J Clin Periodontol 2013; 40 (Suppl. 14): S170–S180 doi: 10.1111/jcpe.12082

Journal of Clinical Periodontology

# Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms

**Phoebus N. Madianos[1], Yiorgos A. Bobetsis[2] and Steven Offenbacher[3]**

[1]Department of Periodontology, School of Dentistry, University of Athens, Athens, Greece; [2]Department of Periodontology, School of Dentistry, University of Athens, Athens, Greece; [3]Department of Periodontology, University of North Carolina School of Dentistry at Chapel Hill, NC, USA

*Madianos PN, Bobetsis YA, Offenbacher S. Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms. J Clin Periodontol 2013; 40 (Suppl. 14): S170–S180. doi: 10.1111/jcpe.12082.*

***Abstract***

**Aim:** To evaluate the evidence on potential biological pathways underlying the possible association between periodontal disease (PD) and adverse pregnancy outcomes (APOs).

**Material & Methods:** Human, experimental and in vitro studies were evaluated.

**Results:** Periodontal pathogens/byproducts may reach the placenta and spread to the foetal circulation and amniotic fluid. Their presence in the foeto-placental compartment can stimulate a foetal immune/inflammatory response characterized by the production of IgM antibodies against the pathogens and the secretion of elevated levels of inflammatory mediators, which in turn may cause miscarriage or premature birth. Moreover, infection/inflammation may cause placental structural changes leading to pre-eclampsia and impaired nutrient transport causing low birthweight. Foetal exposure may also result in tissue damage, increasing the risk for perinatal mortality/morbidity. Finally, the elicited systemic inflammatory response may exacerbate local inflammatory responses at the foeto-placental unit and further increase the risk for APOs.

**Conclusions:** Further investigation is still necessary to fully translate the findings of basic research into clinical studies and practice. Understanding the systemic virulence potential of the individual's oral microbiome and immune response may be a distinctly different issue from categorizing the nature of the challenge using clinical signs of PD. Therefore, a more personalized targeted therapy could be a more predictive answer to the current "one-size-fits-all" interventions.

Key words: adverse pregnancy outcomes; experimental studies; in vitro studies; pathogenic mechanisms; periodontal disease

Accepted for publication 14 November 2012

The proceedings of the workshop were jointly and simultaneously published in the Journal of Clinical Periodontology and Journal of Periodontology.

In 1891, Miller published the theory of "focal infection" which hypothesized that oral foci of infection were responsible for a number of regional and systemic diseases, such as tonsillitis, pneumonia, endocarditis and septicaemia. (Miller 1891) However, the lack of scientific evidence condemned this theory to dormancy. One hundred years later, in the early 1990s, Offenbacher's group using a bacteremia model and a "chamber" model to mimic a focal infection on pregnant hamsters demonstrated that periodontal bacteria and inflammatory mediators have the ability to disseminate systematically to the foetal-placenta unit, via the blood circulation and induce pregnancy complications. Hence, the authors proposed that oral infection, such as periodontitis, may act as a distant infectious reservoir and affect pregnancy outcomes. (Collins et al. 1994a,b) Since these first landmark series of animal studies, many investigators have tried to elucidate whether this causal pathway of an oral pathogen inducing adverse pregnancy outcomes (APOs) in an animal model has analogy with periodontal disease and APOs in humans.

Although a large number of epidemiological and intervention studies demonstrate a positive association

**Conflict of interest and source of funding statement**

The authors declare no conflict of interest. The workshop was funded by an unrestricted educational grant from Colgate-Palmolive to the European Federation of Periodontology and the American Academy of Periodontology.

between periodontal disease (PD) and APOs the results are not always consistent (Xiong et al. 2007, Polyzos et al. 2010). Systematic reviews and meta-analyses suggest that this diversity is, in part, due to methodological inconsistencies which also render these studies hard to be compared. Alternative explanations include population differences, relative obstetric risk and other factors which are known to vary the prevalence of APOs, irrespective of oral status. This may explain why after all these years of research the scientific community cannot address with certainty the generalizability of this association and whether it is uniformly aetiological in nature.

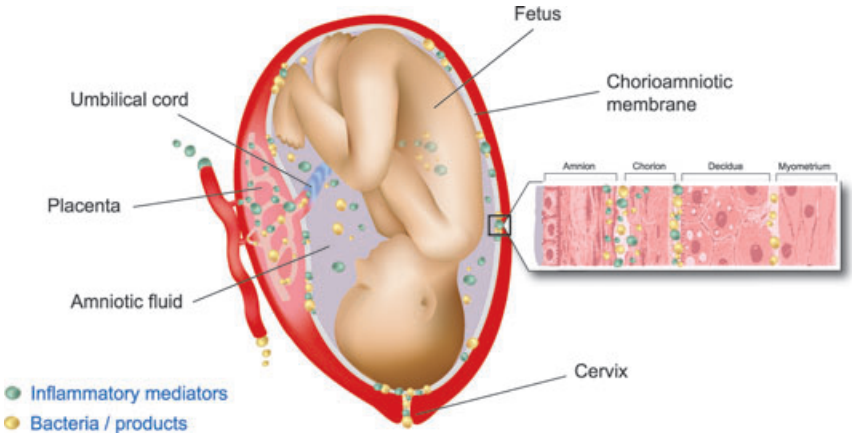


*Fig. 1.* Anatomical structure of the foeto-placental unit and locations where infection may take place.

#### Physiology of normal pregnancy

To better understand how periodontal pathogens may affect pregnancy outcomes the physiology of normal pregnancy and the changes that occur during pregnancy complications need to be briefly described first.

After conception, the placenta that is totally derived from the foetus invades and grows supported completely by the maternal uterine tissue. Through the vessel-rich placenta, there is exchange of nutrients and waste between the mother and the foetus. This transportation occurs via the umbilical cord that connects the foetus with the placenta. Having the necessary resources, the foetus grows in the amniotic fluid which is contained by the amniotic sac. The walls of this cavity consist of the amnion and the chorion and like the placenta are attached to the uterus through the decidua and the myometrium (Fig. 1). As the foetus grows the increasing needs for nutrients and the decreasing space become critical parameters for the survival of both mother and foetus. Hence, as pregnancy progresses, amniotic fluid levels of prostaglandin E2 (PGE2) and inflammatory cytokines, such as TNF-$\alpha$ and IL-1$\beta$ rise steadily until a critical threshold level is reached to induce rupture of the amniotic sac membranes, uterine contraction, cervical dilation and delivery. (Haram et al. 2003). Thus, normal parturition is controlled by inflammatory signalling and this process represents a triggering mechanism that can be modified by external stimuli including infection and inflammatory stressors.

#### Pathogenic mechanisms of APOs

A large number of studies associate an increase in the levels of local and systemic markers of inflammation with APOs. Hence, elevated levels of IL-1$\beta$, IL-6, TNF-$\alpha$, PGE2, fibronectin and $\alpha$-foetoprotein in the amniotic fluid have been associated with PB, while other biomarkers such as MMPs, estriol, elastase, protease, phospholipase, prolactin myeloperoxidase and tissue inhibitor of MMP (TIMP)-1 have been evaluated but with inconclusive results. (Inglis 1997, Gürsoy et al. 2010). Increased maternal serum levels of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF-$\alpha$, have also been reported to be associated with prematurity or low birthweight (PLBW) (Greig et al. 1997, von Minckwitz et al. 2000, Turhan et al. 2000, Gücer et al. 2001, Hitti et al. 2001). Moreover, C-reactive protein (CRP), which is an acute phase reactant synthesized by the liver in response to pro-inflammatory cytokines, and hence a marker of systemic inflammation is also associated with PB (Pitiphat et al. 2005).

Besides PB, elevated levels of CRP have been shown to be associated with an increased risk for other pregnancy complications, such as intrauterine growth restriction (IUGR) (Tjoa et al. 2003) and pre-eclampsia. (Teran et al. 2001). Pre-eclampsia, which may occur only during pregnancy, affects primarily the pregnant woman instead of the foetus. It manifests mainly with increased blood pressure and maternal proteinuria and is also associated with high levels of pro-inflammatory cytokines. (Herrera et al. 2007). Finally, elevated serum CRP, IL-6 and TNF-$\alpha$ in the women with gestational diabetes mellitus (GDM) suggest a role of inflammation in the aetiology of this pregnancy complication. It is known that IL-6 and TNF-$\alpha$ interfere with insulin signalling and are also insulin antagonists. Therefore, sustained elevated levels of IL-6 and TNF-$\alpha$ can interfere with carbohydrate metabolism, and consequently cause glucose intolerance that can result in GDM.

