

Biological mechanisms between periodontal diseases and pregnancy complications

A narrative review

Report co-authored by
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Report co-authored by

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Clinical Relevance

Scientific rationale for the study: Chronic periodontitis has been acknowledged as a potential risk factor for adverse pregnancy outcomes (APOs). The aim was to review the current evidence about the plausible biological mechanisms between periodontal inflammation and pregnancy complications.

Principal findings: Commensal and pathogenic oral bacterial species are able to colonise the foeto-placental unit. The oral microorganism transmission between the periodontium and placenta is supported by the haematogenous dissemination. The presence of periodontal bacteria and by-products in the foeto-placental unit activate a local immune/inflammatory response, which in turn might contribute to APOs.

Practical implications: The current evidence supports the biological plausibility within the association between periodontal inflammatory burden and APOs. However, as there are many potential mechanisms affecting the outcome, and their roles alone or together are inconclusive, further clarification and in-depth investigation are still warranted.

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Biological mechanisms between periodontal diseases and **pregnancy complications**: A narrative review

Abstract

Aim: This narrative review evaluated the current evidence in relation to the plausible biological mechanisms between periodontal disease and pregnancy complications.

Material and methods: Data available mainly from clinical-association and intervention studies, along with microbiological and immunological findings, were included.

Results & conclusions: The current data support the role of periodontal inflammation along with mainly Gram-negative disease-provoking microbes in the etiopathogenesis of APOs. However, because of their multi-factorial nature, the outcomes are not yet conclusive and the association between bacterial colonisation and maternal immunological responses remains speculative. Furthermore, understanding the exact mechanisms – why some women develop APOs in the presence of a periodontal inflammatory burden while others do not – remains to be achieved.

Introduction

In 1891, the American dentist W. D. Miller published the article “The human mouth as a focus of infection”, which hypothesised that oral focal sepsis (infection) could be attributed to infected non-oral sites and pathological conditions elsewhere in the body. Since then, there have been latent periods within the focal-infection theory, but various hypotheses have been proposed to explain the association and potential pathogenic pathways between the oral infection and its systemic afflictions – i.e. secondary non-oral infections, systemic conditions, and diseases (see review by Thoden van Velzen *et al.*, 1984). The ground-breaking appraisal for the idea of focal-infection was reached during the 1990s, when the term “periodontal medicine” was launched at the World Workshop in Periodontics (Offenbacher, 1996) and chronic periodontitis was acknowledged as a potential risk factor for several systemic diseases and conditions, such as adverse pregnancy outcomes (APOs), atherosclerosis and cardiovascular disease, bacteraemia, diabetes, infective endocarditis, and respiratory diseases (Scannapieco, 1998). Of these, the epidemiology of the associations between APOs and maternal periodontal disease has been reviewed and discussed in detail in an accompanying systematic review (Petrini *et al.*, 2017).

In the context of existing knowledge, the focal-infection concept underlies three potential mechanisms: metastatic infection, injury, and inflammation (Thoden van Velzen *et al.*, 1984). These postulated pathways, alone or together, may play a role in the interaction between periodontal infection and various systemic diseases/conditions, including pregnancy complications (Van Dyke & Van Winkelhoff, 2013; Madianos *et al.*, 2013). Based on the focal-infection-hypothesis model, periodontitis could be linked with APOs via the following biological pathways. Firstly, the metastatic infection at the non-oral body sites (e.g. placenta and amniotic sac) could be established by haematogenous bacterial dissemination, as the periodontal pathogens may leak from the infected periodontal pocket sites to the blood circulation (Gauthier *et al.*, 2011; Han *et al.*, 2006, 2009, 2010). Secondly, circulating microbes – together with their toxins and other by-products – may cause harm and initiate inflammatory response at the foetal-placental unit as a metastatic injury (Arce *et al.*, 2009; Bobetsis *et al.*, 2006; Han *et al.*, 2009). Thirdly, the metastatic inflammation – in which inflammatory mediators of a periodontal origin and/or acute-phase reactants from the maternal liver – could initiate a secondary reaction at the foetal-placental unit (Herrera *et al.*, 2007; Park *et al.*, 2013; Taylor *et al.*, 2016). The purpose of the current article is to review the updated literature and evaluate in detail these plausible biological mechanisms between periodontal diseases and pregnancy complications. With this intention, the periodontal etiopathogenesis, together with the physiology of healthy gestation and various pregnancy complications, are briefly introduced.

Etiopathogenesis of periodontitis

The balance of the host-microbial interaction needs to be interrupted before the initiation and proceeding of periodontal disease, which is a multifactorial process and accompanied by several modifiable and non-modifiable risk factors. On one hand, a microbial dysbiosis from health-associated microbiota towards polymicrobial diversity with enriched number of disease-provoking microbes is required for the activation of this cascade (Hajishengallis & Lamont, 2012). For example, *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Filifactor alocis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* (Kumar *et al.*, 2006; Könönen *et al.*, 2007; Stingu *et al.*, 2012), and species belonging to the other genera – such as *Dialister*, *Eubacterium*, *Selenomonas*, and *Synergistetes* (Meuric *et al.*, 2017; Sousa *et al.*, 2017; You *et al.*, 2013) – have been linked to periodontitis-associated microbiota. On the other hand, dysregulated host immune response

