Journal Pre-proof

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PII: S1043-6618(19)32884-1

DOI: https://doi.org/10.1016/j.phrs.2020.104692

Reference: YPHRS 104692

To appear in: *Pharmacological Research*

Received Date: 13 December 2019

Revised Date: 23 January 2020

Accepted Date: 10 February 2020

Please cite this article as: Ahmadian E, Rahbar Saadat Y, Mahdi Hosseiniyan Khatibi S, Ziba N-Saleh-Fam, Milad B, Fatemeh Zununi V, Mohammadreza A, Sepideh Zununi V, Pre-Eclampsia: microbiota possibly playing a role, *Pharmacological Research* (2020), doi: https://doi.org/10.1016/j.phrs.2020.104692

Pre-Eclampsia: microbiota possibly playing a role

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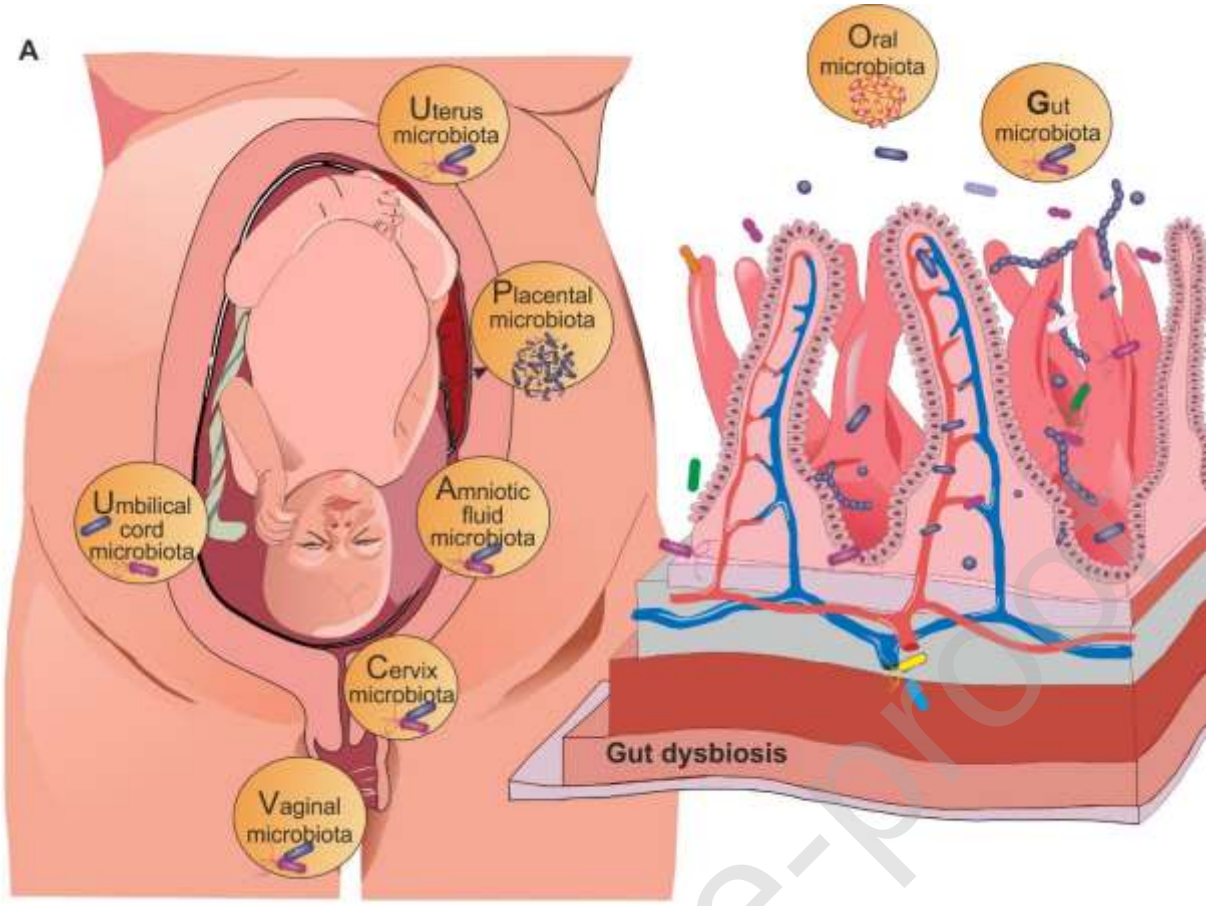
Graphical abstract



Abstract

Pre-eclampsia (PE) is a complication of pregnancy that is associated with mortality and morbidity in mothers and fetuses worldwide. Oxygen dysregulation in the placenta, abnormal remodeling of the spiral artery, defective placentation, oxidative stress at the fetal-maternal border, inflammation and angiogenic impairment in the maternal circulation are the main causes of this syndrome. These events result in a systemic and diffuse endothelial cell dysfunction, an essential pathophysiological feature of PE. The impact of bacteria on the multifactorial pathway of PE is the recent focus of scientific inquiry since microbes may cause each of the aforementioned features. Microbes and their derivatives by producing antigens and other inflammatory factors may trigger infection and inflammatory responses. A mother's bacterial communities in the oral cavity, gut, vagina, cervix and uterine along with the placenta and amniotic fluid microbiota may be involved in the development of PE. Here,

we review the mechanistic and pathogenic role of bacteria in the development of PE. Then, we highlight the impact of alterations in a set of maternal microbiota (dysbiosis) on the pathogenesis of PE.

Keyword: Preeclampsia, Microbiota, Dysbiotic microbiota, Bacteria, Placental dysfunction.

