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MICROBIOTA AND PREGNANCY

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Abstract: This article reviews the pregnancy microbiota literature published between April 2019 and March 2020. The literature this past year has been far reaching including research into pathological pregnancy outcomes, predominately; preeclampsia (PE), gestational diabetes mellitus (GDM) and preterm birth (PTB). Other conditions covered included preterm pre-labour rupture of membranes, bacterial vaginosis, miscarriage, fetal growth, allergies in the offspring, psychiatric disorders, and pregnancy after bariatric surgery.

Of 94 identified papers, 21 were review papers and 19 papers were of mechanistic animal studies in mice, rats, cows and various other animals, including an attempt at elucidating the presence of a placental microbiome in animals and placental/endometrial microbiota in humans.

Three human interventional trials were published, two probiotic interventions for vaginal microbiota alteration and one investigating whether antimicrobial toothpaste can change the oral microbiota. These were published along with two protocols for randomised control trials (RCT).

Most papers focused on the bacterial component of the gut microbiota through 16S ribosomal RNA (rRNA) gene sequencing of the V4 hypervariable region. Three studies included metagenomic and multi-omic analysis, and 24 included vaginal, oral or blood microbiota analysis.

Keywords: Microbiota, Pregnancy, Preeclampsia, Gestational diabetes mellitus, Pre-term birth.

INTRODUCTION

Pregnancy induces many immunological, metabolic, and hormonal changes that are necessary to support this unique physiological state. The maternal microbiota (the population of microbes occupying various body sites) both influences, and is affected by, physiological and pathological changes in pregnancy[1]. This relationship has only been recently appreciated and there continues to be a lack of consensus regarding the nature of pregnancy microbiota composition and alterations. Changes in the microbiota throughout pregnancy have been documented in studies primarily examining the gut, the vaginal and the oral microbiota.

Prior to this year in review, changes in the gut microbiota in pregnancy were known to be characterised by decreased α-diversity (diversity within a sample) and increased β-diversity (diversity between samples)[2]. Also previously noted were specific increases in Actinobacteria and Proteobacteria and decreased *Faecalibacterium*, a butyrate-producing bacterium which is also depleted in metabolic syndrome[3,4]: reflecting the overarching sizable shift in metabolism of the pregnant state. The vaginal microbiota is characterised by the same pattern of diversity shift as well as enrichment with Lactobacillus[5]. The classical paradigm of the feto-placental unit as a sterile unit has been challenged, with studies suggesting that there exists distinct microbiota in the placenta and amniotic fluid of healthy pregnancies[6,7]. However, this issue remains in debate due to methodological difficulties[8].

The influence of maternal microbiota on adverse perinatal outcomes has been a focus of study, particularly in conditions whereby the pathophysiology is not fully understood such as preeclampsia, preterm birth, and gestational diabetes[9-11]. Research in this field is largely

aimed at identifying and characterising any microbial imbalance (dysbiosis) associated with the disease state and exploring avenues towards potential interventions to modulate the pathophysiology.

This review summaries the latest literature available related the study of the microbiota in relation to pregnancy.

PREECLAMPSIA/HYPERTENSION

Preeclampsia (PE) is persistent *de novo* hypertension, accompanied by proteinuria, maternal organ dysfunction and/or uteroplacental dysfunction, with onset at or after 20 weeks' gestation[12]. The aetiology of PE is multifactorial and not fully elucidated. Focal areas in microbiota studies include examining placental vascular dysfunction, systemic inflammation and features of metabolic syndrome[13]. Four case-control studies investigated the gut microbiota of PE women compared to healthy controls using 16S ribosomal RNA (rRNA) sequencing[14-17]. Findings suggest a role of microbiota in mediating preeclampsia however the causative relationship is uncertain.