is another essential component in the disease initiation. An enhanced load of dysbiotic and disease-provoking microbiota, together with their virulent by-products – including proteolytic enzymes, leukotoxins, and endotoxins (e.g. lipopolysaccharide [LPS]) – interact with the host immune system (see reviews by Hajishengallis, 2015; Van Dyke & Van Winkelhoff, 2013). Host cell receptors recognise the pathogenic intruders and trigger specific signalling pathways, which activate the immune system in order to eliminate the pathogens and resume the host-microbe homeostasis. In susceptible individuals, however, the host immunoregulatory defects and/or the active bacterial subversion of the host response enable pathogen persistence in the local inflammatory environment (Hajishengallis, 2015). Thus, the dysregulated immune response leads to periodontal-tissue breakdown at the cost of protection.

Several signalling molecules, inflammatory mediators, and enzymes related to tissue destruction are produced locally in the periodontium. Proinflammatory cytokines, such as interleukin (IL)-1 and -6, are secreted not only by epithelial cells, fibroblasts, neutrophils, and macrophages, but also by lymphocytes in the persisting phase of inflammation (Van Dyke & van Winkelhoff, 2013). IL-1 and -6 play a role in the inflammatory cell migration and osteoclastogenesis. Their production is up-regulated by tumour necrosis factor (TNF)- α , another cytokine, which also participates in the extracellular matrix degradation and bone resorption by endorsing matrix metalloproteinase (MMP) and receptor activator of nuclear factor kappa-B ligand (RANKL) secretion. In addition, prostaglandin E2 (PGE2) is involved in systemic inflammation, endothelial cell activation, vascular endothelial growth factor expression, platelet aggravation, and alveolar bone resorption (Van Dyke & Van Winkelhoff, 2013). Several resident and inflammatory cells of the periodontium – such as keratinocytes, fibroblasts, periodontal ligament cells, osteoblasts, osteoclasts, neutrophils, monocytes, macrophages, and plasma cells – produce different types of MMPs (Sorsa *et al.*, 2006). Secretion and activation of MMPs are regulated mainly by bacterial products or by host cytokines, while their activation can be inhibited by tissue inhibitor of matrix metalloproteinases (TIMPs).

In the case of acute periodontal lesion persistence, macrophages and dendritic cells introduce the bacterial antigens to the adaptive immune system, which includes the B- and T-cell lymphocytes (Sykes *et al.*, 2012, Van Dyke & Van Winkelhoff, 2013). While B cells are responsible for the humoral immunity via the immunoglobulin (Ig) secretion, T cells are considered to be the effectors of cell-mediated immunity. Of these, CD8⁺ T cells (cytotoxic/killer T cells), are mainly classical immune-effector cells, whereas CD4⁺ T cells are divided into different subsets based on their discrete cytokine-production profile. These are: 1) T helper 1 (Th1) cells responsible for IL-2 and interferon (IFN)- γ production; 2) Th2 cells producing IL-4, IL-5 and IL-13; 3) Th17 cells producing IL-17 and IL-22; and 4) T regulatory (Treg) cells producing IL-10 and transforming growth factor (TGF)- β . Moreover, various T-cell subsets activate macrophages, B cells, and other T cells as well (Van Dyke & Van Winkelhoff, 2013).

Establishment and maintenance of gestation

Progesterone modulates stromal cell proliferation, decidual growth, and expression of adhesion molecules while suppressing the maternal immune system to allow embryo implantation (Halasz & Szekeres-Bartho, 2013). The corpus luteum is responsible for the increased progesterone and oestrogen production while waiting for the development of the placenta, which then takes over the role of the corpus luteum and continues raising the production of female sex hormones from the second trimester to delivery (Nakajima *et al.*, 1991). In addition, as a vessel-rich organ, the placenta provides favourable circumstances for foetal growth by permitting gas exchange,

nutrient uptake, and waste disposal through the mother's blood circulation. This transportation is enabled via the umbilical cord, which connects the foetus to the placenta. The two-layered amniotic sac, filled with amniotic fluid, surrounds and protects the developing and growing foetus within the uterus. The inner membrane is called "amnion" and the outer membrane is known as "chorium". Like the placenta, the chorioamniotic membrane connects to the uterus walls through the maternal decidua and myometrium.

During gestation, the maternal immune system has to defend both the mother and the foetus from external pathogens, but simultaneously it needs to be suppressed in order to tolerate the foetal components inherited from the father (La Rocca *et al.*, 2014). Thus, in successful gestation, a trend from Th1 towards the Th2 cytokine profile seems to occur both in the peripheral blood and at the foeto-maternal interface as well (Sykes *et al.*, 2012). Furthermore, Treg cells act as suppressors of immune responses enabling pregnancy to continue without foetal rejection (La Rocca *et al.*, 2014). Other potential mechanisms implicated in foeto-maternal tolerance are summarised in Table 1. Progesterone plays in a major role by regulating not only the embryo-implantation period and immune responses throughout gestation, but also the induction of parturition and cervical ripening (Halasz & Szekeres-Bartho, 2013).