Introduction

Pre-eclampsia (PE), a specific pregnancy complication, is described by new-onset hypertension and proteinuria that can progress to organs dysfunction, after 20 weeks of gestation [1]. PE remains the main cause of morbidity and mortality in mothers and fetuses [2, 3]. The etiology of PE is multifactorial and comprises maternal (a family history of PE, previous PE, multiple pregnancies, chronic kidney disease, obesity, genetic susceptibility, higher age, hypertension and pre-pregnancy endothelial damage) and fetal (uteroplacental insufficiency and increased-placental volume/mass) related risk factors [1, 3, 4]. Generally, oxygen dysregulation in the placenta and angiogenic impairment are the main biochemical causes that are associated with PE [5, 6]. Beyond fetal/maternal factors and endothelial dysfunction, the activation of inflammation in different maternal organs plays an essential role in the attainment of PE, Figure 1. The immune system plays a fundamental role in maintaining a balance between fetal allograft and mother in pregnancy [7]. Immune imbalance and chronic immune activation stimulate an inflammatory state in PE. It is evident that a common source of endothelial dysfunction and inflammation is the bacterial infection; acute maternal infection in periodontal disease and urinary tract infection (UTI) increases the risk of PE [8, 9].

For decades, the presence of microbes has been associated with inflammation and unfavorable outcomes during pregnancy [10]. This statement was under the assumption that the placenta and uterus were sterile and the fetus develops in a sterile womb [11]. However, based on molecular approaches, recent evidence suggests that there is a distinctive set of

microbiota in the uterine cavity, vagina and placenta of healthy pregnant women that challenges this old "sterile womb paradigm" [11-13]. The human body hosts more than 100 trillion prokaryotic micro-organisms that establish our "new organ" (the so-called microbiota). Emerging evidence suggests that the human body has coevolved with a set of microbiota that can change the mother's metabolism to support the fetus's growth [14]. Any alteration in the structure and composition of maternal microbiota (dysbiosis) can be involved in the pathogenesis of PE [15, 16]. On the other hand, chronic intake of milk-based probiotics seems to be beneficial in decreasing PE incidence since they can alter the inflammation-basis of severe PE [17]. Therefore, exploring the impact of bacteria on the multifactorial pathway of PE is the recent focus of scientific inquiry.

It is suggested that 'gut–placenta' axis can have an essential role in the aetiology of PE and gut dysbiosis can provoke pre-eclamptic systems and impact the host's blood pressure in women with PE [16]. The underlying mechanism is that translocation of bacteria or their components into the intrauterine environment can prompt inflammatory and abnormal immune responses in the placenta and maternal circulation [16, 18].

Although the underlying mechanism leading to the microbiota modification is not understood, alterations in the mucosal surfaces of the immune system may change the microbiota [19]. The particular micro-organism(s) that may initiate PE can be controversial. A single bacterium and infectious event may be strong enough to promote the inflammation and lead to PE. On the other hand, the burden of multiple micro-organisms infection may trigger PE. These events may lead to irregular function of trophoblast; causing endothelial dysfunction and nutrients, and oxygen impairment to the placenta that finally increases the maternal blood pressure and PE development [20, 21].

Since the underlying mechanisms of microbiota in PE remain to be unclarified, understanding how alteration in a set of mother's microbiota impact PE can be helpful to develop rational

therapy. The present review aims to highlight the possible effects of a set of maternal microbiota on the development of PE.

Microbiota and hypertension

Hypertension that arises in PE seems to be mediated through agonistic angiotensin II type 1 receptor autoantibody (AT1-AA) and antiangiogenic factors rather than renin-angiotensin-aldosterone system (RAAS) [22]. In rodent models of PE, AT1-AA induces the production of reactive oxygen species (ROS), inflammatory factors and hypertensive mechanisms including endothelin (ET) and soluble fms-like tyrosine kinase 1 (sFLT1) [23]. Soluble endoglin (sENG) and sFLT1 are antiangiogenic factors releasing from the ischemic placenta. sFLT1, also known as a soluble vascular endothelial growth factor (sVEGFR1), binds to circulating and local VEGF and placental growth factor (PlGF) and inhibits their cellular signaling that is essential for angiogenic signaling and vascular homeostasis [1]. The inhibition of PlGF and VEGF signaling results in the release of procoagulant proteins and decreases the production of nitric oxide (NO) and prostacyclin, and finally, leads to endothelial cell dysfunction. Decreased levels of circulating hydrogen sulfide (H_2S) and NO along with an increased level of RT-1 [24-26], as a vasoconstrictor in women with PE, have been shown to mediate hypertension. Despite the aforementioned differences, endothelial dysfunction is a common pathway shared by essential hypertension and PE. Existing knowledge also supports a role for the microbiota in the generation of essential hypertension. The chronic low-grade inflammatory conditions can be a cause or consequence of the development of hypertension [27], further, it may occur as a result of a reduction in microbial gene richness [28].

The gut microbiota impacts host blood pressure via different mechanisms [29] [30]. It regulates the host's genes related to proliferation, immune response and metabolism that may ultimately impact blood pressure [31]. Gut microbiota can also impact the inflammatory response of the host and alter the function of endothelial that influences the host blood