As the increased release of inflammatory cytokines and mediators plays a critical role in the pathogenesis of APOs, infections of the genitourinary (GU) tract have been evaluated. (Hillier et al. 1995, Nigro et al. 2011) Indeed, intra-uterine infection can be confined to the decidua (deciduitis), extend to the space between the amnion and the chorion (chorioamnionitis), and reach the amniotic fluid (amniotic fluid infection). Moreover, it may involve the placenta (villitis), the connective tissue of the umbilical cord (funisitis) and the foetus (sepsis) (Fig. 1). Microorganisms can gain access to the amniotic cavity: (1) by ascending from the vagina and the cervix, (2) by haematogenous dissemination through the placenta, (3) by accidental introduction at the time of invasive procedures (amniocentesis) and (4) by retrograde spread through the fallopian tubes. (Goldenberg

et al. 2008). This microbial invasion is frequently associated with intra-amniotic inflammation and a foetal inflammatory response which are linked to APOs. (Romero & Mazor 1988a) Interestingly, intra-uterine infection might account for 25–40% of PB. However, this percentage may be underestimated due to difficulties in culturing techniques and the presence of subclinical infections. (Goldenberg et al. 2000, 2008, Han et al. 2009). Finally, studies on the use of systemic antibiotics such as metronidazole and clindamycin to control early infections during pregnancy and their effects on APOs, although not always consistent, further support an infectious aetiology of PD and LBW (Lamont & Sawant 2005, Lamont et al. 2007, McDonald et al. 2007). Besides PB, early treatment of urinary and vaginal infections has been shown to decrease the incidence of pre-eclampsia suggesting that infection may play a role in the pathology of this disease too (Herrera et al. 2001).

It is worth noting that elevated levels of PGE2 have been observed in the amniotic fluid, in consistent and reproducible association with PLBW, even in the absence of clinical or subclinical genital tract infection, and it has been postulated that the majority of PB cases are probably caused by an infection of unknown origin. (Romero et al. 1988b) Thus, several non-genital tract infections, such as pyelonephritis, asymptomatic bacteriuria, pneumonia and appendicitis, have been associated with, and probably predispose to PB (Romero et al. 1989, Goldenberg et al. 2005).

#### Hypothetical mechanistic model

PD is also an infectious disease that occurs distant to the foeto-placental unit. In an attempt to contain or eliminate this infection, host cells activate a local inflammatory response against these bacteria and their numerous virulence factors (e.g. lipopolysaccharides, LPS). However, several studies report that inflammatory cytokines, periodontal bacteria and/or their virulence factors may enter the blood circulation and disseminate throughout the body, triggering the induction of systemic inflammatory responses and/or ectopic infections (Guntheroth 1984, Han et al. 2006, 2010, Témoin et al. 2012).

In this context, a mechanistic model that could potentially explain the biological association between PD and APOs has been proposed (Bobetsis et al. 2006). The main arms of this hypothetical model include the following: (1) Direct pathway: Periodontal bacteria and/or their pathogenic products disseminate to the foeto-placental unit where they initiate an ectopic infection and/or trigger a local inflammatory response that results in the elevation of inflammatory cytokines and mediators that contribute to pregnancy complications. (2) Indirect pathway: Inflammatory cytokines and mediators produced at the gingival level in response to periodontal pathogens, enter the blood circulation and reach i) the foeto-placental unit and enhance the accumulation of larger amounts of these mediators in this compartment and ii) the liver where they stimulate a systemic inflammatory response by the production of acute phase reactants. These products gain access to the blood circulation and may enter the foeto-placenal unit exacerbating intra-uterine inflammation.

To date, many studies have strived to confirm different aspects of this hypothetical model. In addition, it remains clear that these mechanistic studies that investigate plausible biological pathways add credit to a potential association between PD and APOs. In this Supplementary article, the putative mechanisms that have been examined will be reviewed. For practical reasons, the evidence generated will be discussed by dividing the data to those occurring from human studies and those originating from experiments in animal models and in vitro experiments.

### Evidence from Human Studies

PD is caused mainly by anaerobic Gram-negative bacteria. Socransky et al. divided these bacteria into microbial complexes or clusters. The "blue," "green," "yellow" and "purple" clusters include mainly bacteria that colonize the periodontal sulcus in the early stages of dental plaque formation. As the biofilm matures and becomes more pathogenic, organisms of the "orange" cluster *(C. rectus, F. nucleatum, Peptostreptococcus micros, Prevotella intermedia* and *Prevotella nigrescens)* appear and provide the necessary habitat for the subsequent colonization and establishment of the more aggressive bacteria of the "red" cluster *(Porphyromonas gingivalis, Tannerella forsythia* and *Treponema-denticola)*. Although the exact role of each of these bacterial species in the progression of PD is not fully understood, it remains clear that the presence of a large group of bacteria somehow is necessary for the overall pathogenic effect (Socransky et al. 1998).

During pregnancy, due to physiological hormonal changes, there is a systematic inclination to periodontal disease. In particular, there is a global rise in anaerobic Gram-negative agents such as *F. nucleatum*, *T. denticola*, *T. forsythia*, *C. rectus*, *Eikenella corrodens* and *Selenomonas sputigena* (Carta et al. 2004). Several studies have tried to evaluate whether the presence and number of specific periodontal pathogens in periodontal tissues are associated with APOs (Contreras et al. 2006, Skuldbøl et al. 2006, Lin et al. 2007). Although certain oral organisms have clear foeto-placenta virulence traits, due to the large number and diversity of the microbiome, it is difficult to effectively identify the pathogenic potential of all oral organisms in APOs, either when examined as individual species or as the entire microbiome.

From a mechanistic aspect, maybe a more critical question is whether specific periodontal pathogens/byproducts reach the foeto-placental unit. It is established that Gram-negative periodontal bacteria and/or their virulence factors (e.g. endotoxins) may enter the systemic circulation and in the case of bacteria produce a low-grade bacteremia (Guntheroth 1984, Jarjoura et al. 2005). Especially during pregnancy, the hormonal changes due to elevated levels of estrogens and progesterone increase vascular permeability in the gingival tissues and, as a consequence, bacteria and/or their products can diffuse through the tissues more readily than normal.

Evidence indicating that periodontal pathogens/byproducts may reach the foeto-placental unit derives, in part, from immunological data. Once the mother is exposed to bacterial pathogens, the host's innate immune response will attempt to contain and resolve the infection. If this is not successful, then, the more efficient

adaptive immune response will be initiated by the production of bacterial-specific antibodies. First, IgM specific antibodies are formed and then, through isotype switching, IgM are converted to IgG. Since periodontitis is a chronic infection, maternal IgG antibodies can pass to the foetus via the placenta. In utero, the foetus is not immunocompetent and can only mount an IgM antibody response when challenged.

In this context, Madianos et al. found that maternal serum IgG specific to oral organisms was associated with decreased PB and increased birthweight. There was, also, a general trend for mothers with term babies to have higher prevalence of serum IgG antibody to orange and red complex organisms compared to mothers with PB. Moreover, there was a higher prevalence of umbilical cord IgM seropositivity for one or more organisms of the red or orange complex in PB. Furthermore, the lack of maternal anti-red IgG was associated with PB similarly to the presence of foetal anti-orange IgM. Interestingly, the highest rate of PB occurred in mothers with low or no anti-red IgG and high anti-orange IgM. In the presence of maternal oral organisms, the lack of a protective maternal IgG response could increase foetal exposure which in turn may contribute to a foetal immune response that could lead to PB (Madianos et al. 2001).

Other investigators have also demonstrated comparable results. Boggess et al. found that one-third of pregnant women were positive for umbilical cord IgM against at least one of the five oral organisms tested and this was further associated with increased risk for PB and vaginal bleeding. Whether vaginal bleeding facilitates the translocation of oral bacteria to the foeto-placental unit or is induced by the foetal exposure to the oral pathogens could not be determined (Boggess et al. 2005, 2006). Finally, similarly to a pilot study by Lin et al., data from the large OPT study revealed that mothers who deliver preterm have significantly lower serum IgG levels against *P. gingivalis* compared to term mothers (Lin et al. 2007, Ebersole et al. 2009).

On the contrary, there are some studies that demonstrate no differences or even the opposite results in antibody levels in relation to pregnancy complications. Hence, Jarjoura et al. found similar levels of serum antibodies against oral pathogens between women with or without PB or LBW (Jarjoura et al. 2005). However, Ebersole et al. found elevated serum antibodies against *F. nucleatum* in women who suffered a foetal loss, and Desanayake et al. observed that higher IgG levels against *P. gingivalis* were associated with more LBW infants (Dasanayake et al. 2008).

From the above results, it is interesting that no study has questioned, so far, the biological possibility of foetal exposure to oral pathogens in women with pregnancy complications. However, the role of maternal serum antibodies against these bacteria remains a debate with a double interpretation. Thus, low levels of serum IgG antibodies could suggest inadequate protection against the disseminating oral pathogens that may translocate easier 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.

