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Through the Microbial Looking Glass: Premature Labor, Preeclampsia, and Gestational Diabetes

A Scoping Review

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Abstract

The influence of microbial factors on adverse perinatal outcomes has become the focal point of recent investigations, with particular interest in the role of the microbiome and probiotic interventions. The purpose of this scoping review was to identify and critique the most recent evidence about these factors as they relate to pregnancies complicated by preeclampsia (PEC), preterm birth (PTB), and gestational diabetes mellitus (GDM). Four databases (PubMed, EMBASE, Web of Science, and Cochrane) were searched for articles published in English in the last 10 years with the concepts of the microbiome, probiotics, and PEC, PTB, or GDM. Forty-nine articles were eligible for full-text review. Five articles were excluded, leaving 44 articles that met all the eligibility criteria. The relationships between the microbiome and the risk for PEC, PTB, and GDM are not fully elucidated, although probiotic interventions seem beneficial in decreasing PEC and GDM risk. Probiotic interventions targeting bacterial vaginosis and elimination of infection in women at risk for PTB appear to be beneficial. More research is needed to understand the contributions of the microbiome to adverse perinatal outcomes. Probiotic interventions appear to be effective in reducing risk for select outcomes.

Keywords

gestational diabetes, microbiome, prebiotics, preeclampsia, preterm birth, probiotics

Introduction

Pregnancy is characterized by a variety of microbiological, immunologic, and inflammatory changes that promote the health of both the mother and the developing fetus.[1,2] The microbiome composition of the maternal gut changes dramatically between the first and third trimesters of pregnancy. These changes include increased β-diversity (differences between pregnant women), an overall increase in proteobacteria and actinobacteria, and reduced richness (lower number of species).[3] Similarly, the vaginal microbiome composition changes substantially during the course of normal pregnancy. Diversity decreases, stability increases, and the vagina is enriched with *Lactobacillus* species.[4] Vaginal pH becomes more acidic, while vaginal secretions are increased. However, by the third trimester, the vaginal microbiota resembles that of the nonpregnant state.[4]

Host microbial interactions between the mother and the infant during pregnancy and the timing of the first exposure of the fetus to maternal microbes are not fully understood, although the largest exposure is thought to occur at birth.[5] Historically, the intrauterine environment was thought to be germ-free; however, newer evidence suggests that a bacterial milieu may be present in the fetoplacental circuit.[4,6,7] Microbial exposures and alterations have been implicated in the etiology of several adverse perinatal outcomes, including preeclampsia (PEC), preterm birth (PTB), and gestational diabetes mellitus (GDM).[8,9] Recent investigations exploring the contributions of the human microbiome (defined as the total collection of microbes and their genetic characteristics)[10] have become the focus of recent scientific inquiry in understanding the etiologic mechanisms of these obstetrical syndromes.[11–13]

Background

Completed in 2012, the Human Microbiome Project characterized microbial communities and their respective physiologic activities in a variety of body sites, including the nasal passages, oral cavity, urogenital system, and gastrointestinal tract.[14] The initiative generated health reference data for a variety of microbiome sites, including those associated with normal pregnancy.[3,4] Utilizing advanced molecular genetics and genomics technology, a variety of DNA-sequencing technologies including *16S* (gene)-based analysis, as well as whole genome shotgun sequencing (entire bacterial DNA sequence of an organism), have allowed for both characterization of the types of microbes present in select body sites, as well as their genetic coding patterns and metabolic capacity.[15,16] Advances in genomic analyses and the development of bioinformatics communication channels, such as Quantitative Insights into Microbial Ecology and Mothur (a microbiome software platform), have streamlined the process of sharing the taxonomic information identified in these

analyses.[17,18] Furthermore, these bioinformatic resources provide detailed information about the composition of body sites and the influence on host physiology, which are key in understanding health outcomes.

The closely related area of microbial interventions has received considerable attention worldwide. Probiotic interventions contain live, usually freeze-dried bacterial microbes, often from the *Lactobacillus* and/or *Bifidobacterium* genera.[19] When given in sufficient quantities, probiotic bacteria confer health benefits on the host.[20] Probiotics are considered food. For use in antenatal applications, probiotic interventions can be administered either orally or vaginally. In most studies involving pregnant women, the oral route was used.[19] Prebiotic interventions administered orally are not live bacteria but rather comprise indigestible food substances, such as dietary fibers, certain starches, and oligosaccharides that are selectively fermentable.[21] Prebiotics are food for the probiotic bacteria and therefore increase microbial numbers. Synbiotics are a combination of probiotic and prebiotic interventions.[22] Synbiotics produce beneficial effects by promoting the survival of the live microbes in the gut by stimulating growth and/or metabolic activity of one or more probiotic bacteria. Probiotic interventions and, to a lesser extent, prebiotics have been applied during pregnancy to improve maternal and fetal outcomes.[19] More recently, synbiotic antenatal interventions have been studied.[23,24]

The purpose of this scoping review is to explore and synthesize the scientific evidence about the maternal and neonatal consequences of microbial dysbiosis, with a focus on the role of the microbiome during pregnancy, as well as the influence of prebiotic and probiotic exposures. Specifically, this review outlines the most recent literature on perinatal microbiome and probiotics, prebiotics, and synbiotics specific to the maternal and neonatal outcomes in pregnancies that are complicated by PEC, PTB, or GDM. A brief review of the implications for the neonate as well as future clinical and research implications is discussed.

Methods

A comprehensive literature search of 4 databases (PubMed, EMBASE.com, Web of Science Core Collection, and the Cochrane Database of Systematic Reviews) was undertaken to identify relevant articles. A scoping review strategy was chosen as the review method, as research studies investigating the impact of microbiome and probiotics on adverse perinatal outcomes have not been comprehensively explored.[25] To ensure that the full breadth and depth of the literature were explored, the searches were developed and conducted by an experienced medical librarian with input from the research team. This review was conducted using the standards established by the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines, which advocate for systematic and reproducible methods. Search strategy details are provided in Table 1. The results were exported to Endnote and reviewed by the clinical authors.