pressure. The aromatic-L-amino acid decarboxylases of the gut bacteria (i.e. *Lactobacillus plantarum, L. bulgaricus* and *Enterococcus faecalis*) convert the amino acid tyrosine into the tyramine. Tyramine may accumulate in the body and result in excessive release of norepinephrine (NE) and epinephrine (E) from the sympathetic nerve endings and the adrenal medulla, respectively. High amounts of the E and NE (catecholamine family members), consequently, result in the cardiac output increase, peripheral vasoconstriction and hypertension. This condition may be a probable reason for hypertension during pregnancy. The function of gut microbiota is also involved in hypertension by breaking the dietary fiber and producing short-chain fatty acids (SCFAs) that affect vascular tone [32]. SCFA-producing microbes may impact host blood pressure via plasminogen activator inhibitor-1 (PAI-1) or SCFA' direct effects on vasodilation [29]. SCFAs (propionate, acetate and butyrate) are absorbed in the systemic circulation and sent to target tissues to modify cellular responses and regulate blood pressure. These actions are mediated through G protein-coupled receptors (GPCR) including Olfr78, Gpr109a, Gpr43 and Gpr41 [33]. By binding to Olfr78, SCFAs increase renin secretion while through Gpr43 and Gpr109a, they induce vasodilation. Moreover, SCFAs can elevate the activity of the sympathetic nervous system via binding to GPR41 [34, 35]. As mentioned, tyramine results in catecholamine synthesis which in turn, leads to imbalances in gut microbiota via stimulating the growth of some strains. Subsequently, an imbalance results in the growth of butyrate-producing bacteria and excess amounts of butyrate. Lower SCFAs production may contribute to hypertension, and accordingly, increase the PE risk in pregnant women [36]. Furthermore, gut microbiota can impact kidney biology to expel sodium load and control blood pressure [33].

Recent studies indicate that gut dysbiosis mainly through dysregulation of PAI-1, butyrate and SCFA can lead to blood pressure elevation and PE development [16] [29]. In obese and overweight pregnant females, the abundance of bacteria belonging to *Clostridiales*

(Christensellaceae, Clostridiidiaceae and *Blautia* families) and *Bacteroidales (Bacteroides* and *Rikenellaceae*) orders were correlated with an increased blood pressure negatively [29]. In these women, the richness of gut butyrate-producing bacteria and levels of the butyrate-producing *Buk* enzyme (butyrate kinase) were negatively connected with inflammatory marker PAI-1 and diastolic and systolic blood pressure [29]. It is also reported that an inverse correlation has existed between body weight, fat mass, blood pressure and *Bacteroides* [37]. *Odoribacter* abundance induces Buk expression and butyrate production that increase the PAI-1 and consequently, elevates blood pressure in obese pregnant women. It may be possible to maintain a normal blood pressure by increasing the butyrate-producing capability of gut microbiota in obese pregnant women [29]. Hence, to prevent the incidence of hypertensive diseases during pregnancy, the characterization of the gut microbiota before the pregnancy is of great importance.

Probiotics are recommended to potentially modify all aspects of PE including blood pressure, systemic and placental trophoblast inflammation [17, 38]. Consistent intake of probiotic (*L. acidophilus, B. lactis* and *L. rhamnosus* GG*)* food may decrease the risk of PE in primiparous women [17]. It is reported that the *L. rhamnosus* GR-1supernatant could increase IL-10 and alter the inflammatory response of lipopolysaccharide (LPS) in placental trophoblast cells [39]. It is significant to note that the hypertensive effect of gut dysbiosis may not depend on pregnancy since mice receiving fecal material from PE patients developed hypertension before their pregnancy [16]. The microbiota dysbiosis patterns of non-pregnant hypertensive women were similar to women with PE; microbiota profile was enriched in *Fusobacterium* and *Veillonella* and depleted from *Akkermansia* and *Faecalibacterium* [16].

Bacterial infection and PE

Numerous lines of evidence support the involvement of bacterial infection in PE. Women with asymptomatic bacteriuria, periodontal disease and UTI are likely to develop PE than

normal pregnant women. A meta-analysis of epidemiologic studies indicated that any viral or bacterial infection is connected with a higher risk of PE (two-fold) [40]. Another meta-analysis specified that periodontal disease and UTI during pregnancy elevates the risk of PE, with the odds ratios of 1.76 and 1.57, respectively [9]. This association may offer an explanation for PE-related inflammation.

Various studies detected an interplay between the presence of bacterial communities and PE. For instance, the presence of *Prevotella intermedia, Treponema denticola, Porphyromonas gingivalis, F. nucleatum, Actinobacillus actinomycetemcomitans* and *Tannerella forsythensis* was detected in placentas from PE women [41]. A different study detected *Ureaplasma, Lactobacillus iners, Leptotrichia, Sneathia* and *Streptococcus* in amniotic fluid (AF) of cases with PE [42]. Further, analysis of the pre-eclamptic placentas demonstrated the presence of respiratory tract infections (*K. pneumoniae* and *Anoxybacillus*), gastrointestinal tract infections (*Salmonella, Bacillus, Listeria* and *Escherichia*) and periodontal infections (*Prevotella, Dialister, Variovorax* and *Porphyromonas*) [21]. All these reports confirm the involvement of bacterial infection in the development of PE.

Bacteria and their mechanistic explanation in PE

Given the alteration of the bacteria and its correlation with PE, the link may be attributable to the presence of several infectious factors rather than the existence of a specific pathogen. A logical proposal for the relationship between the bacterial community and PE is the activation of the antiangiogenic and inflammatory pathways that impairs endothelial and trophoblast functions and increases the blood pressure.

The inflammatory effect of LPS is a well-known phenomenon that has been reported in previous knowledge. The critical role of LPS in natural or *in vivo* induction of PE has also been proposed. The induction of PE-like conditions through LPS in rodents caused a deficient spiral artery remodeling of the placenta and local and systemic inflammatory responses [43].

In spite of the PE-induced models, the accuracy of these reports is challenging since only humans are afflicted by PE [43-45]. Recently, in plasma samples of PE patients, the elevated levels of LPS and trimethylamine-N-oxide (TMAO) were reported that highlight the impact of gut microbiota-derived LPS in PE. In this study, a functional analysis indicated that the microbial gene functions were mostly associated with LPS biosynthesis pathways in the fecal microbiota of women with PE [46].