Another more direct assessment that periodontal pathogens may translocate to the foeto-placental unit derives from data generated by other laboratory techniques such as PCR and immunochemistry. Specifically, Leon et al. found that from the nine bacteria tested only *P. gingivalis* seemed to invade the amniotic cavity since it was detected in the amniotic fluid of women with threatened preterm labour (TLP) (León et al. 2007). *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* have also been found in the placenta of women with PB or pre-eclampsia (Barak et al. 2007, Katz et al. 2009, Swati et al. 2012). Moreover, in a case of PB, the oral species *Bergeyella* was identified in the amniotic fluid (Han et al. 2006). *F. nucleatum* is also one of the most common isolates from the amniotic fluid of patients with PLBW and intact membranes, but has also been detected in chorionic tissues of high-risk pregnant women (Romero et al. 1988b, Cahill et al. 2005, Tateishi et al. 2012). Finally, *P. gingivalis*, *F. nucleatum* and *Capnocytophaga* have been found in neonatal gastric aspirates obtained from complicated pregnancies indicating a possible bacterial translocation to foetal organs (Mayatepek et al. 1991, Gonzales-Marin et al. 2011).

Interestingly, periodontal pathogens have also been detected in amniotic/foeto-placental tissues of women with normal pregnancies (Katz et al. 2009). Moreover, studies have shown that it is possible to isolate bacteria considered as periodontal pathogens from periodontally healthy persons (Ximénez-Fyvie et al. 2000). On this basis, it remains unknown: a) what factors determine whether the translocation of these pathogens to the foeto-placental unit will contribute to pregnancy complications and b) whether the clinical parameters of PD are always good indicators of the risk for pregnancy complications.

In the case of haematogenous dissemination, although various studies have demonstrated that Gram-negative anaerobes are present and alive in the aerobic environment of the blood stream during transient bacteremias after dental procedures, it is not clear whether these bacteria can reach and colonize the foeto-placental tissues (Lafaurie et al. 2007). Hence, to date, although *F. nucleatum* is commonly isolated from the foeto-placental unit of complicated pregnancies, only one study has managed to culture an oral strain of this bacterium from the placenta, lung and stomach of a term stillbirth infant (Han et al. 2010). Definitely, difficulties in the culturing techniques that still are unable to grow the majority of the microbes living on and within our bodies may be a critical parameter (Han et al. 2009). It is worth mentioning that similar attempts have been performed by researchers studying the association of PD with cardiovascular diseases. To our knowledge, only one study has been able to detect live *P. gingivalis* and *A. actinomycetemcomitans* from atherosclerotic plaques (Kozarov et al. 2005). The results from both these studies support the biological plausibility of a haematogenous translocation and establishment of a new focus of infection with live bacteria distant to periodontal tissues.

Although the key periodontal pathogens possess specific virulence

factors that enable them to colonize, evade host defences and invade host tissues (Madianos et al. 2005), in the case that periodontal pathogens reach alive the foeto-placental unit the question that arises is whether they will remain in a planktonic form or they will develop a biofilm, as in the case of dental plaque. Recently, the presence of a particulate matter in the amniotic fluid, recognized as "amniotic fluid sludge," was associated with the presence of bacteria and intra-amniotic inflammation. The use of electron microscopy provided evidence that this sludge was indeed a biofilm (Romero et al. 2008). Moreover, transmission electron microscope analysis of murine placentas infected with *F. nucleatum* revealed the formation of bacterial colonies within the placenta further supporting the theory of a biofilm formation (Han et al. 2004). Whether several different periodontal pathogens are necessary to translocate to the foeto-placental unit and form a biofilm or whether periodontal pathogens could colonize a biofilm formed by other extra-oral bacteria remains unknown.

As there is evidence indicating that periodontal pathogens, dead or alive, may reach the foetal-placenta unit the question that arises is what is the origin of these pathogens? The current paradigm indicates that the majority of intra-uterine infections originate in the lower genital tract, with the infectious agents ascending into the otherwise sterile womb. Usually, the amniotic membranes and the placenta are the first to be infected followed by the foetus via the blood vessels of the umbilical cord or the aspiration of the infected amniotic fluid (Goldenberg et al. 2000, Romero et al. 2001). Hence, one could suggest that periodontal pathogens may enter the amniotic space as a result of ascending infection following oral-genital transfer. However, to date, there is no evidence to support this hypothetical pathway. From an ecological point of view, it is not that easy for oral bacteria to establish colonization in the vagina as a result of oral-genital contact, due to "colonization resistance." Moreover, to change the flora, a persistently high dose of exogenous inoculums would be needed. Hence, further investigation would be necessary to evaluate this theoretical, yet, biological plausibility. On the other hand, since not all women who experience pregnancy complications have genital infections, and periodontal pathogens/byproducts may enter the blood circulation, it is very likely that these pathogens may translocate to the foeto-placental unit via haematogenous spread. To date in humans, there are two studies that provide evidence of this route of infection. In one case, the same clonal type of uncultivated oral *Bergeyella* was identified in the subgingival plaque and the amniotic fluid of a woman with PB, while no *Bergeyella* cells were detected in the mother's vaginal tract (Han et al. 2006). In the second case, oral *F. nucleatum* was identified as the cause of a term stillbirth and was isolated from the lung and stomach of the infant. Interestingly, the same clonal type of *F. nucleatum* was present in the subgingival plaque of the mother, but not in her vaginal or rectal microflora (Han et al. 2010).

Besides periodontal pathogens, the adverse effects on pregnancy could be mediated by the release of major components of the bacterial cell wall, that is, LPS or other virulence factors. As it will be described later on, local host cells may interact with these byproducts and produce inflammatory cytokines (TNF-α, IL-1, IL-6) and mediators (PGE2). Since during pregnancy, the innate pro-inflammatory immune response is strictly regulated within the uterus to prevent immunological rejection of the foetal allograph, the local increase in pro-inflammatory mediators may disrupt this delicate balance and elicit an inflammatory burden that may contribute to preterm rupture of the membranes and uterine contraction that may, in turn, lead to miscarriage or PB (Africa 2011).

In PD patients, there is also an increase in the production of pro-inflammatory cytokines and mediators from periodontal tissues. Once released, they may diffuse in the gingival crevicular fluid (GCF) or enter the blood circulation and reach the placenta-foetus interface. IL-1, IL-6 and TNF-α could stimulate the production of prostaglandins in the chorion and in conjunction with the maternally derived PGE2 generated at the gingival level and released in the circulation may exacerbate cervical ripening and uterine contraction leading to an increased risk for PB. However, although elevated serum and amniotic fluid levels of these mediators have been associated with various APOs (Greig et al. 1997, Inglis 1997, von Minckwitz et al. 2000, Gücer et al. 2001), there is limited and mostly negative evidence that the elevation of these mediators in GCF, serum and amniotic fluid are associated with pregnancy complications in periodontitis patients (Bearfield et al. 2002, Carta et al. 2004, Dörtbudak et al. 2005, Goepfert et al. 2004, Sert et al. 2011, Tarannum et al. 2011, Fiorini et al. 2012).

Pro-inflammatory cytokines released in the maternal circulation along with transient bacteremia from periodontal tissues may also induce a low-grade systemic inflammation by stimulating the production of acute phase reactants, such as CRP and fibrinogen, by the liver. Elevated levels of plasma CRP could amplify the inflammatory response at the foeto-placental interface through complement activation, tissue damage and induction of pro-inflammatory cytokines. Thus, CRP has been associated not only with periodontal disease but also with pregnancy complications such as PB, IUGR, pre-eclampsia and GDM (Teran et al. 2001, Tjoa et al. 2003, Pitiphat et al. 2005, Dasanayake et al. 2008, Paraskevas et al. 2008).

Although pregnant women with moderate to severe PD have elevated levels of serum CRP (Horton et al. 2008), to date, there are only a few studies evaluating the association between pregnancy complications and CRP levels in periodontitis patients. Thus, several reports indicate that elevated CRP levels in pregnant women with periodontitis appear to be associated with PB and pre-eclampsia (Offenbacher et al. 2006, Ruma et al. 2008, Sharma et al. 2009), while others found no such association (Ghezzi et al. 2002).

### Evidence from Animal Models and in vitro Experiments

Most studies of the biological mechanisms behind the possible association between PD and APOs used hamsters, mice and rabbits. Several experimental models have been employed to simulate in a simplified and reproducible manner a periodontal infection in these pregnant

animals. Thus, periodontal bacteria or their pathogenic products (LPS) have been injected in the blood circulation mimicking bacteremia. In other experiments, a metal chamber/cylinder was placed subcutaneously and periodontal bacteria were injected in the chamber creating a distant site of infection. Due to the open ends of the chamber, bacteria and/or their released byproducts could leave the chamber and enter the systemic circulation.

