadotropin are associated with the dilation of endothelial cells, increased adhesion of platelets and leukocytes to vascular walls, and vascular proliferation [6], [7], [8], [9], [10]. This change affects also periodontal vasculature, leading to crucial alterations [11], [12], [13]. Estrogens and progesterone, that are associated with inflammatory mediators, can lead to changes in vascular response and turnover in periodontal connective tissue [14], [15], [16], [17], [18]. The

microbiome plays an important role in sustaining a healthy pregnancy and contributes to protecting from preterm birth [19], [20], [21], as pregnant women are particularly susceptible to alterations in oral health because of a variety of factors, such as hormonal changes, alteration in dietary habits, and stomach acids from nausea and vomiting [22], [23]. Although most preterm births are due to infection resulting from the bacterial pathogens that rise from the vaginal microbiome, hematogenous spread (bacteremia) due to untreated PD can contribute to an adverse pregnancy outcomes, such as preterm birth and low-birth weight, intrauterine growth restriction (IUGR), and fetal loss [18], [24], [25]. [26]. Biofilms have been implicated as a sine qua non etiological factor for the development of periodontal disease. In a healthy body, the oral microbiota maintains a symbiotic relationship with the host. However, an imbalance or maladaptation within the oral microbial community (dysbiosis) evokes not only a local inflammation but also a significant systemic inflammatory response [6], [7], [8]. The oral cavity encompasses

a spectrum of bacteria that share several virulence characteristics and that may be capable of causing a low-grade systemic inflammation. However, instead of contributing to systemic alterations, some periodontal pathogens seem to disseminate overcoming the placental barrier leading to localized placental inflammation, and triggering preterm membrane rupture [27], [28]. Specifically, the addition with microbial imbalance due to pre-existing periodontal infection might intensify a pathological alteration in the phylogenetic community structure and composition in the oral cavity, exacerbating an inflammatory status, and becoming a potential risk factor for preterm birth [29]. As emphasized frequently in the clinical and epidemiological research, which exalts the processes of biological interactions between alteration of oral microbioma and several systemic pathological conditions [10], [17], periodontitis has been also linked to adverse pregnancy outcomes [27], [28], [29], [30], [31]. Recently, an accumulating body of literature has provided evidence for the potential pathogenic role of specific keystone oral microbes in preterm birth risk, such as *Fusobacterium nucleatum*, resulting in the hypothesis that may be independently capable of contributing to localized placental inflammation, leading to preterm rupture of fetal membranes [29], [30], [31]. Human gestation is characterized by naturally release of high levels of steroid sex hormones, such as estrogen and progesterone. According to Lindhe *et al.*, gum inflammation linked to biofilms is exacerbated by hormonal changes, especially in the second and third trimesters [30], [31].

There is evidence to support a link between the dissemination of pathogenic bacteria – associated with moderate and severe periodontitis – and extraoral infections and inflammation through a hematogenous spread (bacteremia) of periodontal pathogens [32].

From the empirical findings about the relationship between periodontal pathogens and systemic diseases, a clear interest focused on the potential impact of some periodontal pathogens on the preterm birth risk has emerged [32], [33], [34], [35]. As stressed above, the capacity of translocation of pathogen bacteria is the prerequisite for establishing this relationship. In the close emerging link, the potential interdependence existing between dysbiosis of oral microbiome and changes in maternal-fetal barrier in premature rupture of membranes was explored.

Materials and Methods

Literature search strategy

An electronic Medline database (Ovid) search, including original articles from 2011 up to and including 2019, was carried out in accordance with Preferred Reporting Items for Systematic Reviews and

Meta-Analyses recommendations (Moher *et al.*, 2009). To expand this, the electronic search was complemented by a manual search, checking the references of the retrieved articles, from 2004 up to 2019. All titles were checked for relevant clinical and laboratory studies. English language limitation was applied. The following search string was applied: “mouth” OR “mouth” OR “oral” AND “microbiota” OR “microbiota” OR “microbiome” AND “microbiota” OR “microbiota” OR “microbiome” AND “premature birth” OR “premature” AND “birth” OR “premature birth” OR “preterm” AND “birth” OR “preterm birth” AND periodontal AND “pathogens” AND “periodontitis” OR “periodontitis.”