Table 1. PubMed.gov search strategiesa

Preeclampsia	((("microbiota"[MeSH Terms] OR "microbiota"[tiab] OR "microbiome"[tiab] OR pre-biotic[tiab] OR pre-biotics[tiab] OR "prebiotics"[MeSH Terms] OR "prebiotics"[tiab] OR "prebiotic"[tiab] OR "probiotics"[MeSH Terms] OR "probiotics"[tiab] OR "probiotic"[tiab]) AND (preeclampsia[tiab] OR pre-eclampsia[tiab] OR "Pre-Eclampsia"[Mesh])) AND ("2008/08/10"[PDAT] : "2018/08/10" [PDAT]) AND english[All Fields])
Preterm birth	((("microbiota"[MeSH Terms] OR "microbiota"[tiab] OR "microbiome"[tiab] OR pre-biotic[tiab] OR pre-biotics[tiab] OR "prebiotics"[MeSH Terms] OR "prebiotics"[tiab] OR "prebiotic"[tiab] OR "probiotics"[MeSH Terms] OR "probiotics"[tiab] OR "probiotic"[tiab]) AND ("Premature Birth"[Mesh Terms] OR “premature birth"[tiab] OR “preterm birth"[tiab] OR “pre-term birth"[tiab])) AND ("2008/08/10"[PDAT] : "2018/08/10"[PDAT]) AND english[All Fields])
Gestational diabetes mellitus	((("microbiota"[MeSH Terms] OR "microbiota"[tiab] OR "microbiome"[tiab] OR pre-biotic[tiab] OR pre-biotics[tiab] OR "prebiotics"[MeSH Terms] OR "prebiotics"[tiab] OR "prebiotic"[tiab] OR "probiotics"[MeSH Terms] OR "probiotics"[tiab] OR "probiotic"[tiab])

	AND ("Diabetes, Gestational"[Mesh] OR “gestational diabetes”[tiab])) AND ("2008/08/10"[PDAT] : "2018/08/10"[PDAT]) AND english[All Fields])

Data searched August 10, 2018, Chrome browser.

Articles were included if published in English between January 1, 2008, and August 10, 2018, to capture the most recent evidence in line with the Human Microbiome Project that launched in 2008. The searches combined controlled vocabulary supplemented with key words related to the concepts of the microbiome (eg, microbiota, prebiotics, probiotics, synbiotics); PEC (eg, preeclampsia, eclampsia); GDM (eg, pregnancy, diabetes mellitus); and PTB (eg, preterm birth, premature birth). Gray literature (eg, scholarly but not commercially published documents such as conference abstracts or proceedings, government reports, white papers, and theses) was not included.

Following the database searches, articles were selected if they met the inclusion criteria: (*a*) reported findings about PEC, GDM, or PTB AND; (*b*) reported outcomes of prebiotic and probiotic interventions; or (*c*) evaluated the composition of the maternal microbiome. Article quality was not assessed, but rather the types of studies were categorized to offer the most comprehensive review of the state of the science on the topics. The articles included in this scoping review were limited to level 1 experimental designs (randomized controlled trials [RCTs] or systematic reviews of RCTs); level 2 quasi-experimental designs; level 3 observational analytic designs (systematic review of cohort study, cohort study with control group, case-control study); and level 4 (observational descriptive and cross-sectional studies).[26] Titles and abstracts were initially reviewed to determine first round inclusion; full text was later reviewed for final decisions about inclusions. Articles were excluded if not directly related to the select perinatal outcomes (PEC, GDM, PTB), or if the study did not clearly measure the microbiome, prebiotic, probiotic, or symbiotic-related concepts. The PRISMA flow diagram is presented in Figure 1. A series of tables are used to present the literature. Table 2 contains the systematic reviews and meta-analyses.[28–36]Table 3 presents the findings of individual trials: RCTs, prospective cohort studies, retrospective cohort studies, and a case-control study.[23,24,37–50] Studies of the microbiome and the perinatal outcomes of PEC, GDM, and PTB are presented in Table 4.[12,51–67]

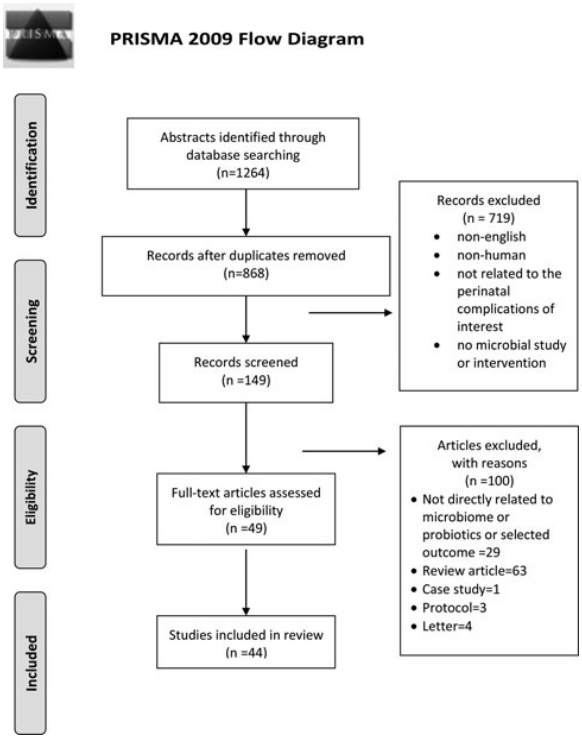

Figure 1. PRISMA 2009 flow diagram. For more information, visit www.prisma-statement.org. From Moher et al.[27]