Chen et al[14] compared the faecal microbiome of 67 PE and 85 normotensive pregnant women in the third trimester[14]. They found *Faecalibacterium* and *Akkermansia* species to be depleted in PE whereas *Fusobacterium* and *Veillonella* were enriched. These findings were supported by Lv et al[18], who reported enrichment of *Fusobacterium, Blautia, Ruminococcus*, and *Bilophila* and depletion of *Faecalibacterium, Gemmiger, Akkermansia, Dialister* and *Methanobrevibacter* in PE women[18]. *Faecalibacterium* and *Akkermansia* are involved in producing intestinal epithelial nutrition, maintaining immune homeostasis and strengthening intestinal barrier functions[19,20]. *Fusobacterium* has been implicated in colorectal cancer pathogenesis and has been found in higher numbers of placenta of women and mice with PE[21]. Faecal microbiota from PE patients, transplanted into mice by Chen et al[14] induced a pre-eclamptic phenotype including hypertension, proteinuria, IUGR and placental dysfunction.

Chang et al[15] compared the faecal gut microbiota and faecal short-chain fatty acids of 27 women with severe PE and 36 healthy controls[15]. They demonstrated altered bacterial abundance in severe PE subjects, with a predominance at the phylum level of Proteobacteria and decrease in Firmicutes, suggesting a difference in microbial signature according to geography or disease severity. Wang and colleagues also reported similar findings of increased abundance of Proteobacteria, Actinobacteria and Bacteroides and a decrease in Firmicutes compared to healthy controls[16]. Some of these changes have previously been identified by Koren et al[3] as occurring in normal pregnancies, and the exaggeration of the shift may be reflective of the amplified inflammatory state in PE.

Both Chen et al[14] and Chang et al[15] showed women with PE had significantly reduced alpha-diversity, however this was not supported by Lv et al[18] and Wang et al[16], although the latter two studies had smaller sample sizes. Further, Lv et al[18] did not detect significant differences in alpha-diversity during the postpartum period either.

Wang et al[3] reported microbial gene functional changes related to lipopolysaccharide (LPS) synthesis was higher in PE, and faecal and plasma LPS concentrations and plasma trimethylamine-N-oxide (TMAO) concentrations were higher in women with PE. Meanwhile, Chang et al[15] reported significantly decreased faecal levels of butyric and valeric acids in PE, which was significantly correlated with differential gut microbiota abundance. Butyrate has been reported to downregulate LPS-mediated macrophage activation and pro-inflammatory cytokine production[22,23]. These study findings together suggest a gut microbial dysbiosis with significant functional implications which occurs in the setting of PE in late pregnancy.

A Taiwanese publication by Hsu et al[17] investigated hypertension in the offspring of rats consuming a high-fat diet. They highlighted the fact that gut microbes and their short-chain fatty acids (SCFA), trimethylamine and trimethylamine N-oxide metabolites are involved in the development of hypertension. They presented a rat model whereby maternal prebiotic and probiotic therapy had beneficial effects by alteration in gut microbial communities, modulation of microbial-derived metabolites and meditation of the renin-angiotensin system, proposing an avenue to hypertension prevention.

Of importance regarding interpretation of this year's preeclampsia microbiota data is all human studies occurred in Chinese populations, so influence of ethnicity cannot be determined, and results may not be able to be extrapolated to populations of other ethnicities.

GESTATIONAL DIABETES MELLITUS

The link between GDM and the microbiota, previously the link with maternal gut microbiota, has previously been a focus of study. Prior studies have demonstrated a dysbiosis in the gut microbiota of women with GDM, and replicated these findings in animal studies using faecal microbial transplant (FMT)[2]. The latest studies (four in the last 12 months) have increased the breadth of research into microbiota signatures in other locations of the body, including oral and vaginal, in the context of GDM.

Ma et al[24] performed a nested case-control study to determine whether the alterations in gut microbial composition during early pregnancy were predictive of GDM development in later pregnancy. Gut microbial profiling of women with GDM (n=98) and normoglycaemic pregnancies (n=98) was performed on stool samples obtained in early pregnancy (10-15 weeks gestation). Using 16SrRNA gene amplicon sequencing of the V4 region they showed significant differences in relative abundance between the groups, specifically, *Eisenberiella, Tyzzerella*, and *Lachnospiraceae* NK4A136 which were enriched in the GDM group. The identification of early gestation gut microbiota differences raised the possibility of gut microbiota-targeted biomarkers as future potential predictors of GDM[24].