As gestation progresses, progesterone affects MMP activity by transcriptional modulation either directly or indirectly via local mediators, such as cytokines and leptin (Halasz & Szekeres-Bartho, 2013). MMPs are responsible for the continual collagenous extracellular matrix remodulation, which ensure the cervix and chorioamniotic-membrane adaptation to uterine and foetal growth (Weiss *et al.*, 2007). Especially MMP-1, MMP-2, and MMP-3 are constitutively expressed during gestation, whereas MMP-9 production is induced mainly in the implantation and again in the delivery (Cockle *et al.*, 2007). MMPs are essential in parturition, because they have a significant role in cervical ripening and dilatation, as well as in membrane weakening and rupture (Weiss *et al.*, 2007).

Overall, progesterone is one of the key elements in the establishment and maintenance of pregnancy, taking part in complex activities, as mentioned above. Any triggering mechanisms, including infection and inflammatory mediators, that interrupt these multifaceted physiological actions may contribute to pathological pregnancy complications, such as spontaneous abortion, recurrent miscarriages, and preterm deliveries (Halasz & Szekeres-Bartho, 2013).

Adverse pregnancy outcomes (APOs)

Pre-eclampsia

Pre-eclampsia – a complication resulting from placental ischemia and clinically characterised by high blood pressure with proteinuria or pulmonary edema, oliguria, or convulsions at ≥ 20 weeks of gestation – is one of the leading causes of maternal and perinatal morbidity and mortality (Chaiworapongsa *et al.*, 2014; Taylor *et al.*, 2016). Within a multifactorial etiopathogenesis, an imbalance between angiogenic and anti-angiogenic factors has emerged as a central pathogenic mechanism of this disorder (Chaiworapongsa *et al.*, 2014). In contrast to the predominance of the Th2 profile in successful pregnancy, pre-eclamptic patients seem to harbour an opposite shift in their cytokine profile because of the diminished Th2 and Treg activities in respective relation to Th1 and Th17 (Peixoto *et al.*, 2016; Sykes *et al.*, 2012; Vargas-Rojas *et al.*, 2016). Changes in cytokine levels have been observed not only in the peripheral blood but also in the placenta and umbilical cord.

Intrauterine infections and inflammation

Micro-organisms, not only from genital but also from oral origins, may invade the intrauterine environment and cause infection within the choriodecidual space, the chorioamniotic membrane, the placenta, the amniotic fluid, the umbilical cord, or the foetus (Bearfield *et al.*, 2002; Blanc *et al.*, 2015; Fardini *et al.*, 2010; Gauthier *et al.*, 2011; Prince *et al.*, 2016; Wang *et al.*, 2013). Acute inflammatory lesions at the various sites of the placenta are involved with diffuse infiltration of neutrophils (reviewed by Kim *et al.*, 2015a). Based on the origin, these lesions representing host response to a chemotactic gradient in the amniotic cavity are divided into: 1) acute chorioamnionitis (an intra-amniotic inflammation with infiltrated maternal neutrophils on the chorion and amnion); 2) funisitis (an inflammatory process involved with the umbilical cord, in which neutrophils are recruited from the foetal origin); and 3) chorionic vasculitis (inflammation affecting the chorionic villous tree as evidence of a foetal host response). In the case of intrauterine infection, chemokines – such IL-8 and granulocyte chemotactic protein – establish a gradient, which favours either the migration of maternal neutrophils into the chorioamniotic membranes or foetal neutrophil passage into the umbilical cord (Kim *et al.*, 2015a).

In chronic chorioamnionitis, maternal CD8+ T cells infiltrate the amniotic sac membranes, induce trophoblast apoptosis, and damage the chorionamniotic membranes (Kim *et al.*, 2015b). It is the most common lesion in late spontaneous preterm birth and the frequency is remarkably high not only in prelabour rupture of membranes (39 %) and preterm delivery (34%), but also in terms of foetal death (60%) (Kim *et al.*, 2015b).

Premature birth and/or low birth weight

Preterm birth (PTB), characterised as any live birth before 37 weeks of gestation, is another major reason for neonatal mortalities and morbidities (World Health Organisation, WHO, 2016). It is also a common reason for low birth weight (LBW), which is characterised as a new-born of <2500g. Infection and/or uncontrolled inflammatory reaction within the uterus may contribute to preterm rupture of the membranes and uterine contraction that may, in turn, lead to miscarriage or PTB (Halasz & Szekeres-Bartho, 2013). This phenomenon is related to aberrant extracellular matrix degradation, a consequence caused by MMPs after an elevated influx of proinflammatory cytokines, such as TNF- α and IL-1 β (Cockle *et al.*, 2007; Sykes *et al.*, 2012). In addition, serum TIMP-1 and -2 levels are lower in preterm gestations compared to those at term, irrespective of labour status (Tency *et al.*, 2012).