The activation of inflammation by LPS occurs, in particular, via toll-like receptor (TLR) signaling and cytokine release during PE [47-49]. Similarly, the activation of TLR-3 and TLR-7/8 via double-strand RNA (dsRNA) is a coincidence event in the progression of PE [50, 51]. Dormant bacteria that are normally invisible in conventional microbiology assessments have been revealed to produce antigens and other inflammatory factors [52-54]. The cross-reactivity of these antigens with the host epitopes can result in the generation of autoantibodies, which attack the host via termination of the placentation by trophoblasts [4]. This process is defined as molecular mimicry. The LPS in Gram-negative microorganisms induces parturition via the activation of corticotrophin-releasing hormone (CRH) [55] or mitogen-activated protein kinase (MAPK) [49]. Moreover, the antibodies against *Helicobacter pylori* (AnticagA) can interfere with trophoblasts via inhibition of placentation [56].

PE-associated microbiota

Uncovering the relationships between the dysbiotic microbiota and the incidence of PE are of great importance [15]. In the following sections, a review of the effects of bacterial communities in PE is presented (Figure 2).

Oral microbiota

The oral cavity has a large, diverse and characteristic microbiota, harboring more than 700 microbial communities. Under healthly conditions, the oral microbiota preserves a symbiotic

connection with the host; however, under oral microbiota dysbiosis, an oral disease including chronic or aggressive forms of periodontitis, gingivitis and dental caries can be developed [57].

Periodontal diseases are considered as common chronic infections in humans, which affect tooth surrounding tissues. Dental plaques that compromise mainly Gram-negative anaerobic microbes form biofilms as the major causative factors of periodontal diseases. Periodontitis and gingivitis are two important clinical manifestations of plaque-related periodontal diseases. Although the primary phase of the disease is commenced by the microorganism, the host inflammatory response determines the extent of periodontal destructions [58]. In addition, daily exposure of bacteremia or spreading of periodontal-originated bacterial endotoxins might stimulate a systematic inflammatory process [59, 60], which in turn, may induce the liberation of pro-inflammatory cytokines [61]. This event is followed by a chronic systemic enhancement of inflammatory responses including C-reactive protein (CRP) and IL-6 [62-65]. Moreover, this inflammatory cascade can activate endothelial cells; resulting in endothelial dysfunction [66-69].

Periodontal diseases are deemed to increase the risk of PE. In a recent case-control study in India, PE patients had a worse periodontal outcome in comparison with normotensive pregnant women. However, there was no significant link between the detected bacteria in the plaques and placental blood samples of PE women [70]. Sumathy et al. analyzed 200 patients to find the relation between maternal periodontitis and PE. They reported that among 92 patients having periodontitis, 67 patients were pre-eclamptic, which showed a significant association [71]. There is also an important interaction between chronic periodontal disease and the presence of *Tannerella forsythensis*, *Eikenella corrodens*, and *Porphyromonas gingivalis* in the development of PE [72]. Evidence has detected an increased number of *S. haemolyticus* in women with mild PE. Kunnen et al. investigated the possible connection

between periodontal disease and PE in a systematic review. However, it was difficult to evaluate the data due to differences in the definition of the diseases, unclear timelines and insufficient regulation of the confounding factors. According to their result, 8 observational studies exhibited a positive relationship between periodontal diseases and PE, while 4 studies demonstrated no connection. Furthermore, the reduction of PE rate was not observed after the treatment of periodontal diseases in the assessed randomized-controlled trials (RCTs). Thus, it is still challenging whether periodontal diseases act a relevant factor in PE development. The occurrence of periodontal diseases due to the PE condition or the enhanced inflammatory response in pregnancy might stand for the detection of a relationship in 8 observational investigations. Therefore, more extensive RCTs with PE as the initial output and detailed pathophysiological evaluations are needed to study the plausible causality between periodontal diseases and PE [73]. However, the association between the risk of PE and periodontal disease has not been verified in all populations [74-76].

Gut microbiota

Maternal gut microbiota experiences bacterial shift during each trimester of the gestational period [77]. The early stage of pregnancy (first trimester) exhibited an unchanged richness of the Gram-negative *Bacteroidetes* and Gram-positive *Firmicutes* phyla. Nevertheless, during the third trimester of pregnancy, the conformation of the gut microbiota shows a low diversity in bacterial populations and shifts from butyrate-producing species (i.e. *Faecalibacterium*) to *Actinobacteria* and *Proteobacteria* [15, 78] that are involved in gut dysbiosis. Moreover, poor maternal diet quality has been connected with gut microbiota alteration in both mothers and offspring [79, 80]. As it is known from the literature, gut microbiota dysbiosis is linked to hypertension that is characterized by an elevated *Firmicutes/Bacteroidetes* ratio, increased population of lactate-producing bacteria and a

reduced population of butyrate- and acetate producing bacteria [81, 82]. It has been reported that the *Firmicutes/Bacteroidetes* ratio is increased in three groups (i.e. angiotensin II-induced hypertensive rats, spontaneously hypertensive rats (SHR) and small group of humans with essential hypertension), while oral consumption of minocycline normalized the aforementioned bacterial ratio and blood pressure of SHR and angiotensin II-induced hypertensive rats [82]. Angiotensin-converting enzyme type 2 (ACE2) can regulate the gut microbiota and epithelial immunity [31].