Crusell et al[25] performed an observational study on the salivary microbiota of women with GDM (n=50) and women with normal glucose regulation (n=160). Using 16S rRNA gene sequencing, they did not find differences in alpha-diversity indices, i.e., observed OTUs (richness), Shannon's index (overall diversity) or Pielou's index (evenness) between GDM and non-GDM groups in the third trimester of pregnancy. They did identify an association between microbial composition and 2hr stimulated plasma glucose, reflecting the body's ability to handle a glucose load. Also, richness of salivary microbiota composition decreased from a timepoint between 27-33 weeks gestation to 9 months post-partum, which they proposed may be mediated by immunological changes which are not yet fully understood. This study was limited by its design; many of the findings in the study were confounded by pre-pregnancy BMI, and when adjusted for pre-pregnancy BMI, the results lost significance. Also testing women in the third trimester does not give good clinical relevance as women would have already completed screening at that time, hence does not help us predict and indeed design strategies to prevent the onset of GDM. However, it can help us assess the phenomena that GDM is a risk factor for developing T2DM later in life.

Cortez et al[26] performed a cross-sectional study investigating the oral, vaginal and faecal microbiota of women in their third trimester of pregnancy with GDM (n=26) and without GDM (n=42). They also did not find any difference in the oral samples between groups at the phyla and genus level. However, they observed that pregnant women with GDM had increased *Lachnospiraceae, Phascolarctobacterium,* and *Christensenellaceae*. There were no significant differences in genera detected in the gut microbiota between the groups. Within the vaginal microbiota the main genus found in both groups was *Lactobacillus (*considered part of a 'healthy' vaginal microbiota)[27], with some additional genera being more abundant in the control group including *Bifidobacteroaceae* and *Atopobium*. Conversely, an increase of certain dysbiotic genera such as *Shuttleworthia, Enterobacter,* and *Enterococcus* were noted in the GDM group.

With increasing rates of maternal obesity, GDM diagnoses and childhood obesity some hypothesize that pregnancy events/microbial composition may have transgenerational effects. Zhang et al[28] published findings from a murine model of inulin-type prebiotic supplementation in high fat diet fed dams. Through metagenomic shotgun sequencing and glucose metabolic status assessment they showed that the offspring from dams with prebiotic supplementation favourably moderated offspring glucose metabolism and gut dysbiosis. Similarly, the fetal effects of maternal obesity and high fat diet were also studied by Wallace et al[29] in a high-fat diet mice model before and during pregnancy. They showed that

maternal obesity was related to placental hypoxia, increased angiogenesis, and increased transcription levels of glucose and amino acid transporters. The maternal diet-induced obesity impaired maternal bacterial metabolite signaling pathways in the mid-gestation phase contributing to adverse maternal and placental adaptations that, via alterations in fetal hepatic glucose handling, may impart increased risk of metabolic dysfunction in offspring[29].

PRETERM BIRTH

Preterm birth (PTB) and its sequelae are major causes of neonatal and paediatric mortality worldwide and is defined as birth prior to 37 weeks gestation. Intrauterine infection is implicated in 25-40% of pre-term births and the most common pathway of infection is ascent from the vagina and cervix[30]. Ravel et al[31] first described a classification scheme for vaginal bacterial species into five community state types (CST). CST-I, -II, -III, -V are characterised by dominance of specific species of *Lactobacillus*. These are *Lactobacillus acidophilus, Lactobacillus fermentum, Lactobacillus crispatus* and *Lactobacillus jensenii* respectively. CST-IV is characterised by diverse community types, enriched in various anaerobic bacteria and relatively low levels of *Lactobacillus*. Vaginal microbiota has been previously shown to be associated with pregnancy outcome, with *Lactobacillus* -dominated communities thought to be protective of preterm birth[32,33]. In total ten studies were included in this review. Six studies examined the vaginal microbiota, of which four were comparing PTB to term controls, one studied preterm premature rupture of membranes (PPROM), and one studied cervical length (shortened cervix mid-gestation is strongly associated with increased preterm birth[34]. The remaining four studies focused on placental, blood, oral microbiota respectively and one was a study protocol.