Stillbirth

In contrast to miscarriage (defined as loss of the foetus before the 20th week of pregnancy), stillbirth (a baby is born with no signs of life) may occur before the onset of parturition (ante partum) or at delivery (intra partum) (Lawn *et al.*, 2016). Stillbirth is defined based on the gestational age thresholds of ≥ 22 weeks (early stillbirth) or ≥ 28 weeks (late stillbirth). The latter definition covers the third trimester of gestation, and therefore represents the true burden. Several maternal and foetal-related factors – including: demographic, environmental, nutritional, and lifestyle factors; maternal infections and non-communicable diseases; and foetal factors – act as potential factors for stillbirth (Lawn *et al.*, 2016). Among these, other APOs – such as pre-eclampsia, restricted foetal growth (in terms of birthweight and gestational age, the smallest babies being at the highest risk of death), and bacterial infections (e.g., chorioamnionitis) – are important contributors. Likewise, an imbalance of angiogenic/antiangiogenic factors (seen as concentration changes between the placental growth factor, soluble vascular endothelial growth factor receptor-1 and endoglin) in maternal circulation precede the clinical diagnoses of pre-eclampsia, small for gestational age, and stillbirth (Chaiworapongsa *et al.*, 2010).

Potential biological mechanisms

Periodontal pathogens and their by-products

Contrary to former beliefs that foetal development occurs within a sterile environment, recent studies have shown that the foeto-placental unit harbours a unique microbiota even in clinically healthy gestations (Aagaard *et al.*, 2014; Stout *et al.*, 2013). Interestingly, when Aagaard *et al.* (2014) classified and compared the placental microbiomes from 320 tissue specimens, their taxonomic profile resembled the oral microbiome derived from non-pregnant subjects rather than the microbiomes of the urogenital tract or stool samples originated from pregnant and non-pregnant individuals. Indeed, by using 16S ribosomal DNA-based and whole-genome shotgun metagenomics technologies, the placental microbiome was characterised as consisting mainly of non-pathogenic commensal members belonging to the *Firmicutes*, *Tenericutes*, *Bacteroidetes*, and *Fusobacteria phyla* (Aagaard *et al.*, 2014). These similarities confirm in general the potential bacterial transmission between the oral cavity and foeto-placental unit.

Indeed, recent case-control studies using advanced PCR techniques have demonstrated that oral bacteria may be harboured in the placenta independently of the mother's periodontal status, although different periodontal pathogens in the placenta are significantly more prevalent in women with periodontitis compared to those with healthy periodontium (Blanc *et al.*, 2015; Chaparro *et al.*, 2013a; Swati *et al.*, 2012). The periodontitis-related micro-organisms linked with various APOs are summarised in Table 2. Specifically, Chaparro *et al.* (2013a) demonstrated, among pregnant women with periodontitis, an association between gestational hypertensive disorders (i.e., pre-eclampsia and gestational hypertension) and the presence of placental *P. gingivalis* and *T. denticola*.

Furthermore, chronic periodontitis and the subgingival presence of *P. gingivalis*, *T. forsythia*, and *Eikenella corrodens* have been significantly associated with pre-eclampsia (Contreras *et al.*, 2006), whereas the presence of *E. corrodens* and *Capnocytophaga* spp. demonstrated a significant association with PTB and LBW, respectively (Santa Cruz *et al.*, 2013). In a Japanese population, pre-eclampsia associated significantly with the elevated levels of *A. actinomycetemcomitans* in subgingival plaque, but not with periodontitis (Hirano *et al.*, 2012). Likewise, no relationship between periodontitis and PTB was revealed in a Danish population, although a significantly higher presence of subgingival *Capnocytophaga ochracea*, *Parvimonas micra*, *Streptococcus oralis*, *Streptococcus sanguinis*, *T. denticola*, and *T. forsythia* was detected in women with PTB in comparison to women with term labour (Skuldbøl *et al.*, 2006). In contrast, in a Spanish population, periodontitis was more prevalent in women with PTB/LBW than in women with full-term pregnancy, but no significant differences in subgingival bacterial compositions could be seen; *P. micra* and *Capnocytophaga* sp., however, were present only in the case group, whereas *E. corrodens* was detected only in controls (Mesa *et al.*, 2013).

The oral bacteria can be found from various parts of the foeto-placental unit. Of uncultivated species, oral strains of *Bergeyella* sp. were identified in the amniotic fluid in cases of chorioamnionitis leading to PTB (Han *et al.*, 2006, 2009) and PTB with early-onset neonatal sepsis (Wang *et al.*, 2013). Similarly, in eight (30.8%) of 26 pregnant women with threatened PTB, *P. gingivalis* had invaded the amniotic fluid (León *et al.*, 2007). Moreover, the most abundant bacterium, *F. nucleatum*, has been detected from the placenta in pre-eclampsia (Barak *et al.*, 2007) and PTB/LBW (Blanc *et al.*, 2015), from the intra-amniotic fluid in intra-uterus infection and PTB with intact membranes (Bearfield *et al.*, 2002, Gauthier *et al.*, 2011), from the paired amniotic fluid and cord blood in PTB with early-onset neonatal sepsis (Wang *et al.*, 2013), and from the placenta and foetus in a case of stillbirth (Han *et al.*, 2010). Interestingly, the capability of *F. nucleatum* to colonise from circulation into the foeto-placental unit is entirely dependent on its unique surface adhesion protein FadA, which binds

to the vascular endothelium cadherin and alters the endothelial integrity (Fardini *et al.*, 2011). This, in turn, enables the bacterium to cross the endothelium by direct invasion into the cells or through the loosened cell-to-cell junctions.