The first experiments occurred on Golden hamsters. When *P. gingivalis* LPS was injected intravenously (I.V.) before and during pregnancy, there was an increase in foetal malformation, IUGR and resorptions. The frequency and severity of these APOs were dose dependent and similar to those occurring after I.V. injection with *Escherichia coli* LPS (Collins et al. 1994a). Although there is a concern that the experimental levels of these bacterial products may be much higher than what may actually occur under transient bacteremia in patients with periodontal disease, this was the first proof of the principle experiment to suggest a possible association of PD with APOs. Specifically, it demonstrated that when periopathogenic byproducts enter the systemic circulation, they may induce pregnancy complications. Similar results occurred when various periodontal microorganisms were injected in the pregnant animals. Hence, in the chamber model, *P. gingivalis* has been shown to induce IUGR and elevated numbers of resorptions in Golden hamsters. Interestingly, the severity of these complications was dependent on the extent of the inflammatory response in the chamber as evaluated by the increased levels of TNF-α and PGE2 (Collins et al. 1994b). IUGR and increased resorptions (analogous to miscarriages) have also been demonstrated after intra-chamber injection of *P. gingivalis* or *C. rectus* in mice (Lin et al. 2003a, Offenbacher et al. 2005, Yeo et al. 2005). It is worth noting that besides oral *C. rectus* other *campylobacter* species have also been shown to be implicated in the induction of pregnancy complications and especially in abortions of sheep and cattle (Simor et al. 1986). Moreover, in humans *Campylobacter foetus*, *Campylobacter jejuni* and *Campylobacter coli* have been associated with abortion, PB and perinatal sepsis, while in mice intravenous injection of these *campylobacters* results in IUGR, impaired foetal development and increase in resorptions (Guerrant et al. 1978, O'Sullivan et al. 1988). Finally, i.v. injection of *F. nucleatum* in mice resulted in premature delivery, stillbirths and non-sustained live births (Han et al. 2004).

In these animal models, since infection with periodontal pathogens/byproducts could cause pregnancy complications investigators tried to elucidate whether these microorganisms disseminate systemically and translocate to the foetal-placental unit. In the chamber model in mice *C. rectus,* DNA was found in the maternal liver and placenta of infected dams (Yeo et al. 2005). Similarly, *P. gingivalis* DNA was detected in maternal liver, uterus and placenta of the IUGR foetuses. Furthermore, infected dams with IUGR foetuses presented with elevated levels of serum TNF-α and *P. gingivalis*-specific IgG antibodies and reduced levels of IL-10 (Lin et al. 2003a,b). However, i.v. infection of mice with *F. nucleatum* was restricted inside the uterus, without spreading systemically (Han et al. 2004).

Although both the chamber and the i.v. infection models indicate that periodontal pathogens may translocate to the foetal-placenta compartment, there is controversy concerning the activation of the systemic inflammatory/immune response and dissemination of pathogens to other maternal organs. It has been suggested that the chamber model represents a chronic infection, since *P. gingivalis* is repeatedly inoculated systemically through the chamber, while the i.v. infection resembles more an acute infection which mimics, predominatly, bacteremia. In the case of the acute infection bacterial translocation is organ-specific, that is, only in the placenta and this is likely due to the immune suppression in the placenta, which allows the bacteria to proliferate freely, whereas the bacteria are killed by the immune cells in the liver (Han 2011). Periodontitis is, usually, a chronic infection where transient bacteremias occur and hence both models may coexist and explain the somewhat diverse results in systemic inflammation demonstrated by pregnant women.

It is also clear that both models have a serious limitation: they simulate more a monoinfection rather than infection organized in a biofilm, where the presence and collaboration of different types of microorganisms are necessary. However, the results suggest periodontal pathogens/byproducts may not need to be part of a biofilm to have a deleterious effect on pregnancy. Interestingly, in a recent study, a diverse group of oral bacteria were found to translocate to the mouse placenta following i.v. injection with pooled human saliva or subgingival plaque from the deep pockets of periodontitis patients. Many of these bacteria have been associated with pregnancy complications in humans and the majority of them are oral commensal organisms (Fardini et al. 2010). This is consistent with studies in humans where the foetus may be exposed to various periodontal pathogens (Madianos et al. 2001). Also, it demonstrates the possibility that these pathogens may organize in a biofilm, as indicated by the recent finding of a bacterial biofilm formed in the amniotic fluid from complicated pregnancies in humans (Romero et al. 2008). The tropism of oral organisms to the nutrient-rich foetal-placental unit may be analogous to the concept of anachoresis first describe by Ascoli in the early 1900s which describes the fact that organisms will disseminate systemically to seek out nutrient-rich environments, such as those associated with inflammation.

In mice, the pattern of foeto-placental interface infection by *F. nucleatum* parallels that in humans. First, *F. nucleatum* is detected in placental blood vessels. Maybe, due to the slow blood flow in the venous sinuses, bacteria have the opportunity to invade the endothelial cells lining the vessels, cross the endothelium, proliferate in the surrounding tissues and finally spread to the amniotic fluid (Han et al. 2004). These data suggest that bacteria can survive in the oxygen-rich blood circulation and reach the placenta. Moreover, they demonstrate that invasion may be an important virulence mechanism for placental infection. In fact, it has been shown that *F. nucleatum's* FadA adhesin plays a critical role in this process (Ikegami

et al. 2009). i.v. injection of *P. gingivalis* into pregnant rats also caused strain-dependent colonization in the placenta, while *C. rectus* is able to invade human trophoblast cells in vitro (Bélanger et al. 2008, Arce et al. 2010). Placental colonization by all three bacteria has been associated with intra-uterine infections in humans, but, perhaps, other invasive periodontal pathogens may have similar properties too (Madianos et al. 2005, Han et al. 2009, Katz et al. 2009).

Colonization of periodontal pathogens in the placenta results in the induction of local inflammatory responses. Specifically, in IUGR placenta infected with *P. gingivalis,* there is an increase in IL-2 and IFN-$\gamma$, while IL-10 is reduced, indicating a shift in Th1/Th2 cytokine balance. IL-6 and TNF-$\alpha$ are also elevated after stimulation of human decidual cells with *F. nucleatum, A. actinomycetemcomitans* and *P. gingivalis* and this is consistent with intra-uterine infections in humans that lead to APOs (Keelan et al. 2010). In murine placenta, inflammatory responses induced by *F. nucleatum* and *C. rectus* seem to be mediated via TLR-4 and this is consistent with the known TLR4 activation in human placental inflammation and preterm pathogenesis (Liu et al. 2007, Arce et al. 2009).

Histologically, this inflammatory response is accompanied by an increase in the inflammatory infiltrate, predominatly by neutrophils, in the decidua, while *F. nucleatum* also stimulates decidual necrosis (Offenbacher et al. 2005, Liu et al. 2007). Interestingly, *C. rectus* infection induces major alterations in the structure of the placenta as indicated by the decrease in the size of the labyrinth (Offenbacher et al. 2005). The labyrinth is the area of the placenta where the exchange of nutrients and waste between the mother and the foetus takes place. Hence, its diminished volume may imply insufficient nutrition of the foetus and consequently impaired growth and LBW. Furthermore, structural damage in the placenta may disrupt the normal blood flow between the foetus and the mother, affecting maternal blood pressure and leading to pre-eclampsia.

Using mRNA expression microarray technology, *C. rectus* infection in mice has been demonstrated to attenuate the expression of genes related to placental and foetal growth, such as *Pgf*, which is the main placental angiogenic factor, and *Igf-2* (Bobetsis et al. 2010). The question, however, is whether infection reduced the size of the labyrinth which led to a decreased expression of these genes or whether infection induced attenuation in gene expression that resulted in impaired placental development. The question was partially answered by the examination of the expression of the murine *igf2* gene. This gene belongs to the group of "imprinted genes" whose expression is dictated, mainly, by the methylation status of their promoter. *C. rectus* infection induced hypermethylation of the promoter of *igf2,* which was associated with the attenuation of its expression (Bobetsis et al. 2007). As these epigenetic changes are inherited in somatic cells, infection of the foetus could alter the expression of imprinted genes and, possibly, affect the offspring throughout life.

Finally, *C. rectus* infection elevated the rates of neonatal mortality in mice. In the surviving pups, *C. rectus* was detected in the brain and induced a local inflammatory response which was accompanied by an increase in apoptosis and defects in nerve myelination (Offenbacher et al. 2005). Similarly, human neonates exposed to both *C. rectus* and *P. gingivalis* are found to be twice as likely to be admitted to the NICU, although none of the orange cluster organisms when considered individually was associated with a prolonged stay in the NICU (Jared et al. 2009). In addition, it is well documented that preterm infants have an increased risk in developing neurodevelopmental, behavioural and learning problems (Angelidou et al. 2012, Barde et al. 2012).

## Conclusions

The described studies in humans and animal models provide mechanistic insight as well as strong argument for the biological plausibility of association by causality between PD and APOs. Based on the available data, a possible biological model could be proposed (Fig. 2).