Inclusion and exclusion criteria

To be included in the review, studies were required to meet the following criteria: Articles reporting the role of the oral microbiome dysbiosis in pregnancy and its contribution as a risk factor for preterm birth. Abstracts, editorials, case reports, prospective studies, review articles, and studies reporting only vaginal microbiome discussion or microbiome conditions in infants were excluded from the study.

Selection of studies and data extraction

Two reviewers independently selected titles based on the inclusion criteria. Consequently, abstracts of all articles agreed on by both authors were screened. Data retrieved were recorded, on flow sheets including: (a) Author, (b) year of publication, (c) type of study, (d) details of participants. Extracted data were examined in a standardized unblinded procedure. Disagreements were resolved by consensus.

Results

Study characteristics

The electronic search identified 66 titles with abstract. In addition, a manual search provided three articles. Six duplicates were excluded from the study. The screening and evaluations of these titles resulted in number of 27 full texts, of which five were finally included. The study selection flow-chart is shown in Figure 1 documents this process.

Exclusion of studies

The reasons for excluding studies after full text was obtained, as summarized in Table 1, (n = 56) were (1) a retrospective study, (2) literature review, (3) not reporting on oral microbiome, (4) multiple publications on the same sample study, (5) and studied conducted

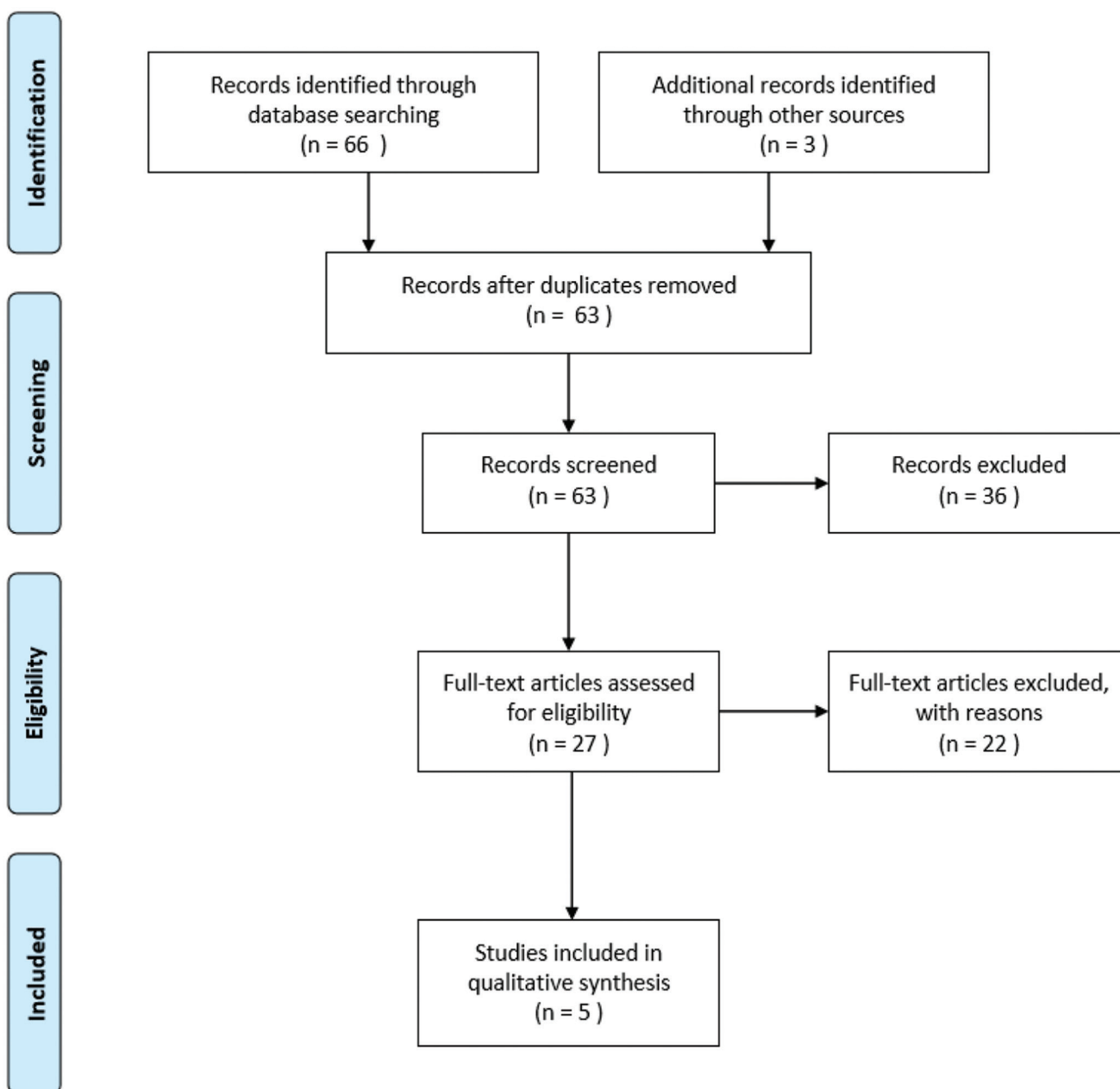


Figure 1: Process of identifying the studies included in the review