Study of microbiota communities indicated alterations in richness, diversity and structure of gut microbiota in the feces of PE cases. A reduction in *Ruminococcus, Clostridiales* and *Clostridia* and an elevation in gamma-*Proteobacteria* and *Enterobacteriaceae* was observed in women with PE in comparison to healthy controls [46]. Liue et al. investigated the gut microbiota in Chinese women with PE and detected a decrease in probiotic bacteria *Coprococcus catus* and an increase in *Clostridium perfringens* and *Bulleidia moorei* (pathogenic bacteria) [83]. In another study, an increased number of *Blautia, Ruminococcus, Bilophila* and *Fusobacterium* as well as a decrease in *Faecalibacterium, Gemmiger, Akkermansia, Dialister* and *Methanobrevibacter* populations in the antepartum specimens of PE women in comparison to healthy controls (Figure 3). Further, they reported that liver enzyme levels and maternal blood pressure were correlated to *Anaerococcus, Ruminococcus, Oribacterium,* and *Bilophila* positively. In addition, maternal blood LPS level was associated with *Akkermansia* negatively, whereas IL-6 level was positively correlated with gut *Oribacterium* and *Bilophila* [84].

During PE, *Fusobacterium* can be an important regulator of the gut–placenta axis by affecting cellular proliferation, DNA repair and apoptosis. These events are mediated by bacterial attachment to epithelial cells, disruption of intestinal epithelia integrity, leading to bacterial translocation into placenta [16, 85]. *Fusobacterium* can also activate the immune

response through the nuclear factor-κB (NF- κB) signaling pathways. Extreme maternal inflammation through a systematic and mucosal imbalance of Treg/Th17 is participated in PE pathogenesis as a supposed consequence of gut dysbiosis. Higher inflammatory cytokines in the placenta results in oxidative stress in placenta and vascular dysfunction that reject the fetus and develops the hypertension [16, 86].

Vaginal Microbiota

The majority of microbial communities resided in the vaginal tract of healthy women have a broad impact on inhibition of microbial infections, sexually transmitted disease (STD), UTI and HIV (human immunodeficiency virus). The vaginal microbial communities also exert antimicrobial and cytotoxic activity as well. It has been obvious that the protective role of the vaginal microbiota mainly attributed to the *Lactobacillus* sp., which prohibits the growth of pathogenic microbes [87, 88]. Vaginal microbes are possibly important for the programming of neonatal immunity and they may reach the placenta, AF and fetus [89].

It is hypothesized that the high levels of estrogen during pregnancy may result in augmented vaginal glycogen deposition, which in turn, promotes *Lactobacilli* proliferation. Additional advantages of the dominance of *Lactobacillus* in the gravid vaginal microbiota include protection from ascending genital tract infections which lead to spontaneous preterm births and forming the neonatal upper gastrointestinal microbiota [90]. A study detected a significant decrease in *Lactobacillus delbrueckii* and *Lactobacillus spp*. as well as a rise in *S. haemolyticus, S. aureus,* and *ß-hemolytic streptococcus* populations in vaginal mucosa of women with PE [91].

Cervix Microbiota

The concept of cervical microbiota independent of the vaginal microbiota seems to receive research attention. Evidence has suggested that the cervical microbiota is resembled the

vaginal microbial populations (predominantly *lactobacilli* and *Gardnerella*), further, consists of viral and bacterial micro-organisms. The anatomical location of the uterine cervix makes it as an impediment between uterine sterile environments. Further dysfunction of the cervical sphincter (cervical insufficiency) may lead to preterm birth, which can be treated by surgery. Microbes induce activation of pro-inflammatory factors that recruit matrix metalloproteinases (MMPs), hyaluronidases, which in turn, promote collagenolysis in the cervical stroma, results in cervical insufficiency. Furthermore, intra-amniotic activation of pro-inflammatory factors such as IL-6 and monocyte chemotactic protein-1 (MCP-1) occur when the cervical length is shorter than normal. There is still doubt whether the cervical microbiota or transmission of microbial communities from other parts, contribute to such inflammatory responses [92].

Uterine microbiota

The concept of the "sterile womb" which was coined by Henry Tissier, was overturned currently, based on increasing evidence focused on the characterization of microbial communities colonizing uterus [93, 94]. Owing to the improvements in our knowledge in microbial characterization and sampling methods, it has been evident that detection of microbial populations in uterine environment do not attributed to the pathological conditions like infections [93]. Identification of a native uterine microbiota and its clinical effects open new horizon to evaluate its effect on reproductive function [95].

Presence of commensal at mucus layer of uterus epithelium induces the production of regulatory factors by decidual macrophages and trophoblast [96]. Accordingly, antimicrobial products of macrophages regulate the overgrowth of commensal and invasion of pathogens. Trophoblast expands T regulatory cells (Treg), increases the anti-inflammatory factors when recognizes bacterial products. Existence of any infection at implantation site, prohibits the ability of macrophage to control bacterial growth, causes dysbiosis and inflammatory condition. There are a few reports on the uterine microbiota, especially bacteria that exists in placenta, the AF and fetal membranes [97].

Placental Microbiota

Recently, placental vascular dysfunction is a pivotal area in microbiota investigations of PE [3]. Placenta harbors a distinctive microbiota composed of nonpathogenic bacteria in the *Bacteroidetes, Proteobacteria, Tenericutes, Firmicutes* and *Fusobacteria* phyla [13]. The origin of placental bacteria can be from the oral, gastrointestinal and genitourinary systems [98]. Factors, such as vaginal infection, maternal obesity, periodontitis, diet, gestational diabetes mellitus and antibiotic therapy can change the composition of placental microbiota [98]. It is also reported that excess gestational gain weight may cause dysbiosis in the placenta that is associated with a decrease in butanoate metabolism and bacterial folate biosynthesis pathways, a risk factor for preterm birth [99]. In a study, the placental tissues of PE women were compared with normotensive women. The placental microbiota of women with PE (12.7%) had a variety of pathogenic (*Escherichia, Salmonella*, and *Listeria*) and commensal bacteria [21].