During pregnancy, due to hormonal changes, there is an increase in vascular permeability that facilitates entrance of periodontal pathogens/byproducts into the blood circulation and translocation to the placenta. Progression of PD with increased inflammation likely contributes to the accessibility of oral organisms to the circulation. In addition, the ability of certain periodontal pathogens, such as the FadA adhesin from *F. nucleatum*, to actively permeabilize the endothelium and allow even other bacteria to penetrate through may also increase the potential of bacteremia. The possibility of an ascending translocation of periodontal pathogens from the lower genital tract due to an oral-genital transfer although cannot be excluded still needs to be verified. Once in the blood stream and due of the slow blood flow in the placental venous sinuses and the invading properties of the bacteria, the pathogens have the opportunity to invade the endothelial cells lining the vessels, cross the endothelium, proliferate in the surrounding tissues and finally spread to the foetal systemic circulation and amniotic fluid. The presence of bacteria/byproducts in the placenta and the foetal compartment stimulates a foetal immune and inflammatory response characterized by the production of IgM antibodies against the pathogens and the secretion of elevated levels of pro-inflammatory cytokines. If the maternal infection is contained, then foetal exposure is minimized and no pregnancy complication occurs. However, when exposure occurs, the local release of inflammatory cytokines by the foetal tissues may elicit premature rupture of the membranes and uterine contraction which, in turn, may cause miscarriage or PB. Accumulation of pro-inflammatory cytokines in the uterine circulation may also be enhanced by the cytokines released from the gingival tissues although the evidence for this plausible pathway is still weak. Moreover, infection/inflammation in the placenta will down-regulate the expression of genes related to growth and development of the placenta and the foetus. This will contribute to a significant alteration in the architecture of the placenta, especially in areas that are critical for the exchange of nutrients between the mother and the foetus. Impaired

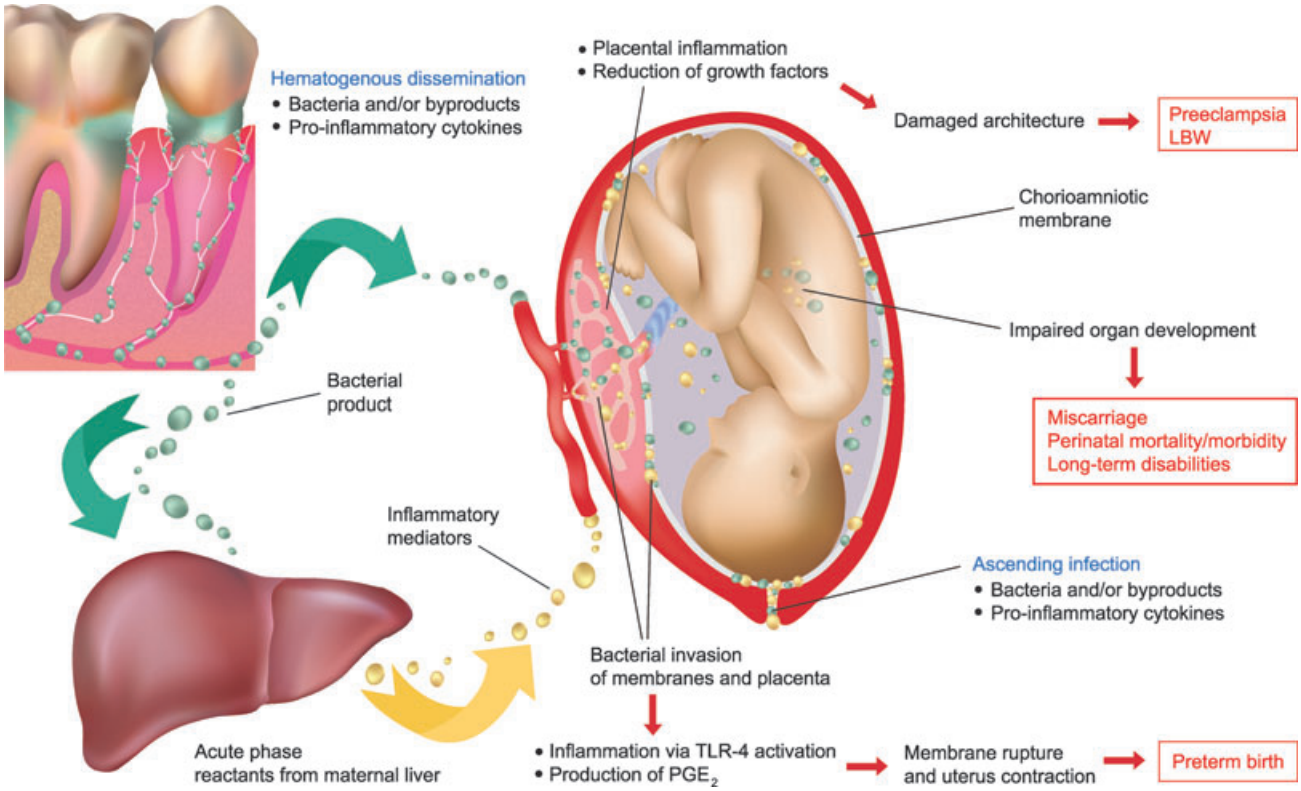


*Fig. 2.* Possible biological mechanisms/pathways associating periodontal disease and pregnancy complications.

nutrient transportation via the placenta may lead to LBW, while structural damage of the blood vessel-rich placenta may disrupt normal blood flow and increase maternal blood pressure initiating pre-eclampsia.

Foetal exposure to periodontal pathogens and/or their byproducts may also result in tissue damage. Depending on the extent of the damage the foetus may die or the neonate may demonstrate an increased risk for perinatal mortality or morbidity. Moreover, some defects that happen during foetal development as a consequence of exposure may follow the offspring and could be expressed later in life.

Besides the direct infectious effect, a systemic inflammatory response that is elicited in response to PD may regulate, in part, the risk for pregnancy complications. A protective antibody response may reduce the risk of bacterial translocation and colonization. On the other hand, acute phase reactants produced by the maternal liver may reach, through the circulation, the foeto-placental interface and exacerbate the local inflammatory responses which are responsible for the APOs.

It should be kept in mind that this basic biological model that connects PD and APOs has some limitations since part of the data were generated in animal models or by in vitro experiments and may not completely apply to in vivo situations in humans. Moreover, it is evident that many aspects of these biological pathways need further clarification and in-depth investigation.

Currently, the emphasis in research has been artificially placed on PD and the importance of clinical signs and responses to treatment rather that understanding the role of these commensal organisms in pregnancy. Specifically, the data presented in this review suggest that probably the most important biological pathway that determines whether an APO will occur is the challenge/establishment of an infection in the foeto-placental unit by periodontal pathogens/byproducts. So, the real question that would help our understanding of the possible association of PD with APOs is what are the conditions and parameters that allow periodontal bacteria to gain systemic access and expose the foeto-placental unit. Definitely, bacteremia is a key player in this process. The inflamed periodontal tissues with the increased vascular permeability are the main port of entry of periodontal pathogens in the blood stream. However in reality, how many bacteria are necessary to enter the blood circulation to be capable of inducing APOs? If the number of bacteria is critical then an increase in bacteremia would increase the risk for APOs. Studies by Former and co-workers have demonstrated that in periodontitis patients, the magnitude of bacteremia was associated with gingival index, plaque index and number of sites with bleeding on probing, but not with pocket depth measurements (Forner et al. 2006). Hence, increments of PD clinical parameters, such as probing pocket depth, that determine the severity of the disease do not necessarily correspond to a linear incrementation of bacteremia. Furthermore, the ability of certain periodontal pathogens, such as the FadA adhesin from *F. nucleatum*, to actively permeabilize the endothelium and allow even other bacteria to penetrate through may also increase

the magnitude of bacteremia irrespective of the severity of PD.

But again, once in the circulation what bacteria are important and how do these correlate with the periodontal status? To date, unfortunately, only few selected oral pathogens have been the focus of investigation, while it is known that periodontal pockets may harbour more than 700 microbial species. However, some answers may derive from the periodontal pathogens mostly studied. The oral organisms, *C. rectus* and *F. nucleatum,* belong to the "Orange Complex" and the overgrowth of these organisms is associated with early gingivitis, bleeding on probing and persist in moderate and severe periodontitis. In studies of animal infection models and in observational studies in humans, the evidence supporting the pathogenic potential and possible role of *C. rectus and F. nucleatum* infection in APOs is strong (Madianos et al. 2001, Han et al. 2004, 2006, Offenbacher et al. 2005). However, these organisms are highly prevalent in the subgingival flora in both periodontal health and in disease and persist following treatment. What distinguishes *C. rectus* and *F. nucleatum* as potential foetal pathogens is that they are a threat only when placental exposure occurs – an event which is largely independent of the periodontal status. Hence, comprehending the systemic virulence potential of the oral microbiome may be a distinctly different issue than categorizing the nature of the challenge using clinical signs of periodontal disease. Thus, for the purposes of moving our understanding forward, we should view the potential linkage as one that connects the organism, which has foeto-placental virulence potential, to the foetal-placental exposure. By analogy, many vaginal commensal organisms can elicit pregnancy complications by ascension, but they do not necessarily result in clinical signs of vaginitis or chorionamniotis.

In this context, treatments which are designed to manage the clinical condition may not be disruptive of the organism-placental exposure pathway. This may explain, in part, why intervention studies have not been as effective as anticipated. It is reasonable to suggest that periodontal therapy may have occurred too late in gestation. At that point, improvement of the periodontal clinical parameters may not correlate with the decreased risk for APOs if prior to treatment bacteria have already gained access to the foeto-placental unit and have exerted a pathogenic challenge.