Several different bacteria were enriched in PTB and PPROM however five studies agreed with current thinking that vaginal microbiota enriched with Lactobacillus is protective of PTB. This may reflect different aetiologies for PTB/PPROM and differences in clinical criteria of studies and ethnicity. Increased bacterial diversity in the vagina, as seen in bacterial vaginosis[35], has also been thought to be pathogenic and the following studies support this theory. The findings of these studies support the current theory that preterm birth is linked to the vaginal microbiota; however, the phenotype may vary depending on the population and environmental factors.

Elovitz et al[36] performed a nested case-control study comparing the vaginal microbiota of women with spontaneous preterm birth (n=107) and controls with term deliveries (n=432). Seven bacterial taxa were significantly associated with increased risk of spontaneous PTB, with the strongest association observed with *Mobiluncus curtsii/mulieris* and *Sneathia sanguinegens*. Interestingly, the risk associated with *M. curtsii/mulieris* was eliminated when *Lactobacillus* relative abundances were high. Higher levels of the host-derived anti-microbial peptide β-defensin-2 was demonstrated to lower the risk of sPTB, independent of the presence of *Lactobacillus*-dominated microbiota communities, which presents a novel avenue for further investigation.

Chang et al[37] identified CST-I, CST-III and CST-IV to be the dominant clusters in vaginal samples from 126 pregnant Korean women. Within their study, six of the eight women who experienced PTB had vaginal microbiota profiles classified as CST IV (non-*Lactobacillus* dominant). Reads specific to *Gardnerella vaginalis* and *Atopobium vaginae* were found in 6/8 and 5/8 of the PTB vaginal samples, respectively. These results support existing literature suggesting the protective role of *Lactobacillus* sp. and the role of pathogenic vaginal infection in PTB; however, it is important to note the relatively small sample size.

Fettweis et al[38] delved deeper by performing 16S rRNA, metagenomic, metatrascriptomic and cytokine profiles on a cohort derived from the Multi-Omic Microbiome Study: Pregnancy Initiative (MOMS-PI). They used shotgun metagenomic and metatranscriptomic sequencing to profile the maternal vaginal microbiota longitudinally in women, of predominantly African ancestry, with preterm (n=45) and term (n=90) births. In this comprehensive multi-omic study, women with preterm births again exhibited significantly lower vaginal levels of *Lactobacillus crispatus*. They also found increased proinflammatory cytokines were correlated with preterm-birth-associated taxa; these were eotaxin, IL-1β, IL-6 and MIP-1β. However, in a study by Costa et al[39] lower serum levels of IL-10 and TGF-β were associated with increased risk of

PTB. Based on the differing reported findings, the role of inflammatory cytokines in mediating PTB remains unclear, however the bulk of current literature points to a positive association, as labour, including preterm labour, is associated with proinflammatory cytokine expression.

Blostein et al[40] compared the vaginal microbiota of women with preterm births (n=25) compared to women with term births (n=100). 16S rRNA amplicon gene sequencing was used to characterise vaginal microbiota composition and Dirichlet multinomial mixture models to group into community state types (CST). They reported no association between vaginal microbial CST with preterm birth overall. This contradicts the findings by Elovitz et al[36] and several other studies[34,36-38], however the current study was limited by its design as a nested case-control study. The timing of vaginal swab collection varied within the first two trimesters and no repeated measures were performed. After stratifying for timing of swabbing, *Lactobacillus*-dominated communities were negatively associated with preterm birth in women swabbed prior to 12 weeks (n=15). However, in women swabbed after 12 weeks (n=10), *Lactobacillus*-dominated communities were positively associated with preterm birth. Since this was not a longitudinal study, the women who were swabbed prior to or after 12 weeks may not be interchangeable. Although the sample size was small for this study, this raises questions about the *Lactobacillus* hypothesis and longitudinal studies would give us more valuable information.