on infants. The grey literature and articles written in languages other than English were not included in the review, recognizing this as a limitation.

Included studies

Five studies for inclusion in a systematic review were selected. Table 2 summarizes the general characteristics of these studies.

Three studies conducted on animal models, and two human researches explored the potential role of critical periodontal pathogen in inducing preterm birth [36], [37], [38], [39], [40]. Specifically, a new mechanistic link between oral pathogens and preterm birth was proposed where placental inflammation

was potentially the result of the local inflammatory response elicited by the crossing of specific periodontal bacteria into placental barrier [36], [37]. The most commonly pathogen identified was the *F. nucleatum*, an opportunistic Gram-negative oral anaerobe, having a specific role in pathogenesis of different periodontal conditions [36], [37], [38], [39], [40]. An initial detection of these bacteria from the amniotic fluid, placenta, and chorioamniotic membranes of women delivering prematurely, led to this concept [12]. The first evidence that *F. nucleatum* may be transmitted through hematogenous dissemination derives from a study conducted by Han *et al.* [39] on experimental pregnant mice. The direct injection of *F. nucleatum* into the bloodstream resulted in adverse pregnancy outcomes, manifested as premature births and term stillbirths.

Furthermore, the results of immunohistochemical analysis showed that *F. nucleatum* infection started in the decidua basalis of the placenta. Due to its capability to migrate through the capillary endothelium, it was suggested that *F. nucleatum* might migrate into the