Table 2. Systematic review and meta-analyses

					Findings related to intervention if applicable	
Author (year)	**Systematic review**	**Meta-analysis**	**Articles included**	**Intervention if applicable**	**Significant**	**Nonsignificant of (NA) statistics not provided**
Preeclampsia (PEC)						
Lindsay et al[28] (2013)[a]	x		7	Probiotics	↓ preeclampsia (OR = 0.80, 95% CI: 0.66-0.96) and severe preeclampsia (OR = 0.61, 95% CI: 0.43-0.89)	NA: ↓preeclampsia
Gestational diabetes mellitus (GDM)						
Barrett et al[29] (2014)	x		1	Probiotics	↓ diagnosis of GDM (*P* = .03)	
Dallanora et al30 (2018)	x		7	Probiotics		NA: Probiotics may improve glycemic control, ↓ VLD Cholesterol, ↓ inflammatory marker
Lindsay et al[28] (2013)	x		7	Probiotics		NA: ↓ fasting blood glucose, incidence of GDM
Pan et al[31] (2017)	x	x	6	Probiotics	↓ fasting serum insulin (*P* = .00001), HOMA (*P* = .02)	FBS (*P* = .9), gestational age (*P* = .63), birth weight (*P* = .9)
Taylor et al[32] (2017)	x	x	4	Probiotic	↓ HOMA (*P* = .01)	FBS (*P* = .96), LDL-cholesterol (*P* = .67), weight gain, mode of birth, neonatal outcomes, adverse events (P levels not reported)
Zheng et al[33] (2018)		x	10	Probiotics	↓ fasting serum insulin (*P* = .0001), HOMA (*P* = .03)	FBS (*P* = .26), lipid levels (*P* = .71), HDL cholesterol (*P* = .87), LDL cholesterol (*P* = .97), triglycerides (0.29)
Spontaneous premature labor (sPTL) and/or premature birth (sPTB)						
Jarde et al[34] (2018)	x	x	49	Probiotic Prebiotic		PTL <34 wk (*P* = .96), or <37 wk (*P* = .96); PTL <37 wk (*P* = .73), or <37 wk (*P* = .83)

Mendz et al[35] (2013)	x		13	N/A	↓ genital infection with probiotics (*P* = .00096)	NA: Vaginal bacteria were the most common source of intra-amniotic infection
Othman et al[36] (2007)	X	x	3	Probiotics		PTB <32 wk (*P* = .79), PTB <37 wk (*P* = .26)

Abbreviations: CI, confidence interval; FBS, fasting blood sugar; HDL, high-density lipoprotein; HOMA, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; OR, odds ratio; VLD, very low density.
[a] Same systematic review noted later.

Table 3. Summary of probiotics studies

					Findings related to intervention	
Author (year)	**Study design**	**Total participants**	**Sample and microbial intervention [a]**	**Purpose**	**Significant**	**Nonsignificant**
Dolatkhah et al[40] (2015)	RCT	64-GDM	32-Probiotic 32-Placebo	Glucose metabolism Weight gain	↓pregnancy weight gain study weeks 5 and 6(P < .05), FBS (P < .05), insulin resistance index (P < .05)	Pregnancy weight gain (study weeks 1-4)
Hajifaraji et al[41] (2018)	RCT	GDM	Probiotic Inflammation	Oxidative stress		
Jafarnejad et al[42] (2016)	RCT	82-GDM	41-Probiotic 41-Placebo	Glycemic control Inflammatory status	↓IL-6 (P = .04), TNF-α (P = .04), hs-CRP (P = .03)	FBS, HbA_{1c}, HOMA, IL-10, insulin levels
Karamaliet al[43] (2016)	RCT	60-GDM	30-Probiotic 30-Placebo	Glycemic control Lipid profiles	↓FBS (P < .001), serum insulin levels (P < .001), HOMA (P = .03), insulin sensitivity (P < .0007), triglycerides (P = .03), VLD cholesterol (P = .03)	Lipid profiles

Kijmanawat et al[44] (2018)	RCT	60-GDM	28-Probiotic 29- Placebo	Insulin Resistance	↓FBS (P = .34), fasting plasma insulin (P = .001), HOMA (P = .001)	Pregnancy weight gain
Lindsay et al[45] (2015)	RCT	149-GDM	74-Probiotic 75 Placebo	Metabolic health	↓ Total cholesterol (P = .031), LDL cholesterol (P = .011)	FBS (P = .588), insulin (P = .927), HOMA (P = .875), C-peptide (P = .843), HDL cholesterol (P = .341), triglycerides (P = .687), HDL ratio (P = .704), LDL ratio (P = .244)
Luoto et al[46] (2010)	RCT	256 gravidas	67-Probiotic and dietary intervention 63-Placebo and dietary intervention 85-Control and placebo	Pregnancy outcome and prenatal and postnatal growth	Probiotic plus dietary intervention ↓GDM (P = .003)	
Nabhani et al[23] (2018)	RCT	90-GDM	48-Synbiotic 49-Placebo	Insulin resistance Lipid profile Total antioxidant capacity	The symbiotic intervention resulted in decreased LDLs from baseline (P < .05). ↓ SBP and DBP in symbiotic group compared with placebo (P < .05).	FBS, insulin resistance/sensitivity, lipid profile, TAC indices symbiotic group compared with placebo (P < .05).
Wickens et al[47] (2017)	RCT	Healthy pregnant women	212-Probiotic 211-Placebo	GDM prevalence	↓GDM in women aged ≥35 y(P = .009) and women with history of GDM (P = .004)	Overall GDM prevalence (P = .08)

Spontaneous premature labor (sPTL) and/or spontaneous premature birth (sPTB)						
Kirihara et al[48] (2018)	Retrospective cohort	121	45-Probiotic 76-Controls	Perinatal outcomes sPTB	↑Gestation age (P = .012), ↓ sPTB <32 wk (P = .001), ↓ CAM (P = .03), ↓ vaginal Lactobacillus (P = .052)	Funisitis (P = .052), birth weight (P = .021)
Krauss-Silva et al[49] (2011)	RCT	644 asymptomatic gravidas	320-Probiotic 324-Placebo	Prevention of PTB-associated BV		Preliminary results: sPTD <34 wk (P = .31), sPTD <37 wk (P = .14)
Myhre et al[50] (2011)	Prospective cohort study	23 822 healthy gravidas	950-Probiotic milk 17 938-controls	sPTB risk	↓sPTB with high intake or probiotic milk (P < .035)	
Nordqvist et al[39] (2018)	Prospective cohort	37 050 Norwegian women	Probiotic milk	Intervention timing and incidence of PTL	Probiotic milk intake during early pregnancy intake ↓PTL (P = .03)	Dose response