It is proposed that PE may be also caused by infectious factors in the placenta and a dominant one can initiate the condition. A research studied the presence of bacteria in the placental samples of pregnant women with PE (N=16) and normal controls (N=14) obtained from the cesarean section at the time of delivery. In PE group, β-hemolytic *Streptoccocus* group B (with anal or vaginal origin), *Gamella morbillorum* and *Proprionobacterium acnes* (with skin origin) were found. Furthermore, the number of studied bacteria was higher in the PE group [41]. Moreover, an association between *Prevotella intermedia* and *Porphyromonas gingivalis* and increased levels of NF-κB and TLR-4 were reported in the placenta of PE women with periodontitis [100]. In another study, 12.7% of placental tissues from women with PE (N=55) were positive, while samples from control women (N=55) were significantly negative for the presence of bacteria. However, no evidence was found for the presence of bacteria in the placenta in a cohort of complicated (318 cases) and uncomplicated (219 controls)

pregnancies. Almost all of the infectious signals were associated with either the contamination during delivery or laboratory processes. This study concluded that human placental infection is not a common cause of PE and adverse pregnancy outcomes. Moreover, this research confirmed that placental microbiota does not have a microbiota. Nevertheless, *Streptococcus agalactiae* (group B *Streptococcus*), the main cause of neonatal sepsis, present in the placenta [101]. It is also reported that some carriers of *S. agalactiae* inversely had the risk of PE [102].

Amniotic Fluid Microbiota

Intra-amniotic infection resulted from the microbial invasion of the amniotic cavity (MIAC) is the main cause of global neonatal mortality. Some invader bacterial species including *Ureaplasma* spp., *Mycoplasma* spp., *Prevotella* spp., *Fusobacterium* spp., *Bacteroides* spp., and *Streptococcus* spp. have been associated with MIAC. Some other non-bacterial taxa including parasites, fungi (*Candida* species), viruses, *protozoa* (*Toxoplasma* and *Trypanosoma*) and Archaeal species are also associated with MIAC (reviewed in Ref. [103]). A research group identified a gene in *Mycoplasma hominis* isolates from the placenta and AF that is associated with pathogenic potential of the bacterium [104]. There is a limited set of studies that have examined the link between the MIAC and PE. DiGiulio et al. indicated that 9% of women with PE had MIAC and members of the family *Fusobacteriaceae* (*Sneathia*/*Leptotrichia* spp.) were found in 50% of these cases. Other identified micro-organisms are included *Streptococcus* spp., *Lactobacillus* spp. and *Ureaplasma urealyticum* in AF. Rarely are *Leptotrichia* and *Sneathia* identified as pathogens in pregnancy [105]. Since the occurrence of MIAC is low in PE, it can be speculated that intra-amniotic infection only has a restricted role in PE. Nevertheless, a high number of AF samples with *Leptotrichia* and/or *Sneathia* needs further investigation [42].

Conclusion

The pathophysiology of PE is complex and not fully clarified. However, dysregulation of the maternal immune system and infections increase the risk and severity of PE. Microbes may be involved in an inflammatory response and support the role of microbial interactions in the etiologic pathways of PE. The associations between the different microbiotas and the risk for PE has not been fully understood. Since gut-associated metabolites (butyrate) are correlated with PAI-1 inversely in obese and overweight pregnant women, it is rational to suggest that the microbiota can regulate the blood pressure in pregnancy.

Although a limited number of studies exist, available evidence suggests that gut, oral, placenta and vagina of women with PE harbor dysbiotic microbiota that is associated with inflammation and hypertension. Exploring the associations between the microbiota, bacteria interventions and incidence of PE in high-risk groups would be helpful in the clinic. More research is needed to explore the biological pathways of microbiota in PE pathophysiology. It is reported that probiotics can control gastrointestinal health via different mechanisms, some of which include modulation of the microbial flora, inhibition of pathogenic bacteria and inflammation that consequently affect pathophysiological processes of PE.

PE-prone dysbiosis during pregnancy is an appealing hypothesis that needs future investigation both for its pathophysiologic understanding and its preventive and therapeutic considerations.

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Figures legends

Figure 1. Physiopathology of pre-eclampsia. **(A)** Genetic, environmental and immunologic factors play a central role in the development of PE. In placental PE, poor trophoblast invasion leads to an impaired spiral artery remodeling that results in placental I/R and inflammation. Unusual placentation activates different events that cause chronic placental ischemia. Ischemia releases antiangiogenic (sFlt-1, sEng) and decreases proangiogenic (VEGF, PlGF) factors from placenta and subsequent entrance of them into maternal circulation that ends ensuing maternal endothelial dysfunction. Moreover, in ischemic placenta, the antioxidant defense is decreased, while lipid peroxides, RNS and ROS are increased, resulting in oxidative stress. Systemic oxidative stress causes inflammation, oxidation of LDL, damages in proteins, DNA and lipids, and activation of immune cells mainly neutrophil and monocytes. Angiogenic imbalance along with oxidative stress and inflammation give rise to endothelial dysfunction. **(B)** Different factors contribute to the increased oxidative stress and reduction in NO bioavailability and endothelium-dependent relaxation. Overproduction of the sFlt-1 and sEng antagonize the vasodilatory effect of VEGF and PlGF and TGF-β. Increased levels of oxLDL in circulation leads to the production of superoxide and peroxynitrite in endothelial cells. Moreover, increased activity of TxA2 elevates the ET-1 production and results in higher vascular constriction. The clinical manifestations of PE arise from multi-organ microvascular damages, including the brain, kidneys, heart, liver, pancreas and lung. I/R: ischemia/reperfusion, ET-1: endothelin, TxA2: thromboxane A2, oxLDL: oxidized low-density lipoprotein, ROS: Reactive oxygen species, RNS: reactive nitrogen species, LDL: Low-density lipoprotein, sFlt-1: soluble fms-like tyrosine kinase-1, sEng: soluble endoglin, VEGF: Vascular endothelial growth factor, PlGF: Placental growth factor, NO: nitric oxide, TGF-β: transforming growth factor beta.