placenta, leading to localized inflammation, which may trigger the premature rupture of membranes [37], [38]. This linkage may be determined by the ability of the pathogen to colonize the placenta, through its ability to adhere and invade host epithelial and endothelial cells through a *FadA* adhesin, the only adhesion identified from *F. nucleatum*, which may be involved in pathogenesis of intrauterine infection [39]. *FadA* adhesin is essential process in inter-species interaction, being implicated in *F. nucleatum* attachment and invasion to host cells. *FadA* exists in two forms: A non-secreted form (pre-*FadA*), consisting of non-secreted 129-amino-acid residues, and secreted mature *FadA* (m*FadA*), formed by 111-aminoacid-residues. Pre-*FadA* is binded at the inner membrane, while the secreted m*FadA* is exposed on the bacterial surface. Both components are required for attachment and invasion of the host cells [39], [40]. The crystal structure of m*FadA* characterized by an antiparallel α -helical organization linked by an 8- aa loop, a non-alpha-hairpin loop. The m*FadA* subunits determine the formation of elongated filaments, by linking in a head-to-tail pattern. Intermolecular hydrophobic interactions between the leucine residues confer stabilization of these filaments [40]. Specifically, Leu 53, Leu60, Leu76, Leu84, and Leu87 determine the intramolecular interactions between the two antiparallel α -helices, and the N-terminal leucine residues Leu7, Leu11, Leu14, and Leu21 are responsible for the intermolecular contacts with Leu53, Leu76, and Leu87 of a neighboring molecule in a parallel fashion. The role of *F. nucleatum* virulence factors in placenta invasion was verified by deletion and complementation experiments [39], [40], [41], and the *FadA* function *in vivo* was first demonstrated by Ikegami *et al.* [41]. This was the first genetic complementation study, which confirmed that the promotion of cellular invasion and placental colonization mediated by the exposure of *FadA*, through the construction of *F. nucleatum* 12230 USF81, a *FadA*-complementing clone. The immunofluorescent staining analysis of expression of *FadA* on the *F. nucleatum* 12230 USF81 strain confirmed that the *FadA* is equally exposed on the bacterial cell surface, promoting adherence and invasion of host cells. This

Table 1: Excluded studies

Author	Year	Reason for exclusion
Borgess <i>et al.</i>	2007	Study conducted on infants
Seymour <i>et al.</i>	2007	No data on oral microbiome
Rouga <i>et al.</i>	2009	Study conducted on infants
Ganguli <i>et al.</i>	2011	No data on oral microbiome
Buddington <i>et al.</i>	2011	No data on oral microbiome
Nelun <i>et al.</i>	2011	Study conducted on infants
Vitali <i>et al.</i>	2012	No data on oral microbiome
Nogueira <i>et al.</i>	2012	No specific data on oral microbiome
Mendz <i>et al.</i>	2013	Review
Madianos <i>et al.</i>	2013	Review
Costello <i>et al.</i>	2013	Study conducted on infants
Keski-Nisula <i>et al.</i>	2013	No data on oral microbiome
Mysorekar <i>et al.</i>	2014	Review
Luoto	2014	No data on oral microbiome
Aagaard <i>et al.</i>	2014	No data on oral microbiome
Jensen <i>et al.</i>	2014	No data on oral microbiome
Payne <i>et al.</i>	2014	Review
Hendricks-Mua <i>et al.</i>	2015	Study conducted on infants
DiGiulio	2015	No data on oral microbiome
He <i>et al.</i>	2015	Review
Solt <i>et al.</i>	2015	Review
Yang <i>et al.</i>	2015	Review
Rodriguez <i>et al.</i>	2015	No data on oral microbiome
Patel <i>et al.</i>	2015	No data on oral microbiome
Herrera <i>et al.</i>	2015	Review
A stergaard <i>et al.</i>	2016	Data on newborn preterm animals
Sherman <i>et al.</i>	2016	No data on oral microbiome
Mending	2016	No data on oral microbiome
Singhi <i>et al.</i>	2016	No data on oral microbiome
Vinturache <i>et al.</i>	2016	Review
Sohn <i>et al.</i>	2016	Study conducted on premature infants
Gille <i>et al.</i>	2016	No data on oral microbiome
Romano-Keeler <i>et al.</i>	2017	No data on oral microbiome
Hill <i>et al.</i>	2017	No data on oral microbiome
Kurath-Koller <i>et al.</i>	2017	No data on oral microbiome
Tettamanti <i>et al.</i>	2017	Review
Cobb <i>et al.</i>	2017	Review
Corwin <i>et al.</i>	2017	Not available data
Olm <i>et al.</i>	2017	Study conducted on premature infants
Pelzer <i>et al.</i>	2017	No data on oral microbiome
Carinci <i>et al.</i>	2018	No data on oral microbiome
Kirihara <i>et al.</i>	2018	Retrospective study
Strus <i>et al.</i>	2018	Study conducted on infants
Ormesher <i>et al.</i>	2018	No data on oral microbiome
Chu <i>et al.</i>	2018	Review
Xu <i>et al.</i>	2018	No data on oral microbiome
Biagi <i>et al.</i>	2018	Study conducted on premature infants
Grev <i>et al.</i>	2018	Review
Davis <i>et al.</i>	2018	No data on oral microbiome
Younge <i>et al.</i>	2018	Study conducted on infants
Pan <i>et al.</i>	2018	Study conducted on preterm animals
Ca tA <i>et al.</i>	2018	Review
Brunse <i>et al.</i>	2019	No data on oral microbiome
You <i>et al.</i>	2019	No data on oral microbiome
Fisher <i>et al.</i>	2019	No data on preterm birth
Baqui <i>et al.</i>	2019	No data on oral microbiome