Table 4. Summary Table of microbiome studies, often metagenomic-based

Author, year	Study design	Total participation	Microbiome site studied	Findings
Preeclampsia				
DiGiulio et al[51] (2010)	Retrospective cohort	62	Intra-amniotic	Women with PEC with MIAC demonstrated a ↑ mean amniotic fluid IL-6 level (P = .002).
Jaramillo et al[52] (2012)	Secondary analysis of RCT	57	Oral	RCT was on subgingival scaling and planning vs supragingival prophylaxis No differences in periodontopathic organisms between the 2 groups.
Amarasekara et al[12] (2015)	Nested case-control	110: 55 cases, 55 controls	Placenta	12.7% of the placenta samples of women with PEC were PCR positive for the 16S rRNA gene. Pathogenic microbiome identified: Listeria, Salmonella, and Escherichia.
Nizyaeva et al[53] (2017)	Case-control	32: 20 cases, 12 controls	Placenta	25% of the PEC group demonstrated pathologic microbial growth Control group placentas were culture negative. Placental microbes were identified only in women with PEC who delivered after 34 wk of gestation
Liu et al[54] (2017)	Case-control	100: 26 first trimester 24 second trimester 24 third trimester 26 controls	Gut	Women with PEC: there was an overall ↑ in pathogenic bacteria. Clostridium perfringens (P = .03) and Bulleidia moorei (P = .00) ↑ in women with PEC decrease noted in the probiotic bacteria Coprococcus catus (P = .03).
Gestational diabetes				
Crusell et al[55] (2018)	Prospective cohort	50 GDM, 157 controls	Gut	GDM associated with significant gut microflora disruption similar to that found in nonpregnant adults with type 2 diabetes
Kuang et al[56] (2017)	Exploratory descriptive	124: 43 GDM, 81 healthy	Stool in second trimester	Significant differences in microbiomes of women with or without GDM. ↓ diversity noted with GDM women.
Wang et al[57] (2018)	Descriptive	346 women with GDM and 140 of their NBs to total of 581 women, 248 NBs = 1062 samples	Oral, pharynx, GI, vagina, meconium, amniotic fluid	Lactobacillus iners strains were significantly greater in those with GDM (P< .05). Also ↑ viral load in meconium of NBs whose mothers have GDM. Microbial variations showed convergence across body sites with

				more similar community structure in those with GDM.
Zheng et al[58] (2013)	Descriptive comparison	105, in 4 groups: HP = 31, GDM = 39, T1DM = 35, HN = 32	Vagina, for fungal flora	Fasting plasma glucose (FPG), 1- and 2-h plasma glucose levels, & HbA1c were always ↑ in GDM and type 1 DM groups (P < .01). Diversity in fungi greater by pregnancy group, ↑ from 13, 17, and 20 species respectively, in HP, GDM, and TIDM groups
Preterm birth				
Ardissone et al[59] (2014)	Descriptive	52 infants: (23-41 wk at birth)	Meconium	Gestational age prior to 33 wk at birth had largest influence on microbial community structure (P = .029); mode of delivery (C/Sec vs vaginal) also had effect (P = .044). Associated bacteria seen in PTBs: Enterobacter, Enterococcus, Lactobacillus, Photorhabdus, and Tannerella
Avershina et al[60] (2017)	From RCT (about allergy prevention)	335: 256 at 36 wk, 105 in labor	Vagina	Swabs at 36 wk or labor admission: at labor onset had ↑ α-diversity and ↑ closer to labor.
Brown et al[61] (2018)	Prospective cohort	250 women with history of early loss	Vagina	In those with PTBs (38/250 = 15%) compared with term births, Lactobacillus was depleted prior to PPROM (P = .026), which occurred at mean gestational age of 30 wk, and depletion persisted after PPROM (P = .005). Dysbiosis also occurred in women with high amounts of Lactobacillus when treated with erythromycin (P = .00009), as did NB sepsis.
Dahl et al[62] (2017)	Prospective cohort, case control	121 births: 102 term, 19 = PTB	Stool at 4 d postpartum	If PTBs, had lower α-diversity in gut and significantly fewer OTUs in genera of Bifidobacterium and Streptococcus and families in Clostridiales order (stats not available).
Doyle et al[63] (2014) .	Cross-sectional study	989 258 (26.1%) with chorio, 120 (12.1%) had severe	Placenta	Nonsevere chorioamnionitis (defined as ≥25 neutrophils granulocytes on average per 10 high power field) showed difference in community members, ↑ bacterial load, higher phylogenetic diversity, ↓ species richness, and smaller (shorter) newborns

Hyman et al[64] (2014)	Prospective cohort	88: 17 PTBs, 71 term births	Vagina	Significant correlation between race/ethnicity and diversity (used Shannon Diversity Index). Location of sampling matters (prefer posterior fornix). Small sample size with limited statistical analysis.
Subramaniam et al[65] (2016)	Retrospective, stratified by race, BV, and PTB	40 prior taken vaginal swabs at 21–25 wk, and Nugent smears	Vagina	BV samples had greater diversity ($P < .05$), with more abundant BV-associated bacteria. Underpowered to compare PTB with microbiome but may be that changes in communities are linked to sPTBs rather than specific organisms.
Tabatabaei et al[66] (2018)	Nested case-control in cohort	450: 17 early SPTBs, 77 late SPTBs, and 356 controls	Vagina	↑ diversity ($P < .05$) in CST IV. Presence of BV-associated bacteria (Gardnerella vaginalis, Atopobium vaginae, and Veillonellaceae bacterium) also associated with PTB
Wylie et al[67] (2018)	Nested case-control in prospective cohort study	60: 128 swabs through PG	Vagina for virome	24 had PTBs (38.7%). African American women were 65% of sample. Higher viral richness was associated w/ PTB in total sample ($P = .0005$) and African American subgroup ($P = .0003$). Both high diversity of bacteria and viruses in first trimester were associated with ↑ risk for sPTB ($P = .01$); I ↓ combined diversity with term birth ($P < .0001$).

Abbreviations: CST IV, community state type 4; GDM, gestational diabetes mellitus; HN, healthy nonpregnant; HP, healthy pregnant; IL, interleukin; MIAC, microbial invasion of the amniotic cavity; NB, newborn; OTUs, operational taxonomic units are clustered, grouped sequences; PCR, polymerase chain reaction; PEC, preeclampsia; PG, pregnancy; PPROM, preterm premature rupture of membraines; PTB, preterm birth; PTL, preterm labor; RCT, randomized controlled trial; sPTB, spontaneous preterm birth; T1DM, type 1 diabetes mellitus.