Figure 2. The proposed role of microbes on the pathology of pre-eclampsia. (**A**) Macterial communities in a set of maternal microbiota oral cavity, gut, vagina, cervix, uterine, placenta and amniotic fluid may be involved in the development of PE. **(B)** Based on the available data, it is logical to speculate that four biochemical causes of PE included oxygen dysregulation in the placenta, abnormal trophoblast invasion, angiogenic impairment and inflammation may be affected by bacteria and their derivatives (LPS, SCFAs). For more information, please see the main text. oxLDL: oxidized low-density lipoprotein, LDL: Low-density lipoprotein, TLR4: Toll-like receptor 4, ET-1: endothelin, NO: nitric oxide, GPCR: G protein coupled receptors, LPS: lipopolysaccharide.

Figure 3. Comparison of the gut microbiota profiles between PE cases and healthy controls. Gut microbiota profiles were evaluated by 16S rRNA gene amplicon sequencing (A) At the genus level, *Bilophila, Blautia, Ruminococcus, Fusobacterium* genera were dominant in PE microbiomes. In reverse, *Faecalibacterium, Akkermansia, Gemmiger, Methanobrevibacter and Dialister* genera were significantly depleted in antenatal PE specimens. The boxplots represent the median and the interquartile range (IQR). (B)The PE-related bacterial genera and species in samples at antepartum, and 1 and 6 weeks postpartum. The lengths of bars show the Z-score of a genus or species at different time points and the red colors represent enrichment in patients (Z-score > 0) and blue colors represent bacterial enrichment in controls (Z-score < 0). Data were adapted with permission from a work published by Li-Juan Lv [84].