To date, microbial data has been obtained mostly from studies using molecular or culture-based techniques. Within the last few years, however, whole-microbiome analyses through next-generation sequencing have been launched. Among the first of these, Prince *et al.* (2016) demonstrated that the placental microbiome alters in association with PTB, as well as the microbial community between chorion and amnion in relation to clinically diagnosed PTB and histologically proven inflammation. Moreover, these changes varied within the severity of chorioamnionitis: i.e. women with PTB and severe chorioamnionitis had diminished species diversity and ectopically predominant presence of urogenital and oral bacterial – such as *Ureaplasma* sp., *F. nucleatum*, and streptococci – in their placental tissues in comparison to women with term pregnancy (Prince *et al.*, 2016). In the first complete microbiome study of pre-eclampsia, Amarasekara and co-workers (2015) collected 110 placental samples from pregnant women with and without pre-eclampsia. Out of 55 pre-eclampsia patients, seven (12.7%) had PCR-positive samples, whereas all placental samples (n=55) collected from the matched normotensive pregnant women (controls) were negative. Interestingly, the microbiome of the positive samples consisted of several agents with one or more predominant infectious agent(s), which can typically be found in the vagina, the gastrointestinal tract, the respiratory tract, and the periodontium. Moreover, Amarasekara *et al.* (2015) failed to confirm haematogenous dissemination, as none of these organisms was present in venous blood or urine at the time of delivery by caesarean section.

According to experiments on pregnant mice, haematogenous injection of *F. nucleatum* results in specific colonisation and proliferation of this microorganism in the foeto-placental unit, whereas bacterial injection into the decidua, mimicking the chorioamnionitis, eventually leads to preterm and term stillbirth (Han *et al.*, 2004, 2010). Similarly, in mice infected with *P. gingivalis* and *C. rectus*, translocation of these periodontal pathogens to placental tissues causes PTB with LBW (Ao *et al.*, 2015; Arce *et al.*, 2012). *P. gingivalis* infection could induce approximately a twofold increase in the levels of circulating cytokines, such as TNF- α , IL-1 β , IL-6, and IL-17 (Ao *et al.*, 2015). Moreover, intrauterine growth restriction by *C. rectus* as well as foetal death by *F. nucleatum*, are most likely induced via a stimulation of Toll-like receptor 4-mediated placental cytokine activation (Arce *et al.*, 2012; Liu *et al.*, 2007). Based on morphologic and histologic approaches in humans, several reports have been published in relation to bacterial presence in the various locations of the foeto-placental unit (Prince *et al.*, 2016; Romero *et al.*, 2008; Stout *et al.*, 2013). In a recent cross-sectional study, Stout *et al.* (2013) demonstrated for the first time that 53 (27%) of 195 placenta specimens harboured intracellular Gram-positive and negative bacteria in the maternal basal plate (i.e. the surface layer of the placenta anchored to the endometrium and enriched with an extensive vascular network).

Besides an ascending infection from the lower genital tract into the uterus, an alternative route for the intrauterine colonisation with oral microbes is supported by the biological plausibility of their haematogenous translocation (Fardini *et al.*, 2010; Han *et al.*, 2006, 2010). First, periodontal infection accompanies gingival inflammation and bleeding, and second, the elevated concentrations of female sex hormone during pregnancy increase the vascular permeability. These two conditions together may thus enhance the opportunities for haematogenous transmission during gestation.

Another interesting argument to be resolved is the distribution of microbial-associated and non-associated intra-amniotic lesions: why do some women develop APOs, while some do not despite the simultaneous bacterial colonisation? For example, out of 46 amnion fluid samples obtained by transabdominal amniocentesis from women with clinical signs of chorioamnionitis at term

gestation, seven (15%) did not have intra-amniotic inflammation (defined as IL-6 concentration ≥ 2.6 ng/mL) or infection, three (6.5%) had only microbial invasion of the amniotic cavity, 25 (54%) had microbial-associated intra-amniotic inflammation, whereas 11 (24%) had intra-amniotic inflammation without detectable micro-organisms (Romero *et al.*, 2015). On this basis, it remains unidentified which factors will contribute to pregnancy complications in cases where oral microbes are present in the foeto-placental unit. Potential elements could be the species-specific translocation and microbial load – i.e. low vs. high quantities of potential pathogens (Fardini *et al.*, 2010) – as well as intra-species variation within the virulence factors and disease-provoking abilities. Consequently, further studies on these issues are warranted. Moreover, very little is known about the bacterial co-aggregation and biofilm-formation capability in the amniotic cavity (Romero *et al.*, 2008). Indeed, the role of the current “keystone pathogen and polymicrobial synergy” concept in periodontology (Hajishengallis, 2015; Hajishengallis & Lamont, 2012) might be useful to take into account in future studies on etiopathogenesis of APOs.