Table 2: General characteristics of included studies

Author	Year	Purpose	Study design	Subjects	Analysis
Vettore <i>et al.</i>	2008	To compare subgingival biofilm of puerperal women with preterm lowbirthweight and non-preterm low-birth weigh	Case-control	n=116 postpartum women preterm (n=40), low-birth weight (n=35), preterm and/or low-birth weight (n=50) and preterm and low-birth weight (n=25) compared with normal non-preterm low-birth weight controls (n=66) age>30	Analysis of 39 bacterial species using checkerboard DNA-DNA hybridization
Han <i>et al.</i>	2004	To investigate the hypothesis of bacterial transmission from the oral cavity to the uterus	<i>In vivo</i> animal study		immunoistochemical analysis
Ikegami <i>et al.</i>	2009	To explore the ability of <i>F. nucleatum</i> to colonize the mouse placenta	<i>In vivo</i> animal study		Construction of the adhesion <i>FadA</i> -complementing strain
Fardini <i>et al.</i>	2010	To investigate the association between oral pathogen and preterm birth in pregnant mice	<i>In vivo</i> animal study		16S rRNA gene-based PCR and clone analysis
Yang <i>et al.</i>	2019	To determine the differences among the bacterial taxa between the healthy periodontal tissues and the inflamed periodontal tissues in pregnant with preterm birth risk	Comparative descriptive pilot study	n=34 third trimester of pregnancy age range 18 – 40 years	Anal ysis for interleukin-1 matrix metalloproteinase-8, and C-reactive protein

study provided the first evidence that the expression of *FadA* was involved in placental colonization and proliferation *in vivo*. Interestingly, the study conducted by Fardini *et al.* [42], aimed at elucidating the process of *F. nucleatum* translocation into the host cells, included genetic analysis, defining components that are required for *FadA* activity. This was exemplified by the identification of the VE-cadherin as a potential *FadA* receptor. This study showed that *FadA* might be a microbial determinant contributing to placental invasion, causing a localized inflammatory response, responsible for fetal demise prematurely [42].

Discussion

The oral microbioma in pregnancy has been widely investigated to study the contributions of potential periodontal pathogens to increase preterm birth risk. The clinical correlation between PD and preterm birth is well known, but this relationship has not been clearly established. The likely biological mechanism that may link periodontal disease and adverse outcomes in pregnancy has been hypothesized by two evidence-based theories [21], [43], [44], [45], [46], [47], [48], [49], [50], [51], [52], [53], [54], [55]. The first one is based on the possibility that women with PD may be prone to frequent bacteremia and that the uterine cavity would be susceptible to direct colonization of bacteria causing an inflammatory cascade at the level of the fetus and placenta, triggering pre-term birth. In the second hypothesized pathway, the release of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), promoted by chronic inflammation, may explain why periodontal infection can trigger premature uterine contractions, causing pre-term delivery [54].