Gerson et al[34] examined the cervical microbiota and cervical length in early second trimester in cases of sPTB (n=67), medically indicated PTB (n=47) and term births (n=358). They reported a community state type (CST IV) defined by a paucity of *Lactobacillus* spp. and a diverse set of strict and facultative anaerobes was associated with preterm birth and shortened cervix. They also found that women with both CST IV and shortened cervix (n=20) have increased risk of sPTB compared to women with other CST classifications, defined by predomination of various *Lactobacillus* spp., and normal cervical length. This study was performed in a largely African-American population.

Brown et al[41] used metataxonomics to longitudinally characterise the vaginal bacterial microbiota in women with preterm pre-labour rupture of membranes (PPROM) (n=60) and term deliveries (n=36). They demonstrated reduced *Lactobacillus* abundance and increased vaginal bacterial diversity prior to PPROM. Enrichment of potentially pathogenic bacteria including *Prevotella, Streptococcus, Peptoniphilus, Ureaplasma* and *Dialister* spp. occurred later in pregnancy for PPROM. Women with term deliveries had reduced bacterial richness as gestation increased, with a domination of *Lactobacillus* spp. They suggest that further interventional studies are required to study the role of modifying the vaginal microbiota to prevent preterm birth. This group also published results of next generation sequencing of the vaginal microbiota for women pre and post rescue cervical cerclage (placed to prevent miscarriage and preterm birth)[42]. They demonstrated reduced relative abundance of *Lactobacillus* spp. in the group with premature cervical dilatation and high levels of *Gardnerella vaginalis* associated with unsuccessful cerclage (resulting in miscarriage, intrauterine death, neonatal death or significant neonatal morbidity). They also successfully showed that the placement of the monofilament cervical cerclage didn't change the vaginal microbiota composition, which is consistent with previously published work[43].

Seferovic et al[44] successfully observed sparse and very low bio-mass bacteria within the placentas of term and preterm pregnancies, by histological and 16S rRNA gene sequencing methods. The quantitative 16S rRNA signatures observed in PTB placentas (n=13) were not significantly different from term Caesarean (no labour) specimens (n=18), however there was heterogeneity observed between individuals which is an area for further exploration. The results of this study support other previously published findings which have characterised a unique placental microbiota. The presence of a placental microbiota would indeed further the hypothesis of the role of maternal microbial translocation and mediating perinatal outcomes and fetal development.

Little is known about the blood microbiota in preterm birth. You et al[45] conducted a case control study of the blood microbiota of women with PTB (n=21) and term delivery (n=20) using 16S rRNA gene sequencing. They observed several taxa associated with PTB; in women with PTB, Firmicutes and Bacteroidetes were more abundant while Proteobacteria was less prevalent. These are early observational findings and further studies are required to understand the implications.

Lokken et al[46] published a protocol for a Kenyan prospective case-cohort study on the impact of preconception vaginal microbiota on women's risk of spontaneous preterm birth. This study would provide a new perspective on the field with focus on preconception vaginal microbiota as a mediator of preterm birth.

MISCARRIAGE/ENDOMETRIAL MICROBIOTA IN EARLY PREGNANCY

One study by Al-Memar et al[47] focused on miscarriage, defined as pregnancy loss prior to 20 weeks gestation. Their aim was to characterise the vaginal bacterial composition in early pregnancy and investigate its relationship with first and second trimester miscarriages. Main outcome measures were relative vaginal bacterial abundance, diversity, and richness. The findings agreed with the recent PTB literature regarding protective effects of vaginal *Lactobacillus,* with first trimester miscarriage associated with a reduced *Lactobacillus* spp.-dominated vaginal microbiota, higher alpha diversity and higher richness. This suggests vaginal bacterial composition may be an avenue for modifying risk factor for first trimester miscarriage prevention.

A novel and insightful case report was also published by Moreno et al[48]. They detected taxonomic and functional differences between the microbiota found in endometrial fluid collected during an early successful pregnancy and before a spontaneous abortion with euploid embryos in the same patient, suggesting that the difference between pregnancy success and failure was related to the microbiome.

These papers suggest that the microbiota of the human reproductive tract at conception and early pregnancy contribute to successful pregnancy carriage to term and opens the possibility of further research and/or interventional studies.