Proinflammatory cytokines

Increased production of proinflammatory cytokines and mediators during periodontal diseases is a known phenomenon. In a recent case-control study among pregnant women, the serum levels of IL-4 and TNF- α were significantly higher in the subjects with gingivitis and periodontitis than in periodontally healthy women (Kumar *et al.*, 2014). However, serum TNF- α levels were significantly lower among women with periodontitis who developed pre-eclampsia during the beginning of the second trimester than in mothers with healthy gestation (Kumar *et al.*, 2014). Perunovic *et al.* (2016) in turn demonstrated that women who experienced PTB had significantly higher rates of periodontitis, worse periodontal status, and enhanced levels of IL-1 β and PGE2 in their gingival crevicular fluid (GCF) than women who gave birth at full term. However, all tested labour triggers (i.e. IL-1 β , IL-6, TNF- α , and PGE2) in serum were equally distributed between the groups. In contrast, pregnant women with periodontitis harboured significantly higher IL-1, IL-6, and PGE2 levels in GCF, as well as TNF- α and PGE2 levels in serum than periodontally healthy controls (Perunovic *et al.*, 2016). Overall, these findings indicate the potential impact of periodontitis on the onset of pre-eclampsia and PTB (Kumar *et al.*, 2014; Perunovic *et al.*, 2016).

Proinflammatory cytokines – such as IL-1, IL-6 and TNF- α – stimulate the production of prostaglandins in the chorion, and elevated serum and/or amniotic-fluid levels of these mediators have been associated with amniotitis and PTB (Buhimschi *et al.*, 2009; Gücer *et al.*, 2001; Park *et al.*, 2013; Von Minckwitz *et al.*, 2000). On the one hand, PGE2 generated in the periodontal tissues – at least in theory – also contributes to enhanced prostaglandin levels in the chorion. By doing so, it induces cervical ripening and uterine contraction, eventually leading to an increased risk of PTB. On the other hand, conclusive evidence is not yet available to support the idea that elevated levels of certain inflammatory mediators in GCF, serum, and/or amniotic fluid are associated with pregnancy complications in periodontitis patients (Bearfield *et al.*, 2002; Fiorini *et al.*, 2012; Kumar *et al.*, 2014; Perunovic *et al.*, 2016; Politano *et al.*, 2011; Sert *et al.*, 2011; Taylor *et al.*, 2016).

Most of the current clinical evidence related to immunological processes within APOs relies on cross-sectional case-control studies. Bearing in mind that the gestation period involves both proinflammatory and anti-inflammatory phases influenced by fluctuations of the female sex hormone, further studies with longitudinal follow-up settings might be warranted in the future. Moreover, as no single immune biomarker alone is likely to predict any APO (Taylor *et al.*, 2016), studies combining several immune markers together with clinical and microbiological data may be useful when defining the exact biological mechanisms that connect periodontal diseases and APOs.

C-reactive protein

The release of bacteria and proinflammatory cytokines from the infected periodontal tissues into the systemic circulation may induce a low-grade systemic inflammation via the acute-phase response in the liver, which is shown as the enhanced production and release of C-reactive protein (CRP) and fibrinogen (Herrera *et al.*, 2007; Park *et al.*, 2013). The association between the elevated levels of CRP in serum and moderate/severe periodontitis has been demonstrated among pregnant African-American women (Horton *et al.*, 2008). As CRP disseminates via circulation into other body sites, it is able to contribute consecutively to intra-uterine inflammation (Herrera *et al.*, 2007; Park *et al.*, 2013). Thereby, besides periodontitis, enhanced CRP levels are related to several infection-induced inflammatory conditions, such as pre-eclampsia, PTB, restricted intra-uterine growth, and gestational diabetes mellitus (Dasanayake *et al.*, 2008; Glotov *et al.*, 2015; Gogeneni *et al.*, 2015; Paraskevas *et al.*, 2008; Pitiphat *et al.*, 2005; Tjoa *et al.*, 2003).

During pregnancy, the inflammatory response at the foeto-placental interface can be amplified by elevated levels of plasma CRP through complement activation, tissue damage, and the induction of proinflammatory cytokines (Sharma *et al.*, 2009). Therefore, elevated CRP levels in pregnant women with periodontitis may be associated with PTB (Sharma *et al.*, 2009) and pre-eclampsia (Chaparro *et al.*, 2013b; Herrera *et al.*, 2007; Ruma *et al.*, 2008), even though controversial results also have been presented (Ghezzi *et al.*, 2002; Souccar *et al.*, 2010).

Immunoglobulins

In terms of the roles of maternal and foetal immune responses against oral pathogens, few studies have been conducted to elucidate the potential causal pathways and their relationship to APOs (Boggess *et al.*, 2005; Ebersole *et al.*, 2009; Lin *et al.*, 2007; Madianos *et al.*, 2001). As the foetus lacks the ability to produce a normal immune response after being exposed to an antigen, it can mount only an IgM antibody response when challenged. As a part of the large American cohort study of pregnant mothers, maternal postpartum IgG antibody levels in serum together with foetal exposure (defined as foetal cord blood IgM levels against maternal periodontal pathogens) were examined (Madianos *et al.*, 2001). The following organisms were included: *C. rectus*, *F. nucleatum*, *P. micra*, *P. intermedia*, and *P. nigrescens* from the orange complex (as described by Socransky *et al.*, 1998) together with red-complex bacteria (i.e. *T. forsythia*, *T. denticola*, and *P. gingivalis*). Comparison between PTB and full-term babies revealed that PTB neonates represented a 2.9-fold higher prevalence of IgM seropositivity for at least one of the tested organisms. For example, the prevalence of positive foetal IgM for *C. rectus* and *P. intermedia* was significantly higher among PTB than full-term infants (Madianos *et al.*, 2001). In addition, neonates who were lacking maternal serum IgG-specific antibodies against the red-complex organisms had a significantly higher risk of prematurity. Moreover, the highest rate of PTB occurred in mothers who had no red-complex IgG response coupled with an elevated foetal IgM response against orange-complex bacteria.