It is also worth noting that observational studies in humans demonstrate that not all patients with similar microbial profiles present a comparable foetal exposure to periodontal pathogens (Madianos et al. 2001). It remains clear that each microbial species may have various strains with virulence factors of different potencies (Madianos et al. 2005). In this regard, it is obvious that the ability of each one of these strains to translocate via the blood circulation, invade and colonize foetal tissues and evade the host's immune response determines its potential to contribute to APOs. A strong immune response against these specific pathogens will be protective for the mother and the foetus, while an underlying genetic predisposition due to a hyper-inflammatory trait may place pregnant women at higher risk for both PD and APOs. Hence, to reduce the chance for APOs, it may be time for us to depart from the "one-size-fits-all" therapy and design more targeted therapies based on the virulence potential of different individual's oral microbiome and immune response.

## References


Africa, C. W. (2011) Oral colonization of Gram-negative anaerobes as a risk factor for preterm delivery. *Virulence* **2**, 498–508.

Angelidou, A., Asadi, S., Alysandratos, K. D., Karagkouni, A., Kourembanas, S. & Theoharides, T. C. (2012) Perinatal stress, brain inflammation and risk of autism-Review and proposal. *BioMed Central Pediatrics* **12**, 89.

Arce, R. M., Barros, S. P., Wacker, B., Peters, B., Moss, K. & Offenbacher, S. (2009) Increased TLR4 expression in murine placentas after oral infection with periodontal pathogens. *Placenta* **30**, 156–162.

Arce, R. M., Diaz, P. I., Barros, S. P., Galloway, P., Bobetsis, Y., Threadgill, D. & Offenbacher, S. (2010) Characterization of the invasive and inflammatory traits of oral Campylobacter rectus in a murine model of fetoplacental growth restriction and in trophoblast cultures. *Journal of Reproductive Immunology* **84**, 145–153.

Barak, S., Oettinger-Barak, O., Machtei, E. E., Sprecher, H. & Ohel, G. (2007) Evidence of periopathogenic microorganisms in placentas of women with preeclampsia. *Journal of Periodontology* **78**, 670–676.

Barde, L. H., Yeatman, J. D., Lee, E. S., Glover, G. & Feldman, H. M. (2012) Differences in neural activation between preterm and full term born adolescents on a sentence comprehension task: implications for educational accommodations. *Developmental and Cognitive Neuroscience* **15**, S114–128.

Bearfield, C., Davenport, E. S., Sivapathasundaram, V. & Allaker, R. P. (2002) Possible association between amniotic fluid micro-organism infection and microflora in the mouth. *British Journal of Gynaecology* **109**, 527–533.

Bélanger, M., Reyes, L., von Deneen, K., Reinhard, M.K., Progulske-Fox, A. & Brown, M.B. (2008) Colonization of maternal and fetal tissues by Porphyromonas gingivalis is strain-dependent in a rodent animal model. *American Journal of Obstetrics and Gynecology* **199**, 86. e1–7.

Bobetsis, Y. A., Barros, S. P. & Offenbacher, S. (2006) Exploring the relationship between periodontal disease and pregnancy complications. *Journal of the American Dental Association* **137** (Suppl.), 7S–13S.

Bobetsis, Y. A., Barros, S. P., Lin, D. M., Arce, R. M. & Offenbacher, S. (2010) Altered gene expression in murine placentas in an infection-induced intrauterine growth restriction model: a microarray analysis. *Journal of Reproductive Immunology* **85**, 140–148.

Bobetsis, Y. A., Barros, S. P., Lin, D. M., Weidman, J. R., Dolinoy, D. C., Jirtle, R. L., Boggess, K. A., Beck, J. D. & Offenbacher, S. (2007) Bacterial infection promotes DNA hypermethylation. *Journal of Dental Research* **86**, 169–174.

Boggess, K. A., Moss, K., Madianos, P., Murtha, A. P., Beck, J. & Offenbacher, S. (2005) Fetal immune response to oral pathogens and risk of preterm birth. *American Journal of Obstetrics and Gynecology* **193**, 1121–1126.

Boggess, K. A., Moss, K., Murtha, A., Offenbacher, S. & Beck, J. D. (2006) Antepartum vaginal bleeding, fetal exposure to oral pathogens, and risk for preterm birth at <35 weeks of gestation. *American Journal of Obstetrics and Gynecology* **194**, 954–960.

Cahill, R. J., Tan, S., Dougan, G., O'Gaora, P., Pickard, D., Kennea, N., Suilivan, M. H., Feldman, R. G. & Edwards, A. D. (2005) Universal DNA primers amplify bacterial DNA from human fetal membranes and link *Fusobacterium nucleatum* with prolonged preterm membrane rupture. *Molecular human reproduction* **11**, 761–766.

Carta, G., Persia, G., Falciglia, K. & Iovenitti, P. (2004) Periodontal disease and poor obstetrical outcome. *Clinical and Experimental Obstetrics and Gynecology* **31**, 47–49.

Collins, J. G., Smith, M. A., Arnold, R. R. & Offenbacher, S. (1994a) Effects of Escherichia coli and Porphyromonas gingivalis lipopolysaccharide on pregnancy outcome in the golden hamster. *Infection and Immunity* **62**, 4652–4655.

Collins, J. G., Windley, H. W. III, Arnold, R. R. & Offenbacher, S. (1994b) Effects of a Porphyromonas gingivalis infection on inflammatory mediator response and pregnancy outcome in hamsters. *Infection and Immunity* **62**, 4356–4361.

Contreras, A., Herrera, J. A., Soto, J. E., Arce, R. M., Jamarillo, A. & Botero, J. E. (2006) Periodontitis is associated with preeclampsia in pregnant women. *Journal of Periodontology* **77**, 182–188.

Dasanayake, A. P., Chhun, N., Tanner, A. C., Craig, R. G., Lee, M. J., Moore, A. F. & Norman, R. G. (2008) Periodontal pathogens and gestational diabetes mellitus. *Journal of Dental Research* **87**, 328–333.

Dörtbudak, O., Eberhardt, R., Ulm, M. & Persson, G. R. (2005) Periodontitis, a marker of risk in pregnancy for preterm birth. *Journal of Clinical Periodontology* **32**, 45–52.

Ebersole, J. L., Novak, M. J., Michalowicz, B. S., Hodges, J. S., Steffen, M. J., Ferguson, J. E., Diangelis, A., Buchanan, W., Mitchell, D. A. & Papapanou, P. N. (2009) Systemic immune responses in pregnancy and periodontitis: relationship to pregnancy outcomes in the Obstetrics and Periodontal Therapy (OPT) study. *Journal of Periodontology* **80**, 953–960.

Fardini, Y., Chung, P., Dumm, R., Joshi, N. & Han, Y. W. (2010) Transmission of diverse oral bacteria to murine placenta: evidence for the oral microbiome as a potential source of intrauterine infection. *Infection and Immunity* **78**, 1789–1796.

Fiorini, T., Vianna, P., Weidlich, P., Musskopf, M. L., Moreira, C. H., Chies, J. A., Rösing, C. K., Oppermann, R. V. & Susin, C. (2012) Relationship between cytokine levels in serum and gingival crevicular fluid (GCF) in pregnant women. *Cytokine* **58**, 34–39.

Forner, L., Larsen, T., Kilian, M. & Holmstrup, P. (2006) Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. *Journal of Clinical Periodontology* **33**, 401–407.

Ghezzi, F., Franchi, M., Raio, L., Di Naro, E., Bossi, G., D'Eril, G. V. & Bolis, P. (2002) Elevated amniotic fluid C-reactive protein at the time of genetic amniocentesis is a marker for preterm delivery. *American Journal of Obstetrics and Gynecology* **186**, 268–273.

Goepfert, A. R., Jeffcoat, M. K., Andrews, W. W., Faye-Petersen, O., Cliver, S. P., Goldenberg, R. L. & Hauth, J. C. (2004) Periodontal disease and upper genital tract inflammation in early spontaneous preterm birth. *Obstetrics and Gynecology* **104**, 777–783.

Goldenberg, R. L., Hauth, J. C. & Andrews, W. W. (2000) Intrauterine infection and preterm delivery. *New England Journal of Medicine* **342**, 1500–1507.

Goldenberg, R. L., Culhane, J. F. & Johnson, D. C. (2005) Maternal infection and adverse fetal and neonatal outcomes. *Clinics in Perinatology* **32**, 523–559.

Goldenberg, R. L., Culhane, J. F., Iams, J. D. & Romero, R. (2008) Epidemiology and causes of preterm birth. *Lancet* **371**, 75–84.

Gonzales-Marin, C., Spratt, D. A., Millar, M. R., Simmonds, M., Kempley, S. T. & Allaker, R. P. (2011) Levels of periodontal pathogens in neonatal gastric aspirates and possible maternal sites of origin. *Molecular Oral Microbiology* **26**, 277–290.