A total of 1264 articles were identified through the database searches. Duplicates (396) were excluded leaving 868 articles to be screened in the initial abstract-screening phase. Forty-nine were eligible for full-text review. Five articles were excluded during the full-text review phase, leaving 44 articles that met all the eligibility criteria for inclusion in this study. In the following sections, the most recent evidence related to PEC, GDM, and PTB will be presented. Details of the types and dosages of prebiotic, probiotic, and synbiotic interventions are beyond the scope of this review but can be found in the individual studies.

Results

The role of the microbiome in PEC

Preeclampsia is a multisystem pregnancy complication characterized by new-onset hypertension with proteinuria, or evidence of systemic disease primarily in the liver and kidneys, with onset after 20 weeks of gestation.[68] The etiologic pathways of PEC are multifactorial and include maternal and fetal factors; however, the full pathway is not fully elucidated. The disorder is characterized by impaired vascular function and activation of maternal systemic inflammation in several organs, which can be aggravated by many factors, including infection. Studies have shown that women with asymptomatic bacteriuria, urinary tract infection, periodontal disease, and chronic pyelonephritis are at increased risk for PEC.[51,69] As such, the current focus of scientific inquiry has shifted to learning more about the role of the microbiome in the multifactorial pathway of PEC.

One of the focal areas in recent microbiome studies of PEC is vascular dysfunction in the placenta, which has been implicated as one of the leading pathways underpinning the disorder.[68] In a nested case-control study, researchers compared the placental tissue of women with PEC with normotensive women to screen the samples for the presence of bacteria. Using polymerase chain reaction and next-generation sequencing methods, the researchers found that 12.7% of the placenta samples of women with PEC were polymerase chain reaction positive for the *16S rRNA* gene. The microbiome of the samples identified a variety of commensal and pathogenic bacteria, including *Listeria, Salmonella*, and *Escherichia*.[12] Similarly, Nizyaeva et al[53] explored the differences in the histological and microbial features of PEC in a nested case-control study. The sample included 20 reproductive age women with the disorder and 12 normal controls, all of whom were between 26 and 39 weeks of gestation.[53] Forty-five percent of the women with PEC demonstrated chronic villitis ($P < .05$) as compared with the control group at 8%. All placentas were cultured; 25% of the PEC group demonstrated pathologic microbial growth (including *Streptococcus agalactiae* or group B *Streptococcus* and *Staphylococcus warneri)*, while all the control group placentas were culture negative. Placental microbes were identified only in women with PEC who delivered after 34 weeks of gestation, suggesting that microbial pathways may be particularly important to consider in women during later pregnancy. Together, these study findings suggest that microorganisms may contribute to an inflammatory response and support the role of microbial interactions in the etiologic pathway of PEC.

Infection and inflammation have also been implicated in the complex pathway of PEC,[69,70] with some focus on the influence of microbes within the intrauterine cavity. In a retrospective cohort investigation, DiGiulio et al[51] found that women with PEC who had microbial invasion of the amniotic cavity demonstrated a higher mean amniotic fluid IL-6 level ($P = .002$). The prevalence of microbial invasion overall in women with PEC was found to be low, although 3 of the 6 women with microbial invasion were positive for *Sneathia/Leptotrichia* spp,[51] which are microbiota associated with bacterial vaginosis (BV) and isolated cases of maternal bacteremia and fetal demise.[71,72] Although microbial invasion of the intra-amniotic environment has been identified in women with PEC, more studies are needed to elucidate the pathways by which microbes influence the intrauterine physiology.

The association between maternal periodontal disease and PEC risk was explored in 2 studies, a meta-analysis[70] and a secondary analysis of an RCT.[52] In a recent meta-analysis of 11 observational studies including 1118 women with PEC compared with 2798 women, researchers found that women with periodontal disease before 32 weeks of gestation had a 3.6-fold increased risk of PEC (odd ratio [OR] = 3.69; 95% confidence interval [CI] = 2.58-5.27) compared with women without gum disease.[70] The risk was also increased if periodontal disease was present 48 hours prior to delivery (OR = 2.68, 95% CI = 1.39-5.18), as well as within 5 days after delivery (OR = 2.22; 95% CI = 1.16-4.27). In a secondary analysis of an RCT conducted by Jaramillo et al,[52] the subgingival microbiota of 57 women with mild PEC were evaluated for differences related to a periodontal intervention (subgingival scaling and planing vs supragingival prophylaxis). There were no differences found in reduction of

periodontopathic organisms between the 2 groups.[52] Despite these findings, the relationship between the oral microbiome and PEC risk remains hypothetical, as most of the current literature consists of proposed pathways related to hematogenous spread of microbial pathogens from the oral environment.[1,73–76] A variety of oral microbial pathogens have been implicated in the etiology of PEC in previous studies, but not enough evidence has been identified to support a causal link.[77] More research is needed to determine whether there is an association between the oral microbiome and PEC risk.

Preeclampsia has features similar to metabolic syndrome, as women with this condition often develop altered glucose and lipid metabolism, insulin resistance, and endothelial damage.[78] Given the role of the gut microbiota in metabolism, a recent study by Liu et al[54] evaluated the gut microbiome of women with PEC to determine whether intestinal dysbiosis could be detected as a marker. One hundred women were grouped into 4 categories: 26 women with PEC and 3 healthy control groups in the first, second, and third trimesters of pregnancy. The dominant bacterium present in all samples was *Bacteroidetes*; however, in PEC women there was an overall increase in pathogenic bacteria. Specifically, *Clostridium perfringens* (P = .03) and *Bulleidia moorei* (P = .00) increased in women with PEC, with a decrease noted in the probiotic bacteria *Coprococcus catus* (P = .03). These findings suggest that there may be a shift in the gut microbial composition in women with PEC.

Overall, 5 groups of investigators studied the microbiome of PEC.[12,51–54] In 2 studies, the placenta site was analyzed[12,53]; in the remaining 3, the mouth,[52] gut,[54] or an intra-amniotic[51] site was examined. Some findings supported the association between pathogenic bacteria and PEC, but this was not a consistent finding. Furthermore, specific pathogenic organisms were not identified in common between the studies on PEC.