These results are supported by Lin *et al.* (2007) and Ebersole *et al.* (2009), who detected significantly lower serum IgG levels for *P. gingivalis* in mothers with PTB than in women with full-term gestation. Likewise, Boggess *et al.* (2005) demonstrated that foetal IgM seropositivity to the panel of five selected oral pathogens associated with increased risk for PTB was the greatest among those who exhibited simultaneous inflammatory response (i.e. seen as presence of CRP or high levels of TNF- α , PGE₂, and/or 8-isoprostane in umbilical-cord blood specimens). Moreover, there are also some conflicting results, where studies either could not detect any differences in the serum antibody levels in relation to PTB/LBW (Jarjoura *et al.*, 2005) or whose

observations were quite the opposite – i.e. *P. gingivalis*-specific maternal serum IgG levels associated with the LBW deliveries (Dasanayake *et al.*, 2001).

Because of the limited number of studies with double interpretation, the association between bacterial colonisation and maternal immunological responses remain inconclusive. However, these preliminary reports provide hypotheses for future studies of whether low levels of maternal serum IgG antibodies deliver inadequate protection against the disseminating periodontal pathogens that may translocate more easily to the foeto-placental unit and contribute to pregnancy complications. On the other hand, the elevated levels of serum IgG antibodies could indicate either an increase in systemic bacterial exposure or a hyper-inflammatory phenotype which may predispose these women to an increased foetal inflammatory response and injury (Madianos *et al.*, 2001, 2013).

Conclusion

The clinical studies referred to above, supported by some animal models, expand our current knowledge about the plausible biological mechanisms between periodontal inflammation and APOs. Interestingly, both commensal and pathogenic bacterial species originating from the oral cavity seem to colonise the foeto-placental unit of women with term gestation as well as with APOs. The haematogenous spread is the most potent mechanism for their transmission between the periodontium and the foeto-placental unit. Although certain periodontitis-associated micro-organisms – such as *P. gingivalis*, *T. denticola*, *T. forsythia*, and *F. nucleatum* – have been associated with pre-eclampsia, preterm delivery, chorioamniotitis, and/or stillbirth, very little is known about their exact role in the etiopathogenesis of these adverse pregnancy complications.

Overall, the majority of the current clinical evidence is based on cross-sectional case-control studies. In order to understand the complex biological processes throughout pregnancy together with their roles in etiopathogenesis of APOs, a longitudinal follow-up setting might be rewarding but – of course – also challenging to conduct. Moreover, as no single immune biomarker is likely to predict APOs, studies combining several immune markers together with clinical and microbiological data may be useful when defining the exact biological mechanisms that link periodontal diseases and APOs.

Table 1.

Proposed mechanisms implicated in the foeto-maternal tolerance and successful maintenance of pregnancy (adapted from Halasz & Szekeres-Bartho, 2013; Kim *et al.*, 2015b)

At organ level	At cell level	At protein level
Progesterone <ul style="list-style-type: none"> • supports the endometrium • decreases the contractility of the smooth muscle in the uterus • affects the maternal immune response 	Trophoblast cells <ul style="list-style-type: none"> • express non-classical major histocompatibility complex molecules 	Activation of placental exosomes and placenta endogenous retroviral envelope proteins leading to <ul style="list-style-type: none"> • a shift in immune response: from Th1/Th17 toward a Th2/Treg cell response • inhibition of natural cell killer cytotoxicity against trophoblasts • immunosuppressive properties, such as Fas Ligand, programmed death ligand 1, and major histocompatibility complex molecules
Oestrogens <ul style="list-style-type: none"> • increase the size of the uterus • thicken the uterine wall and vaginal mucosa • increase the blood supply by the enlarged number and size of vessels 	Methylation in decidual stromal cells <ul style="list-style-type: none"> • causes chemokine silencing, which in turn limits T-cell access to the placenta Dominance of regulatory T-cells <ul style="list-style-type: none"> • avoids the recognition of foetal semiallogenic tissues (inherited from the father) by the maternal immune system 	High levels complement regulatory protein expression
	Maternal T-cell apoptosis	

Table 2.
Periodontitis-associated microorganisms linked with various adverse pregnancy outcomes.