Greig, P. C., Murtha, A. P., Jimmerson, C. J., Herbert, W. N., Roitman-Johnson, B. & Allen, J. R. (1997) Maternal serum interleukin-6 during pregnancy and during term and preterm labor. *Obstetrics and Gynecololy* **90**, 465–469.

Gücer, F., Balkanli-Kaplan, P., Yüksel, M., Yüce, M. A., Türe, M. & Yardim, T. (2001) Maternal serum tumor necrosis factor-alpha in patients with preterm labor. *Journal of Reproductive Medicine* **46**, 232–236.

Guerrant, R. L., Lahita, R. G., Winn, W. C. Jr & Roberts, R. B. (1978) Campylobacteriosis in man: pathogenic mechanisms and review of 91 bloodstream infections. *American Journal of Medicine* **65**, 584–592.

Guntheroth, W. G. (1984) How important are dental procedures as a cause of infective endocarditis? *American Journal of Cardiology* **54**, 797–801.

Gürsoy, M., Könönen, E., Gürsoy, U. K., Tervahartiala, T., Pajukanta, R. & Sorsa, T. (2010) Periodontal status and neutrophilic enzyme levels in gingival crevicular fluid during pregnancy and postpartum. *Journal of Periodontology* **81**, 1790–1796.

Han, Y. W., Redline, R. W., Li, M., Yin, L., Hill, G. B. & McCormick, T. S. (2004) Fusobacterium nucleatum induces premature and term stillbirths in pregnant mice: implication of oral bacteria in preterm birth. *Infection and Immunity* **72**, 2272–2279.

Han, Y. W., Ikegami, A., Bissada, N. F., Herbst, M., Redline, R. W. & Ashmead, G. G. (2006) Transmission of an uncultivated Bergeyella strain from the oral cavity to amniotic fluid in a case of preterm birth. *Journal of Clinical Microbiology* **44**, 1475–1483.

Han, Y. W., Shen, T., Chung, P., Buhimschi, I. A. & Buhimschi, C. S. (2009) Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth. *Journal of Clinical Microbiology* **47**, 38–47.

Han, Y. W., Fardini, Y., Chen, C., Iacampo, K. G., Peraino, V. A., Shamonki, J. M. & Redline, R. W. (2010) Term stillbirth caused by oral Fusobacterium nucleatum. *Obstetrics and Gynecology* **115**, 442–445.

Han, Y. W. (2011) Oral health and APOS - what's next? *Journal of Dental Research* **90**, 289–293.

Haram, K., Mortensen, J. H. & Wollen, A. L. (2003) Preterm delivery: an overview. *Acta Obstetricia et Gynecologica Scandinavica* **82**, 687–704.

Herrera, J. A., Chaudhuri, G. & López-Jaramillo, P. (2001) Is infection a major risk factor for preeclampsia? *Medical Hypotheses* **57**, 393–397.

Herrera, J. A., Parra, B., Herrera, E., Botero, J. E., Arce, R. M., Contreras, A. & López-Jaramillo, P. (2007) Periodontal disease severity is related to high levels of C-reactive protein in pre-eclampsia. *Journal of Hypertension* **25**, 1459–1464.

Hillier, S. L., Nugent, D. A., Eschenbach, D. A., Krohn, M. A., Gibbs, R. S., Martin, D. H., et al. (1995) Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. *New England Journal of Medicine* **333**, 1737–1742.

Hitti, J., Tarczy-Hornoch, P., Murphy, J., Hillier, S. L., Aura, J., Eschenbach, D. & A., (2001) Amniotic fluid infection, cytokines, and adverse outcome among infants at 34 weeks' gestation or less. *Obstetrics and Gynecology* **98**, 1080–1088.

Horton, A. L., Boggess, K. A., Moss, K. L., Jared, H. L., Beck, J. & Offenbacher, S. (2008) Periodontal disease early in pregnancy is associated with maternal systemic inflammation among African American women. *Journal of Periodontology* **79**, 1127–1132.

Ikegami, A., Chung, P. & Han, Y. W. (2009) Complementation of the fadA mutation in Fusobacterium nucleatum demonstrates that the surface-exposed adhesin promotes cellular invasion and placental colonization. *Infection and Immunity* **77**, 3075–3079.

Inglis, S. R. (1997) Biochemical markers predictive of preterm delivery. *Infectious Diseases in Obstetrics and Gynecology* **5**, 158–164.

Jared, H., Boggess, K. A., Moss, K., Bose, C., Auten, R., Beck, J. & Offenbacher, S. (2009) Fetal exposure to oral pathogens and subsequent risk for neonatal intensive care admission. *Journal of Periodontology* **80**, 878–883.

Jarjoura, K., Devine, P. C., Perez-Delboy, A., Herrera-Abreu, M., D'Alton, M. & Papapanou, P. N. (2005) Markers of periodontal infection and preterm birth. *American Journal of Obstetrics and Gynecology* **192**, 513–519.

Katz, J., Chegini, N., Shiverick, K. T. & Lamont, R. J. (2009) Localization of P. gingivalis in preterm delivery placenta. *Journal of Dental Research* **88**, 575–578.

Keelan, J.A., Wong, P.M., Bird, P.S. & Mitchell, M.D. (2010) Innate inflammatory responses of human decidual cells to periodontopathic bacteria. *American Journal of Obstetrics and Gynecology* **202**, 471. e1–11.

Kozarov, E. V., Dorn, B. R., Shelburne, C. E., Dunn, W. A. Jr & Progulske-Fox, A. (2005) Human atherosclerotic plaque contains viable invasive *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *Arteriosclerosis, thrombosis, and vascular biology* **25**, e17–18.

Lafaurie, G. I., Mayorga-Fayad, I., Torres, M. F., Castillo, D. M., Aya, M. R., Barón, A. & Hurtado, P. A. (2007) *Journal of Clinical Periodontology* **34**, 873–879.

Lamont, R. F. & Sawant, S. R. (2005) Infection in the prediction and antibiotics in the predicrion of spontaneous preterm labour and preterm birth. *Minerva Ginecologica* **57**, 423–433.

Lamont, R.F., Nhan-Chang, C.L., Sobel, J.D, Workowski, K., Conde-Aqudelo, A. & Romero, R. (2007) Treatment of abnormal vaginal flora in early pregnancy with clindamycin for the prevention of spontaneous preterm birth: a systematic review and metaanalysis. *American Journal of Obstetrics and Gynecology* **205**, 177–190.

León, R., Silva, N., Ovalle, A., Chaparro, A., Ahumada, A., Gajardo, M., Martinez, M. & Gamonal, J. (2007) Detection of Porphyromonas gingivalis in the amniotic fluid in pregnant women with a diagnosis of threatened premature labor. *Journal of Periodontology* **78**, 1249–1255.

Lin, D., Smith, M. A., Champagne, C., Elter, J., Beck, J. & Offenbacher, S. (2003a) Porphyromonas gingivalis infection during pregnancy increases maternal tumor necrosis factor alpha, suppresses maternal interleukin-10, and enhances fetal growth restriction and resorption in mice. *Infection and Immunity* **71**, 5156–5162.

Lin, D., Smith, M. A., Elter, J., Champagne, C., Downey, C. L., Beck, J. & Offenbacher, S. (2003b) Porphyromonas gingivalis infection in pregnant mice is associated with placental dissemination, an increase in the placental Th1/Th2 cytokine ratio, and fetal growth restriction. *Infection and Immunity* **71**, 5163–5168.

Lin, D., Moss, K., Beck, J. D., Hefti, A. & Offenbacher, S. (2007) Persistently high levels of periodontal pathogens associated with preterm pregnancy outcome. *Journal of Periodontology* **78**, 833–841.

Liu, H., Redline, R. W. & Han, Y. W. (2007) Fusobacterium nucleatum induces fetal death in mice via stimulation of TLR4-mediated placental inflammatory response. *Journal of Immunology* **179**, 2501–2508.

Madianos, P. N., Lieff, S., Murtha, A. P., Boggess, K. A., Auten, R. L. Jr, Beck, J. D. & Offenbacher, S. (2001) Maternal periodontitis and prematurity. Part II: Maternal infection and fetal exposure. *Annals of Periodontology* **6**, 175–182.

Madianos, P. N., Bobetsis, Y. A. & Kinane, D. F. (2005) Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva. *Journal of Clinical Periodontology* **32** (Suppl.), 57–71.

McDonald, H.M., Brocklehurst, P. & Gordon, A. (2007) Antibiotics for treating bacterial vaginosis in pregnancy. *Cochrane Database Systematic Reviews* **24**, CD000262.