VAGINAL INFECTIONS

Whilst the world of microbial science and analysis is ever expanding; the clinical utility in pregnancy is still to be fully determined. Group-B Streptococci (GBS) is commonplace in reproductive-age women globally, including in the final weeks of pregnancy[49]. Culture-based approaches to diagnose vaginal and rectal carriage is performed to stratify who need to receive intra-partum antibiotic prophylaxis (IAP) in order to attempt to prevent neonatal infection with GBS, which can be life-threatening[50]. Martin et al[51] showed the positive effects from a probiotic intervention trial to eradicate GBS infection. After determining *in vitro* that *Lactobacillus salivarius* CECT 9145 was the best candidate for GBS eradication, a pilot trial involving 57 healthy pregnant women was performed. The intervention arm (n=25) were all initially GBS-positive and consumed the oral probiotic supplement daily from week 26 to week 38. By late gestation, 72% and 68% of the women were GBS negative in rectal and vaginal samples, respectively, raising the possibility that such treatment could be used to decreasing the number of women receiving IAP. However, it should be noted that there was no control group, and it is known that GBS status may fluctuate throughout pregnancy[51].

Husain et al[52] conducted an RCT to examine the rates of bacterial vaginosis at 18-20 weeks' gestation after once daily oral administration of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 versus placebo from recruitment between 9-14 weeks' gestation. The results showed no vaginal microbiota modification using oral probiotics during pregnancy. This paper had a published commentary commending the efforts, but stated that the continued use of *Lactobacillus rhamnosus* and *Lactobacillus reuteri* remains questionable as they are not common vaginal *Lactobacillus* spp[53].

Chen et al[54] looked at the vaginal microbial community in four groups of women, pregnant and non-pregnant in those with and without human papillomavirus (HPV) infection. Their findings showed that both being pregnant and HPV infection independently increased vaginal bacterial microbial richness and diversity, with bacterial composition being most influenced by pregnancy, and being both pregnant and HPV positive was associated with a more complex and diverse microbial environment.

DIGESTIVE DISORDERS DURING PREGNANCY

Gastrointestinal tract (GIT) disorders and complaints such as nausea, vomiting, gastroesophageal reflux, heartburn, and constipation are extremely common amongst pregnant women. Three papers investigated the microbiota changes with relevance to GIT symptoms[55] or GIT disorders, such as inflammatory bowel disease (IBD)[56] or malabsorptive states following previous bariatric surgery[57].

In a cross-sectional study, Jin et al[55] studied 71 women with differing GIT complaints; 21 constipation, 24 excessive vomiting, 20 normal pregnancy, six with the severe pregnancy complication of acute fatty liver of pregnancy and 26 non-pregnant subjects. Whilst there were differences within and between symptom groups, the data suggests that the overall abundance of *Acinetobacter, Enterococci, Paenibacillus, Blautis* and *Collinsella* might be associated with GIT symptoms/diseases of pregnancy.

Two articles were published in relation to pregnancy and GIT disorders. An original article by Torres et al[56] studied 73 mother-infant dyads and demonstrated, with 16S rRNA genome sequencing, aberrant gut microbiota composition and lower microbial diversity in pregnant women with IBD compared to controls. This persisted during pregnancy in women with IBD. The alteration in the bacterial diversity and abundance was identifiable in their infant offspring's stool from one week through to 90 days of life. Maternal IBD was the main predictor of microbial diversity in the infant gut, with enrichment of Gamma Proteobacteria and depletion in *Bifidobacteria*. Torres et al[56] replicated this in a germ-free mouse (GFM) model and showed that inoculation with third trimester IBD maternal stool and stool from 90-day infants born of IBD mothers stools showed significantly reduced microbial diversity. They also demonstrated fewer class-switched memory B cells and regulatory T cells in the colon, suggesting that the dysbiotic microbiota triggers abnormal imprinting of the intestinal immune system in GFM[56].