The microorganism of Group B *Streptococcus* most widely associated with preterm birth has been reported to originate from the reproductive or genitourinary tract, ascending upward through the cervix, or reaching the uterus through a hematogenous route [5], [18], [33]. For this reason, the focus of this review was twofold: First (1), to give a general view, it was intended to discuss the biological plausibility of the mechanistic link between oral microbiome characteristics and preterm birth risk factor, and (2) explain the hypothesis of the causative role of periodontal pathogens in preterm rupture of fetal membranes, based on *in vivo* and *in vitro* studies. As stressed above, the capacity of periodontal pathogens to transmigrate from the oral cavity into the fetoplacental unit is the prerequisite for establishing this relationship and several studies have hypothesized the translocation of bacteria from the oral cavity to the uterus through hematogenous transmission. In the

close emerging link, the potential interdependence existing between *F. nucleatum* and preterm birth was explored. The highest level of importance attributed to this hypothesis found confirmation in several studies. However, the limitations are attributable to the type of study design, including animal models which may not completely mimic the events of human pregnancy.

The colonization characteristics of pathogenic bacteria in the placenta are important to understand their potential role in preterm birth risk. Specifically, it has been investigated the role of the Gram-negative periodontal pathogen *F. nucleatum*, an opportunistic pathogen agent having a specific role in pathogenesis of different periodontal conditions, and it is increasingly recognized as an important agent of preterm birth. This linkage may be determined by the ability of the pathogen to colonize the placenta, through its ability to adhere and invade host epithelial and endothelial cells through a *FadA* adhesin, a virulence factor, which may be involved in pathogenesis of intrauterine infection (1). The role of *F. nucleatum* virulence factors in placenta invasion was verified by deletion and complementation experiments (2). The analysis of the research findings relative to the role of *F. nucleatum* provides useful insights for debate, evidencing research development in embryo stage. However, *F. nucleatum* consists of five subspecies, and this heterogeneity needs analysis at the subspecies/strain level to establish its exact origin. With reference to Han *et al.* a strong impetus underpinning bacteria action emerges. Knowledge of the strategic role that oral microbioma covers in pregnancy, has led to focusing to interdisciplinary intervention to enhance the exitus of pregnancy without risks.

In 1996, Offenbacher *et al.* [56] stated that women with PD had a seven-fold increase in the risk of preterm birth, compared to those without PD. After 1996, many non-experimental studies were conducted on the link between periodontitis in pregnant women and negative neonatal outcomes, with inconsistent results in most cases. Since then, results from several other studies have revealed a link between PD and preterm delivery [18], [23], [57]. Konopka and Paradowska-Stolarz [57] also published a systematic review with meta-analysis to evaluate the average influence of gingivitis on preterm birth or IUGR in 2012. Cobb *et al.* [18] tried to provide some reasons for these conflicting results, such as: Variations in periodontal examination (partial vs. full-mouth examination); variations in the consistency in timing of the periodontal examination with regard to gestational age; variations in treatment protocols; and oral treatment that failed or started too late with different outcomes. During periodontal infection, oral bacteria increase dramatically, accompanied by inflammation and bleeding in the gingival tissue. These conditions enhance bacteremia and increase hematogenous spreading. This would explain why periodontal pathogens have been detected in the placentas of women with preeclampsia [43] and

in the amniotic fluid of pregnant women with preterm labor [51], [58].