Mayatepek, E., Zilow, E. & Pohl, S. (1991) Severe intrauterine infection due to Capnocytophaga ochracea. *Biology of the Neonate* **60**, 184–186.
Miller, W. D. (1891) The human mouth as a focus of infection. *Dental Cosmos* **33**, 689–713.
Nigro, G., Mazzocco, M., Mattia, E., Di Renzo, G. C., Carta, G. & Anceshi, M. M. (2011) Role of infections in recurrent spontaneous abortion. *Journal of Maternal-fetal & neonatal medicine* **24**, 983–989.
Offenbacher, S., Riché, E. L., Barros, S. P., Bobetsis, Y. A., Lin, D. & Beck, J. D. (2005) Effects of maternal Campylobacter rectus infection on murine placenta, fetal and neonatal survival, and brain development. *Journal of Periodontology* **76** (Suppl.), 2133–2143.
Offenbacher, S., Lin, D., Strauss, R., McKaig, R., Irving, J., Barros, S. P., Moss, K., Barrow, D. A., Hefti, A. & Beck, J. D. (2006) Effects of periodontal therapy during pregnancy on periodontal status, biologic parameters, and pregnancy outcomes: a pilot study. *Journal of Periodontology* **77**, 2011–2024.
O'Sullivan, A. M., Doré, C. J. & Coid, C. R. (1988) Campylobacters and impaired fetal development in mice. *Journal of Medical Microbiology* **25**, 7–12.
Paraskevas, S., Huizinga, J. D. & Loos, B. G. (2008) A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *Journal of Clinical Periodontology* **35**, 277–290.
Pitiphat, W., Gillman, M. W., Joshipura, K. J., Williams, P. L., Douglass, C. W. & Rich-Edwards, J. W. (2005) Plasma C-reactive protein in early pregnancy and preterm delivery. *American Journal of Epidemiology* **162**, 1108–1113.
Polyzos, N. P., Polyzos, I. P., Zavos, A., Valachis, A., Mauri, D., Papanikolaou, E. G., Tzioras, S., Weber, D. & Messinis, I. E. (2010) Obstetric outcomes after treatment of periodontal disease during pregnancy: systematic review and meta-analysis. *British Medical Journal* **341**, c7017.
Romero, R. & Mazor, M. (1988a) Infection and preterm labor. *Clinical Obstetrics and Gynecology* **31**, 553–584.
Romero, R., Wu, Y. K., Mazor, M., Hobbins, J. C. & Mitchell, M. D. (1988b) Amniotic fluid prostaglandin E2 in preterm labor. *Prostaglandins Leukotrienes and Essential Fatty Acids* **34**, 141–145.
Romero, R., Oyarzun, E., Mazor, M., Sirtori, M., Hobbins, J. C. & Bracken, M. (1989) Meta-analysis of the relationship between asymptomatic bacteriuria and preterm delivery/low birth weight. *Obstetrics and Gynecology* **73**, 576–582.
Romero, R., Gomez, R., Ghezzi, F., Yoon, B. H., Mazor, M., Edwin, S. S. & Berry, S. M. (1998) A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. *American Journal of Obstetrics and Gynecology* **179**, 186–193.
Romero, R., Gómez, R., ChaiworAPOsngsa, T., Conoscenti, G., Kim, J. C. & Kim, Y. M. (2001) The role of infection in preterm labour and delivery. *Paediatric and Perinatal Epidemiology* **15**, 41–56.
Romero, R., Schaudinn, C., Kusanovic, J.P., Gorur, A., Gotsch, F., Webster, P., Nhan-Chang, C.L., Erez, O., Kim, C.J., Espinoza, J., Gonçalves, L.F., Vaisbuch, E., Mazaki-Tovi, S., Hassan, S.S. & Costerton, J.W. (2008) Detection of a microbial biofilm in intraamniotic infection. *American Journal of Obstetrics and Gynecology* **198**, 135.e1-5.
Ruma, M., Boggess, K., Moss, K., Jared, H., Murtha, A., Beck, J. & Offenbacher, S. (2008) Maternal periodontal disease, systemic inflammation, and risk for preeclampsia. *American Journal of Obstetrics and Gynecology* **198**, 389.e1-5.
Sert, T., Kırzıoğlu, F. Y., Fentoğlu, O., Aylak, F. & Mungan, T. (2011) Serum placental growth factor, vascular endothelial growth factor, soluble vascular endothelial growth factor receptor-1 and -2 levels in periodontal disease, and APOS. *Journal of Periodontology* **82**, 1735–1748.
Sharma, A., Ramesh, A. & Thomas, B. (2009) Evaluation of plasma C-reactive protein levels in pregnant women with and without periodontal disease: a comparative study. *Journal of Indian Society of Periodontology* **13**, 145–149.
Simor, A. E., Karmali, M. A., Jadavji, T. & Roscoe, M. (1986) Abortion and perinatal sepsis associated with campylobacter infection. *Reviews of Infectious Diseases* **8**, 397–402.
Skuldbøl, T., Johansen, K. H., Dahlén, G., Stoltze, K. & Holmstrup, P. (2006) Is pre-term labour associated with periodontitis in a Danish maternity ward? *Journal of Clinical Periodontology* **33**, 177–183.
Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C. & Kent, R. L. Jr (1998) Microbial complexes in subgingival plaque. *Journal of Clinical Periodontology* **25**, 134–144.
Swati, P., Thomas, B., Vahab, S. A., Kapaettu, S. & Kushtagi, P. (2012) Simultaneous detection of periodontal pathogens in subgingival plaque and placenta of women with hypertension in pregnancy. *Archives of Gynecology and Obstetrics* **285**, 613–619.
Tarannum, F., Faizuddin, M. & Madaiah, H. (2011) Gingival crevicular fluid prostaglandin E2 level as a predictor of preterm low birth weight: a pilot investigation. *Journal of Oral Science* **53**, 293–300.
Tateishi, F., Hasegawa-Nakamura, K., Nakamura, T., Oogai, Y., Komatsuzawa, H., Kawamata, K., Douchi, T., Hatae, M. & Noguchi, K. (2012) Detection of Fusobacterium nucleatum in chorionic tissues of high-risk pregnant women. *Journal of Clinical Periodontology* **39**, 417–424.
Témoin, S., Chakaki, A., Askari, A., El-Halaby, A., Fitzgerald, S., Marcus, R. E., Han, Y. W. & Bissada, N. F. (2012) Identification of oral bacterial DNA in synovial fluid of patients with arthritis with native and failed prosthetic joints. *Journal of Clinical Rheumatology* **18**, 117–121.
Teran, E., Escudero, C., Moya, W., Flores, M., Vallance, P. & Lopez-Jaramillo, P. (2001) Elevated C-reactive protein and pro-inflammatory cytokines in Andean women with pre-eclampsia. *International Journal of Gynaecology and Obstetrics* **75**, 243–249.
Tjoa, M. L., van Vugt, J. M., Go, A. T., Blankenstein, M. A., Oudejans, C. B. & van Wijk, I. J. (2003) Elevated C-reactive protein levels during first trimester of pregnancy are indicative of preeclampsia and intrauterine growth restriction. *Journal of Reproductive Immunology* **59**, 29–37.
Turhan, N. O., Karabulut, A. & Adam, B. (2000) Maternal serum interleukin 6 levels in preterm labor: prediction of admission-to-delivery interval. *Journal of Perinatal Medicine* **28**, 133–139.
von Minckwitz, G., Grischke, E. M., Schwab, S., Hettinger, S., Loibl, S., Aulmann, M. & Kaufmann, M. (2000) Predictive value of serum interleukin-6 and -8 levels in preterm labor or rupture of the membranes. *Acta Obstetricia et Gynecologica Scandinavica* **79**, 667–672.
Ximénez-Fyvie, L. A., Haffajee, A. D. & Socransky, S. S. (2000) Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. *Journal of Clinical Periodontology* **27**, 648–657.
Xiong, X., Buekens, P., Vastardis, S. & Yu, S. M. (2007) Periodontal disease and pregnancy outcomes: state-of-the-science. *Obstetrical and Gynecological Survey* **62**, 605–615.
Yeo, A., Smith, M. A., Lin, D., Riché, E. L., Moore, A., Elter, J. & Offenbacher, S. (2005) Campylobacter rectus mediates growth restriction in pregnant mice. *Journal of Periodontology* **76**, 551–557.

Address:
*Phoebus N. Madianos*
*School of Dentistry, University of Athens*
*Thivon 2, 11527 Athens, Greece*
E-mail: pmadian@dent.uoa.gr

**Clinical Relevance**

*Scientific rationale for the study*: PD has been postulated to be a risk factor for APOs. The aim was to review data from human, experimental and in vitro studies and identify potential pathogenic mechanisms that underlie this association.
*Principal findings*: During pregnancy, there is an increase in vascular permeability that facilitates periodontal pathogens/byproducts to enter the circulation and reach the placenta. The presence of bacteria/byproducts in the foeto-placental compartment stimulates/enhances a local immune/inflammatory response that could contribute to APOs.
*Practical implications*: There is evidence to support the biological plausibility of the association between PD and APOs. Many aspects of potential biological pathways need further clarification and in-depth investigation. It seems that understanding the systemic virulence potential of the individual's oral microbiome and immune response may be a distinctly different issue from categorizing the nature of the challenge using clinical signs of periodontal disease. Therefore, maybe, a more personalized targeted therapy could be a more predictive answer to the current "one-size-fits-all" interventions.