Probiotic use and PEC

The pathophysiologic pathways underpinning PEC are complex and not fully elucidated; however, maternal immune system dysregulation and infections were associated with PEC risk and severity.[79] Probiotics are thought to reduce both systemic and placental inflammation, thus reducing PEC risk.[37] Probiotics such as *Lactobacillus acidophilus* and *L rhamnosus* were shown to influence gene regulatory pathways and expression in immune response pathways of the human gut mucosal lining,[80] further supporting the exploration of probiotics as an intervention to delay and reduce the incidence of PEC.

Several studies investigating the association between probiotic products and PEC suggest a beneficial effect of probiotics in reducing PEC risk.[28,37,39] A large prospective Norwegian Mother and Child Cohort Study was conducted from 1999 to 2009 and included data from 108 000 pregnancies. Using these data, Brantsaeter et al[37] conducted a prospective cohort study of the relationship between probiotic ingestion in early pregnancy and the risk of PEC in a sample of 33 399 primigravidas.[37] In Norway, there are 2 main probiotic milk products that are consumed. The probiotic beverages deliver 10^8 probiotic bacteria per milliliter. Therefore, the authors calculated the probiotic dose based on dietary recall of these standard products. The researchers categorized dietary probiotic ingestion as follows: none, low, moderate, and high. Just more than 5% (1755 women) developed PEC. High-level probiotic intake (median = 200 mL/d with daily ingestion or greater) was associated with a significant reduction in all PEC (OR = 0.80, 95% CI: 0.66-0.96) and severe PEC (OR = 0.61, 95% CI: 0.43-0.89). These findings were also reported in a systematic review by Lindsay et al,[28] who explored the use of probiotics during pregnancy with maternal outcomes, including PEC. Seven articles were identified in the review, although only 1 study was identified related to PEC risk. Nordqvist et al[39] conducted a secondary analysis of the Norwegian Mother and Child Cohort Study to explore probiotic intervention timing. Specifically, they analyzed probiotic milk intake before pregnancy, during early pregnancy, and during late pregnancy, with PEC risk in nulliparous women (n = 37 050). The researchers found that probiotic milk intake in late pregnancy, but not before or during early pregnancy, was associated with lower PEC risk (OR = 0.80, 95% CI: 0.68-0.94).[39] Together, these studies suggest that probiotic intake was associated with reduced PEC risk.

While the etiology of PEC remains unclear, the markers of hypoxia, oxidative stress, and hemolytic pathways, including hemolysis, elevated liver enzymes, and low platelets, have been observed in women with PEC.[81] Therefore, the physiologic consequences of PEC extend beyond the maternal environment and contribute to fetal complications. A study by Ekambaram et al[38] investigated the antihemolytic and antioxidant efficiency of 2 probiotic yeasts (*Monascus purpureus* and *Saccharomyces cerevisiae*) on fetal cord blood red blood cells of infants born to PEC mothers as compared with healthy normotensive controls. The red blood cells of the PEC sample were found to have low antioxidant status ($P < .05$), increased oxidative stress, ($P < .05$), increased nitrative stress ($P < .05$), and increased hemolysis ($P < .001$). Oxidative stress hemolysis decreased in the cord blood red blood cells for both groups after incubation with both probiotic yeasts. Specifically, *M purpureus* resulted in significant reactive oxygen radical scavenging (removal) ($P < .001$), and *S cerevisiae* resulted in significant nitric oxide radical scavenging (removal) ($P < .001$). These findings hold promise that probiotics could modulate oxidative stress in offspring.

The role of the microbiome in gestational diabetes mellitus

The normal physiologic adaptations of pregnancy provide for the metabolic needs of the growing fetus. Gestational diabetes mellitus is defined as a carbohydrate intolerance with hyperglycemia with onset during pregnancy that varies in severity.[82] Gestational diabetes mellitus is a heterogeneous group of metabolic disorders that impacts up to 14% of pregnancies.[83] Between 20% and 50% of women with GDM will go on to be diagnosed with type 2 diabetes within 5 years.[84] Evidence has emerged that highlights the link between the gut microbiome and metabolism. This has led researchers to hypothesize that the gut microbiome may impact gestational metabolism and development of GDM.[56] The microorganisms that colonize the gut influence the metabolism of nutrients, hunger, satiety, and both lipid and glucose metabolism. Gestational diabetes mellitus has been associated with subclinical inflammation that may lead to oxidative stress.[41] Therefore, efforts to prevent and treat GDM have included probiotic and/or dietary interventions. Outcomes studied include a variety of metabolic and inflammatory markers.

Four groups of researchers examined the microbiome related to GDM.[55–58] The sites cultured varied widely between studies; stool was cultured in 2 studies,[55,56] vaginal swabs to examine fungi were used in 1 study,[58] and in another study, both viruses and bacteria were examined from 3 sites in women (oral, gastrointestinal, and vagina) and 4 sites from their newborns (oral, pharynx, meconium, and amniotic fluid).[57] Dysbiosis was identified more frequently in women with GDM, who also exhibited less overall microbial diversity.[56] More pathogens were found in women with GDM, including more viruses and fungal species.[57] The relationships between dysbiosis and GDM need to be more fully elucidated.

Probiotic use and gestational diabetes mellitus

Gestational diabetes mellitus probiotic and synbiotic interventions have been addressed in 9 RCTs and 7 systematic reviews and/or meta-analyses. In 7 of the RCTs, participants were randomized after GDM diagnosis to probiotic or placebo groups.[23,24,40,42–45] The effect of a probiotic intervention on inflammatory markers and oxidative stress in GDM was the goal of 1 study.[41] Other outcomes attributable to the probiotic intervention are somewhat variable between RCTs. Both C-reactive protein and tumor necrosis factor were significantly decreased among probiotic group participants compared with placebo in 2 studies.[41,42] Women in the probiotic intervention groups had lower rates of GDM than those taking placebos in 2 studies.[46,47] Fasting blood glucose was significantly decreased in probiotic group participants in 3 RCTs.[40,43,44] A review of the meta-analyses/systematic reviews allowed more patterns to emerge. Two systematic reviews/meta-analyses have near-identical findings.[31,33] Probiotic interventions resulted in a significant decrease in fasting serum insulin and HOMA model assessment for insulin resistance.[29,31,33] Overall in the meta-analyses, outcomes related to lipids, gestational age, and inflammatory markers did not reach statistical significance.