Jeffcoat *et al.* [53] reported that mothers with severe PD were 4–7 times more likely to deliver a preterm newborn compared to those with good oral health; they also stated that successful early routine periodontal treatment with scaling and root planning (SRP) is associated with a decreased incidence of spontaneous preterm birth. Pregnant women who were resistant to SRP were significantly more likely to deliver preterm infants [53]. Xiong *et al.* [59], who conducted a meta-analysis on four randomized studies, suggested that dental prophylaxis and periodontal therapy did not significantly reduce the incidence of preterm birth and the birth of underweight babies, but they may reduce the risk of underweight preterm babies (cumulative risk RR).

Although some studies [23], [31] Nardi *et al.* have reported that PD does not affect pregnant woman more than non-pregnant women and that PD parameters showed no indicative correlation with the progression of pregnancy, many studies [4], [18], [21], [33], [34], [35], [53], [54], [56], [59], [60], [61], [62], [63], [64], [65], [66] have revealed a direct relationship between severe PD and pregnancy. During pregnancy, the classic manifestations of PD (bleeding on probing, increase in periodontal pocket depth) are very common, as estrogen and progesterone lead to an inflammatory response that can exacerbate gingival edema and vasculature [67], [68]. As most of the bacteria in the oral cavity are uncultivated, the involvement of oral bacteria in intrauterine infection has been significantly underestimated; consequently, the proportion of the oral microbiome capable of oral-uterus transmission is unknown [69]. Gingivitis in pregnancy is an early form of PD that has been associated with preterm birth. The percentage of pregnant women in need of periodontal treatment can reach 100% [67]. The oral microbiome plays a significant role in our physiology and health, and represents an exceptionally complex habitat, harboring about 50–100 billion bacteria in the oral cavity with about 200 predominant bacterial species [70]. The ecological balance in the oral cavity is maintained through antagonistic as well as mutualistic interspecies interactions [45], [50], [54]. Specific oral pathogenic bacteria, such as *F. nucleatum*, *Porphyromonas gingivalis*, *Filifactor alocis*, and *Campylobacter rectus*, seem to lead to potential adverse outcomes in pregnancy [18], [44]. Well-documented changes in the microbiome occur during such hormonal alterations, with a rise in the proportion between anaerobes and aerobes, such as *Bacteroides melaninogenicus*, *Prevotella intermedia*, and *P. gingivalis* [68].

Carrillo-de-Albornoz *et al.* [71] reported that a worsening in PD was associated with the increase in “red complex” bacteria as *P. gingivalis* and *P. intermedia*. Adriaens *et al.* [72] measured the bacteria load in pregnant women and its relationship with estradiol levels; they concluded that the level of

C. rectus was higher in pregnant women than in non-pregnant women. Lindhe [9] reported that high levels of common periodontal pathogens *P. gingivalis*, *Tannerella forsythia*, *P. intermedia*, and *Prevotella nigrescens*, as well as a suppressed maternal IgG, are associated with an increased risk of preterm birth.

Longitudinal studies have shown that during pregnancy the depth of periodontal probing increases, while gum inflammation worsens [6], [73]. The increase in probing depth has been attributed to the movement of the coronal gum margin due to inflammation-induced swelling. Gürsoy *et al.* [6] found that, in general, there is no permanent loss of clinical attachment. However, in some pregnant women, especially those who had chronic periodontitis before pregnancy, the progression of periodontitis occurs later [74], [75], [76]. Madianos *et al.* [77] reported that blood samples from the umbilical cord of newborns showed that premature babies had specific IgM against oral pathogens that were significantly higher than term babies. As maternal IgM does not pass through the placental barrier, these results suggest a direct intrauterine fetal exposure to these bacteria that may be responsible for premature birth [76].