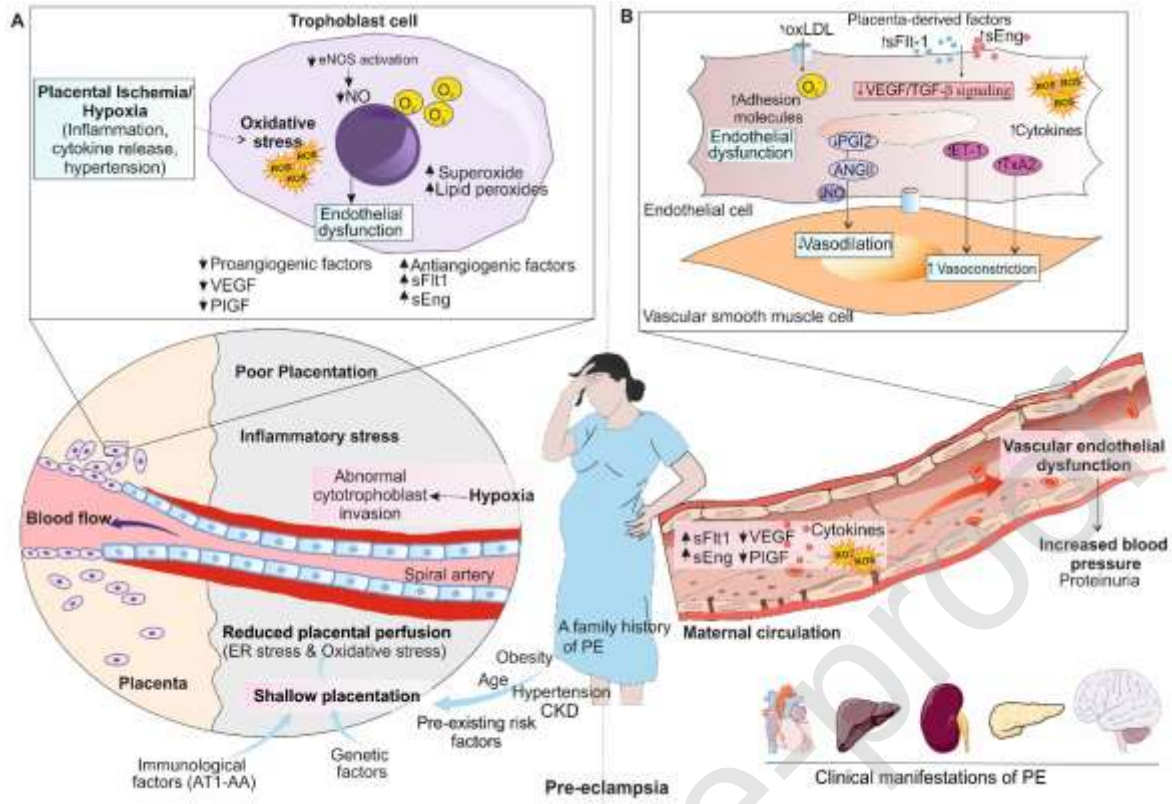

Figure 1

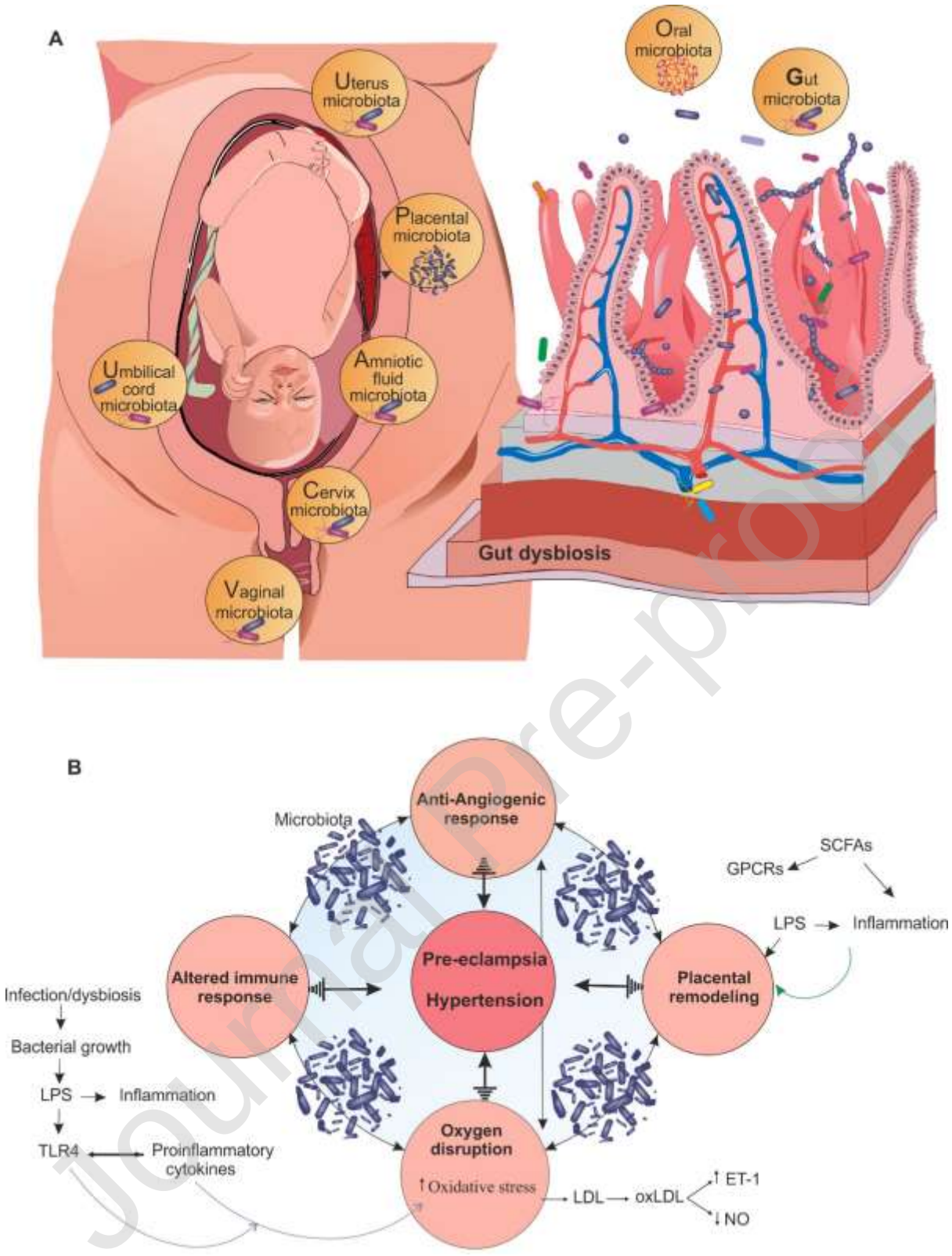
A
Oral microbiota
Gut microbiota
Uterus microbiota
Placental microbiota
Umbilical cord microbiota
Amniotic fluid microbiota
Cervix microbiota
Vaginal microbiota
Gut dysbiosis
B
Microbiota
Anti-Angiogenic response
SCFAs
GPCRs
LPS
Inflammation
Altered immune response
Pre-eclampsia
Hypertension
Placental remodeling
Infection/dysbiosis
Bacterial growth
LPS
Inflammation
TLR4
Proinflammatory cytokines
Oxygen disruption
Oxidative stress
LDL
oxLDL
ET-1
NO

Figure 2

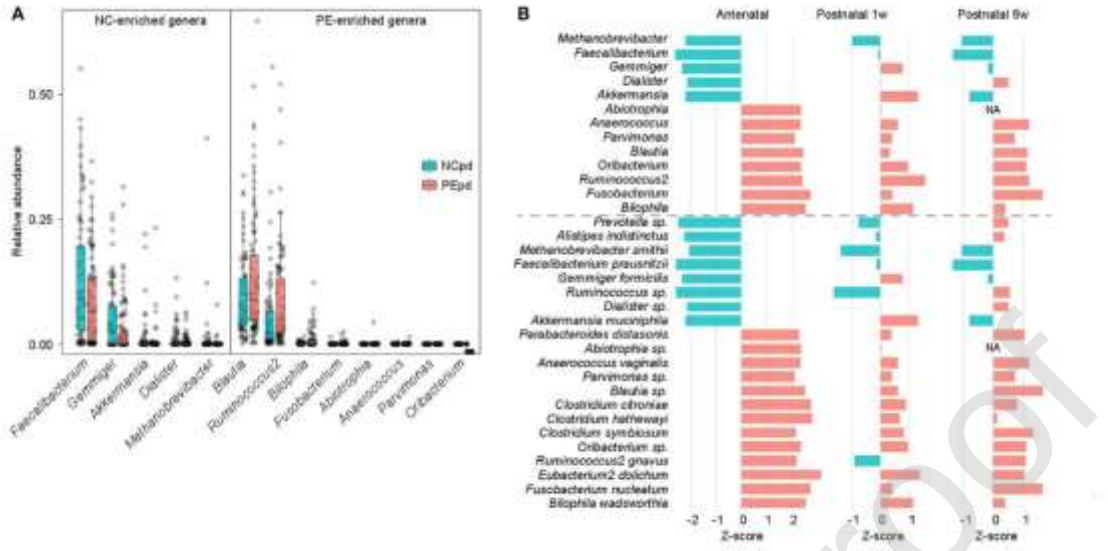

Figure 3