The role of microbiome in preterm birth

During pregnancy, the vagina is dominated by *Lactobacillus* species and characterized by less richness and diversity, but more stability, than the nonpregnant vaginal microbiome.[85] The bacteria of the vaginal microbiome have been classified into 5 community state types (CSTs).[60,86] CST-I to CST-III and CST-V are characterized by high levels of specific species of *Lactobacillus*. Vaginal Lactobacilli species include *L acidophilus, fermentum, crispatus*, and *jensenii*.[87] These Lactobacilli contribute to a protective biofilm on the vaginal mucosa.[88] Some *Lactobacillus* species produce lactic acid that acidifies the vaginal mucosal surface and prevents the adherence of pathogens.[89] Probiotic bacteria also secrete other substances that are associated with vaginal health (bacteriocins, vitamins, and cytokines).[89]

At least 30% of premature labors are associated with maternal infection.[36,85,90,91] Microbial pathogens can reach the uterine cavity through 3 routes that can lead to infection: (*a*) ascending from the vagina and cervix, (*b*) retrograde seeding from the abdominal cavity through the fallopian tubes, and (*c*) hematogenous seeding through the placenta.[90] At the level of the maternal mucosal surface, some microorganisms secrete phospholipase A2 that acts on phospholipids to form arachidonic acid, which ultimately converts to prostaglandins (PG, PGE2, PGF2A). Other microorganisms secrete endotoxins that stimulate a cascade of proinflammatory cytokines.[91] These microbial pathways work together to magnify the inflammatory response and contribute to the degradation of the collagen matrices of the cervix, fetal membranes, placenta, and uterus.[91]

Among the studies reviewed, a number of studies were designed to examine the microbial aspects of these inflammatory pathways to predict PTB. Preterm birth is associated with higher α-diversity (intraindividual) of vaginal flora.[64] The CST-IV[60,86] is associated with BV and is characterized by mixed community types, enriched in various anaerobic bacteria, with low levels of *Lactobacillus*. Bacterial vaginosis is a microbial dysbiosis associated with a 40% increase in the risk of PTB.[36] When BV occurs, there is a shift from a predominance of Lactobacilli populations to a higher proportion of other organisms: *Gardnerella vaginalis*, *Prevotella bivia*, *Mobiluncus sp*, *Mycoplasma hominis*, and *Atopobium vaginae*.[86] This results in the characteristic vaginal secretions with a number of common clinical findings, including pH 5.5 or greater, presence of clue cells, and positive whiff test.[92] Therefore, probiotic interventions have been used to address the BV dysbiosis in an effort to reduce the risk of PTB.

Nine groups of researchers examined the microbiome related to PTB.[59–67] Three of the studies that examined PTB do not lend themselves to comparison because each investigative group sampled a different body site.[59,62,63] In the 6 studies[60,61,64–67] that used vaginal microbial samples, all focused on BV-related assessments, including CST-IV categorization, the presence of BV-associated bacteria, and/or a decrease in *Lactobacillus* dominance. One study reported viruses present.[67] In the systematic review by Mendz et al,[35] the bacterial etiology of intra-amniotic infections was examined. Vaginal organisms were responsible for most intra-amniotic infections.[35]

In 3 investigations, race was explored as a possible factor in PTB, in relationship to the microbiome.[64,65,67] Hyman et al[64] reported that significant correlations were identified between race/ethnicity and diversity in the vaginal microbiome. Using the Shannon Diversity Index as the measure, African American women had the greatest diversity of microbes and Caucasians had the least ($P = .003$). Hispanics had the second most diverse vaginal microbiome ($P = .0082$) compared with Caucasians. Caucasian women who experienced PTB had greater microbial diversity than those who had term births ($P = .00016$).[64] Wylie et al[67] reported higher richness and diversity in the virome of women who experienced PTBs in the total sample ($P = .0005$) and those in the African American subgroup who had PTBs ($P = .0003$). For the entire sample, both high diversity of bacteria and viruses in the first trimester were associated with the highest risk for spontaneous PTB ($P = .01$), while lower combined diversity was associated with term birth ($P < .0001$). Subramaniam et al[65] examined their small sample that was stratified by race and determined that the microbiota did not differ by racial groups.

Probiotic use and preterm birth

Because of the variation in premature labor diagnosis in most studies reviewed, PTBs (<37 weeks and/or <34 weeks) were used as the primary study outcomes. Two systematic reviews and 4 clinical studies explored the efficacy of probiotic interventions to reduce PTB risk. Othman et al[36] conducted a systematic review of probiotic interventions to prevent premature labor. Three studies met their inclusion criteria; the impact of probiotic interventions on vaginal infection was based on 2 trials including a total of 99 participants. The researchers found that probiotic use reduced the risk of genital infection by 81% (relative risk [RR]: 0.19; 95% CI: 0.08-0.48). However, they found insufficient evidence for utility of probiotic intervention for the prevention of premature labor.

Using participants in the Norwegian Mother and Child Cohort Study prospective cohort study, Myhre et al[50] explored the relationship between the ingestion of probiotic containing yogurt products and premature labor, using a sample of 18 888 Norwegian women. Probiotic intake was calculated using dietary histories and operationally defined as low, average, or high. The average probiotic intake in the high group was 138.4 mL/d. Between 2002 and 2007, there were 950 cases of PTB of less than 37 weeks. Women with the "highest" intake of probiotic yogurt experienced significantly lower risk of PTB (P = .035; OR: 0.820; 95% CI: 0.681-0.986).