AQ3

The subjective periodontal health state of women who had recently given birth was investigated by asking women if they thought they had gum bleeding, gum swelling, dental mobility and halitosis, and during and before pregnancy. All the indices consistently increased in pregnancy. This result probably depends on the hormonal variations typical of pregnancy, i.e., the increase in estrogen and progesterone, which can lead to a temporary increase in bleeding on probing and in probing depth, even if there is not an excessive amount of plaque [6]. Yang *et al.* [23] provided an initial description of the relationships among the subgingival microbiome, inflammatory markers of PD, and gestational age at birth in African American women during pregnancy. During pregnancy, a reduction in the antimicrobial activity of peripheral neutrophils leads to a well-documented increase in gum inflammation. The effects of pregnancy on a pre-existing gingival inflammation may be evident from the 2nd month because of the increase in estrogen and progesterone levels in plasma. These levels increase progressively during pregnancy, peaking at the 8th month [77]. Davenport *et al.* [78] examined the mothers of 236 premature or low weight babies (cases) and compared them with the mothers of 507 term and normal weight babies (controls), without finding any link between periodontal status and pregnancy outcome. Surprisingly, the periodontal state of the controls was worse than that of the cases; this result was attributed to the racial composition of their sample compared to the previous studies. Among the reasons behind the conflicting results in the various studies, in addition to the differences in

sample selection, some authors have advanced the hypothesis that the periodontal parameters used to define the state of health or disease are dissimilar and sometimes not overlapping. Manau *et al.* [65] analyzed 23 studies that appeared in the scientific literature and found 14 definitions of periodontitis and more than 50 continuous measurements of periodontal disease. Logistic regression resulted in statistically significant adjusted odds ratios for some adverse pregnancy outcomes in six of the 14 definitions and 17 of the 50 continuous measurements of periodontal disease. Although Figuero *et al.* [79] and Carrillo-de-Albornoz *et al.* [71] found an increase in the gingival index during pregnancy, but no relationship between gingivitis and increased levels of hormones in saliva during pregnancy, Rodrigues *et al.* [2] reported that pregnancy and specific steroid hormones influence normal microflora and induce changes in subgingival ecology. Hormones and local effects on the microbiome play an important role in the process of PD, probably leading to neutrophilic function disorders and change in cellular physiologies. Infection from PD results in the increase in local and systemic prostaglandins (e.g., PGE2) and cytokines (IL-1 and IL-6) that can trigger uterine contractions and promote premature labor. In addition, PD may act as a reservoir of bacterial by-products such as lipopolysaccharide, which also contributes to increasing the levels of pro-inflammatory cytokines and prostaglandins [77]. Although it is thought that vaginal infections that occur during pregnancy are the most common source of systemic inflammation, periodontal infections that initiate systemic inflammation or infectious agents that actually translocate through the bloodstream to the uterus may also increase risk during pregnancy [21], [79], [80]. The underlying link between PD and preterm birth relating to an immune-inflammatory mechanism can be explained in a dysregulation of this anti-inflammatory state [23]. A rise in pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-12, IL-15, IL-18, and interferon- γ), TNF- α , and levels of plasma C-reactive protein caused by PD could then potentially play an important role in this dysregulation of the normal immunologic state during pregnancy [81], [82], [83], [84], [85], [86], [87], [88], [89], [90], [91], [92], [93].

Evidence strongly suggests that periodontal infection has a significant negative impact on pregnancy outcome. Women – especially those in the high-risk category – should be encouraged to achieve a high level of oral hygiene before becoming pregnant and for the duration of their pregnancies. In the presence of gingivitis and related complications during pregnancy, it is important to evaluate the impact of the early administration of a mixture of lactobacillus – also as a daily oral hygiene practice – the use of probiotic toothpaste, and foods high in these nutrients (zinc, calcium) to improve the health of the oral cavity and, at the same time, to evaluate the effect on reducing the risk of preterm birth and low birth weight.

Conclusions

The oral microbiome in pregnancy has been widely investigated to study the contributions of potential translocated periodontal pathogens to preterm birth risk, but the biologic process is not completely understood. The role of *F. nucleatum* in the mechanisms of localized placental inflammation provided crucial insights in its pathogenesis and suggests potential strategies for developing new treatments for pregnant woman at risk to adverse pregnancy outcomes.

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