Periodontal microorganisms	Pre-eclampsia / gestational hypertension	Chorioamniotitis / Intra-amniotic infection	Neonatal sepsis	PTB ± LBW	References
<i>A. actinomycetemcomitans</i>	X				Barak <i>et al.</i> , 2007; Hirano <i>et al.</i> , 2012; Swati <i>et al.</i> , 2012
<i>Bergeyella sp.</i>			X	X	Han <i>et al.</i> , 2006; 2009; Wang <i>et al.</i> , 2013
<i>C. rectus</i>				X	Ercan <i>et al.</i> , 2013
<i>Capnocytophaga spp.</i>		X	X	X	Mekouar <i>et al.</i> , 2012; Santa Cruz <i>et al.</i> , 2013
<i>Dialister sp.</i>	X				Amarasekara <i>et al.</i> , 2015
<i>E. corrodens</i>	X			X	Blanc <i>et al.</i> , 2015; Contreras <i>et al.</i> , 2006; Santa Cruz <i>et al.</i> , 2013
<i>F. nucleatum</i>	X	X	X	X	Barak <i>et al.</i> , 2007; Blanc <i>et al.</i> , 2015; Gauthier <i>et al.</i> , 2011; Han <i>et al.</i> , 2010; Swati <i>et al.</i> , 2012; Wang <i>et al.</i> , 2013
<i>P. micra</i>				X	Blanc <i>et al.</i> , 2015; Skuldbøl <i>et al.</i> , 2006
<i>P. gingivalis</i>	X			X	Amarasekara <i>et al.</i> , 2015; Barak <i>et al.</i> , 2007; Chaparro <i>et al.</i> , 2013a; Contreras <i>et al.</i> , 2006; Lin <i>et al.</i> , 2007; Swati <i>et al.</i> , 2012
<i>P. intermedia</i> / <i>P. nigrescens</i>				X	Barak <i>et al.</i> , 2007; Lin <i>et al.</i> , 2007
<i>P. shahii</i>	X				Amarasekara <i>et al.</i> , 2015
<i>T. forsythia</i>	X			X	Barak <i>et al.</i> , 2007; Chaparro <i>et al.</i> , 2013a; Contreras <i>et al.</i> , 2006; Lin <i>et al.</i> , 2007
<i>T. denticola</i>	X				Barak <i>et al.</i> , 2007; Chaparro <i>et al.</i> , 2013a
<i>Variovorax sp.</i>	X				Amarasekara <i>et al.</i> , 2015

PTB = preterm birth, LBW = low birth weight

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His research activities are focused on periodontal surgical treatment and periodontal medicine. He is the author of more than 60 articles in international journals, a member of the editorial board of the *Journal of Clinical Periodontology*, associate editor of *Minerva Stomatologica*, and a reviewer for numerous scientific journals.

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Prof Graziani has received the second prize for graduate research from the European Federation of Periodontology (2005), the Robinson Award from the American Academy of Periodontology (2013), the Jaccard Prize for Clinical Research from the European Federation of Periodontology (2015), and the HM Goldman Prize from the Italian Society of Periodontology (2017).

He is a member of the executive committee of the European Federation of Periodontology (EFP), and is due to become its president in 2019. He is the EFP delegate of the Italian Society of Periodontology and Implantology (SIdP), of which he is a former secretary general. Prof Graziani was the co-ordinator of European Gum Health Day 2017.

Oral Health and Pregnancy: the project



The aim of the Oral Health and Pregnancy project, a collaboration between the European Federation of Periodontology (EFP) and Oral-B, is to promote women's oral health during pregnancy through guidelines for patients and for healthcare professionals.

The importance of oral health during pregnancy cannot be underestimated. Scientific studies have shown connections between gum disease and adverse pregnancy outcomes such as premature birth, low birth weight, and pre-eclampsia.

The Oral Health and Pregnancy project offers the site oralhealthandpregnancy.efp.org which is full of advice – based on the latest scientific evidence – about the steps that need to be taken to ensure good oral health in pregnant women. The portal includes written, graphical, and video material in three areas:

- *The importance of women's oral health during pregnancy;*
- *The links between periodontal diseases and pregnancy;*
- *Preventing and treating periodontal disease during pregnancy.*

At the heart of the Oral Health and Pregnancy portal are sets of guidelines about oral health in pregnant women for dentists, dental hygienists, other health professionals, and for women themselves. These guidelines have been drawn up by some of the world's leading experts in periodontal science and are based on the results of numerous scientific studies.

The project will also provide a toolkit for the 30 national societies of periodontology which are members of the EFP to enable them to run their own campaigns on oral health and pregnancy, whether through similar portals or through the production and distribution of leaflets based on the guidelines. This toolkit will enable the important information contained in the guidelines to reach health professionals and women across Europe in local languages and adapted to local needs.

oralhealthandpregnancy.efp.org

A joint EFP – Oral-B project



The European Federation of Periodontology (EFP) is the leading global voice on gum health and gum disease and the driving force behind EuroPerio – the most important international periodontal congress – and Perio Workshop, a world-leading meeting on periodontal science. The EFP also edits the Journal of Clinical Periodontology, one of the most authoritative scientific publications in this field.

The EFP comprises 30 national societies of periodontology in Europe, northern Africa, Caucasia, and the Middle East, which together represent about 14,000 periodontists, dentists, researchers, and other members of the dental team focused on improving periodontal science and practice.

www.efp.org



Oral-B is the worldwide leader in the over \$5 billion tooth-brush market. Part of the Procter & Gamble Company, the brand includes manual and electric toothbrushes for children and adults, oral irrigators, interdental products such as dental floss, together with toothpastes and mouth rinses. Oral-B manual toothbrushes are used by more dentists than any other brand in the USA and many international markets.

Oral B has been an EFP partner since 2009 and has participated in many EFP events, including EuroPerio7 (2012) and EuroPerio8 (2015) as a Diamond sponsor, the EFP Postgraduate Symposium in 2013 and 2015, and the European Workshop in Periodontology in 2014. The company will be a Diamond Sponsor of EuroPerio9, which takes place in Amsterdam in June 2018.

www.dentalcare.com



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