Jarde et al[34] conducted a systematic review of pregnancy outcomes in women taking probiotics or prebiotics. In 2 meta-analyses, the authors found no evidence that probiotic interventions altered the incidence of PTB of less than 34 weeks (RR: 1.03, 95%, CI: 0.29-3.64, I^2: 0%, 1017 women in 5 studies) or PTB of less than 37 weeks (RR: 1.08, 95% CI: 0.71-1.63, I^2: 0%, 2484 women in 11 studies). A significant limitation of these meta-analyses is the inclusion of studies of women from high-risk groups, such as obese women[28] and those with GDM.[28,42] The heterogeneity of the studies included in these meta-analyses makes interpretation of the findings difficult. For example, only 1 of the 11 studies in the meta-analyses aimed to reduce PTB using a probiotic intervention.[49]

Krauss-Silva et al[49] enrolled healthy pregnant women in an RCT using 2 *Lactobacillus* species (*L rhamnosus* GR-1 and *L reuteri* RC-14) aimed at treating asymptomatic BV infections and ultimately PTB of less than 34 weeks of gestation. At the time of publication of preliminary findings, the study sample was insufficient to draw conclusions about intervention efficacy. More recently, Kirihara et al[48] studied women at high risk for PTB in an RCT. Bacterial vaginosis was prophylactically treated with a probiotic combination product (*S faecalis, Colstridium butyricum*, and *Bacillus mesentericus*) versus a placebo from 12.5 ± 5 weeks until the time of birth. Findings demonstrated that women in the probiotics group had significantly less spontaneous PTB, increased gestational age, decreased intrauterine infections, and higher rates of normal vaginal flora.

Discussion

There was significant variation in the findings from studies investigating role of the microbiome during pregnancy, as well as the influence of prebiotic, probiotic, and synbiotic exposures in women with PEC, GDM, and PTB. Some findings supported the association between pathogenic bacteria and these perinatal outcomes, but this was not a consistent finding. Furthermore, specific pathogenic organisms were not identified in common between studies. Probiotic exposures appear to have a beneficial effect in decreasing PEC and GDM risk and also appear to decrease the risk for PTB via the reduction of vaginal infections such as BV.

Preeclampsia

The relationships between the microbiome, prebiotic, probiotic, or synbiotic use and the risk for PEC are not fully understood. However, the findings of this review suggest that more research is justified, given the findings that (*a*) placental tissues of women with PEC were found to harbor pathogenic microbes along with signs of placental inflammation and (*b*) probiotic use is associated with a reduction in PEC risk. Although there were a limited number of studies identified, the findings suggest that more research is needed to explore these

biological mechanisms as viable pathways in the complex pathophysiologic pathway underpinning PEC. Future well-controlled RCTs to explore causal relationships between probiotic use and PEC risk are needed.

It may be beneficial to explore the relationships between the microbiome, microbial interventions, and PEC incidence in high-risk groups. Considering the inflammatory state of obesity and the increased risk of hypertensive disorders of pregnancy among obese pregnant women, a recent study associated with an RCT (study of probiotics in GDM) explored whether select gut microbes and metabolites among obese pregnant women influenced gestational blood pressure. Overweight and obese pregnant women were found to have gut-associated metabolites (odoribacter and butyrate) that inversely correlated with systolic blood pressure endothelial markers (plasminogen activator inhibitor 1), suggesting that the microbiome may influence blood pressure regulation in pregnancy.[93] Future microbiome-related studies among obese pregnant women may prove beneficial in understanding the pathology of PEC and GDM, given the increased prevalence of obesity.

Preterm birth

The volume of microbiome studies seeking to identify bacterial communities associated with PTB is extensive. Researchers, utilizing descriptive and correlational studies, attempted to find microbial predictors of premature labor. While all the RCTs sought to reduce PTB risk,[39,48,50] prevention and treatment of infections using probiotic interventions, particularly for BV, were important aims.[36,49] Probiotics have been shown to be an effective independent or adjunctive therapy for BV.[94]

Disparities in perinatal outcomes disproportionately impact African American women and their families. The findings of this scoping review suggest that the vaginal flora of African American women may predispose them to PTB risk. Customized nutritional interventions with prebiotics, probiotics, or synbiotics[95] may offer low-risk strategies for primary prevention.

Premature labor and birth are often the result of polymicrobial infection[96] with organisms of vaginal origin.[35] Vaginal dysbiosis increases the risk of preterm premature rupture of the membranes and neonatal sepsis.[61] Based on the studies reviewed, these findings provide opportunities for effective prevention and/or intervention at the microbial level. Studies on microbial aspects of PTB prevention require adequate statistical power to demonstrate efficacy of interventions.

Gestational diabetes

Since GDM is nonacute and diagnosed at predictable intervals during pregnancy, it is compatible with prospective study. Thus, GDM has received considerable attention in RCTs of probiotic interventions. Healthy women were enrolled in 2 studies.[46,47] Findings from both studies demonstrated that probiotic interventions reduced the incidence of GDM.[46,47] The remaining RCTs used samples of women with GDM and focused on interventions with probiotics to improve metabolic outcomes. In contrast, there is a dearth of research on the microbiome associated with GDM. Since inflammation is associated with GDM, more research in this area is warranted.

Although not detailed in this review, *Bifidobacterium* and *Lactobacillus* were the most studied species of probiotics in GDM RCTs. Probiotic strains and dosages varied greatly between studies. For example, 1 study used only 1 million colony-forming units of a single probiotic strain,[44] while another used a multispecies probiotic with 1 trillion colony-forming units.[42] In their meta-analysis of probiotics and GDM, Zheng et al[33] suggested that a probiotic dose of greater than 10^7 colony-forming units may lead to the most benefit. The authors concluded that antenatal probiotic interventions had a favorable effect on glucose metabolism.[33] More well-controlled trials of the efficacy of various well-characterized probiotic strains and dosages on GDM diagnosis and metabolic outcomes are needed.

Conclusions

The role of the microbiome in PEC, PTB, and GDM is not fully elucidated, although findings from this review suggest that pathogenic microbes in a variety of habitats including the placenta, oral cavity, vagina, and gut may influence the risk for adverse pregnancy outcomes. Although distinct microbial profiles were not identified in select outcomes, probiotics appear to be effective in reducing the risk for PEC, PTB, and GDM in some cases. Probiotic interventions were not associated with adverse events and were well tolerated by the participants. In studies, all 3 entities covered in this review, PEC, GDM, and PTB, were associated with an increase in markers of systemic inflammation.[4,54] Future studies investigating the role of the microbiome on a variety of inflammatory pathways may help elucidate biological pathways underpinning microbial dysbiosis. In addition, laboratory technologies used to study the metagenomics are complex and continually evolving.[64] For example, the analyses can be expensive, time-consuming, and dependent on databases that are imperfect. Newer next-generation sequencing will likely improve accuracy.[64] More research in this area in conjunction with advanced molecular sequencing technologies could significantly impact perinatal outcomes for women who experience any of these high-risk complications.

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