The importance of the GIT and its' absorptive function following bariatric surgery was also highlighted in a study by West et al[57]. They used a parallel metabonomic (molecular phenotyping based on proton nuclear magnetic resonance spectroscopy) and gut bacterial 16S rRNA gene amplicon sequencing to compare pregnant women after two different types of bariatric surgery with malabsorptive/mixed (n=25) and restrictive (n=16) post-operative weight-loss states. Women were of similar early pregnancy body mass index (BMI), as were included controls without bariatric surgery (n=70). Metabolic profiles of their offspring at birth were also included. The study demonstrated that previous malabsorptive, but not restrictive, procedures induced significant changes in maternal metabolic pathways, and a shift in the gut microbiota with a relative increase in *Escherichia/Shigella, Streptococcus* and *Enterococcus* in all higher taxonomic ranks. Whilst bariatric surgery showed altered metabolism in pregnant women which may have beneficial maternal effects the authors suggest that the elevated levels of two of the metabolic compounds, phenolic and indolic, have unknown fetal/infant health implications and require further investigation.

PSYCHIATRY IN PREGNANCY

Acknowledging that self-reported, and likely underreported, rates of maternal anxiety and depression range between 10-20%[58] and Ramsteijn et al[59] stating that up to 10% of women use selective serotonin reuptake inhibitor (SSRI) antidepressants during pregnancy and postpartum, this is an area worthy of further investigation in the microbiota field. Through a rat animal model relevant to depression, gut 16S rRNA gene sequencing and targeted metabolomic analysis Ramsteijn et al[59] were able to demonstrate that the states of pregnancy and that of lactation are different in terms of faecal microbial diversity, the composition which is accompanied by differing metabolite availability[59]. Alongside these findings they were able to demonstrate that SSRI treatment during pregnancy in previously stressed rats altered important features of this transition, with lower faecal amino acid concentrations, which, in turn, correlatively negatively with relative abundance of bacterial taxa including *Prevotella* and *Bacteroides*. Looking further into the impacts of maternal psychological state during pregnancy and the effect on the offspring, Hechler et al[60] in 70 women found an association between maternal anxiety and

faecal microbial composition in late gestation phase, providing the first evidence in a human study that supports a potentially microbially driven initial pathway for the transgenerational behavioural impacts of maternal prenatal psychological stress[60].

One study protocol published in the past year 'Probiotics in pregnancy: protocol of double-blind randomised controlled pilot trial for pregnant women with depression and anxiety (PIP pilot trial) will investigate the role of a commercially available oral probiotic on multiple psychometric scales from enrollment, between 23-25 weeks gestation, to 1 month following birth[58].

FETAL GROWTH

Fetal growth restriction (FGR) is a common and important public health issue which predisposes newborns to inflammatory and metabolic disturbance. Huang et al[61] compared gut microbiota, cytokines and plasma metabolome between FGR and normal birthweight piglets. Raising a cause and effect question, they showed that FGR was associated with significantly impaired small intestinal structure, modified gut microbiota colonization, and disturbed inflammatory and metabolic profiles during the first 12 hours after birth, contributing to the development of inflammation and metabolic diseases. A further study, from Japan, looked at the effects of the human maternal microbiota on fetal growth. Sato et al[62] showed a sex-specific effect of maternal microbiota on fetal growth using 16S rRNA gene amplicon sequencing and short chain fatty acid (SCFA) analysis of maternal faecal samples. They found that maternal gut microbial diversity had a positive association with head circumference in newborn males and identified that *Parabacteroides* and *Eggerthella* genera showed negative associations with newborn head circumference and weight, respectively in males. Conversely, *Streptococcus* showed a negative association in height in female offspring[62].

DIETARY/EXERCISE INTERVENTION AND IMPACT ON PREGNANCY MICROBIOTA SIGNATURES

There is well proven and ongoing evidence that diet/nutrition impacts the microbiota in both human and animal studies. Four animal studies were published in the last year in relation to dietary intake and one exploring exercise during pregnancy. Zhang et al[63] published a paper exploring the use of lactulose, a safe and beneficial molecule, as a form of prebiotic in pregnant mice. Two weeks after daily lactulose administration, a multitude of findings suggested lactulose supplementation benefitted pregnancy performance in mice. These included: a significant increase in *Bifidobacterium* and *Bacteroides* abundance, elevation of beneficial metabolites such as 1-monoolein, glucose-6-phosphate, and short-chain fatty acids, significant reduction in serum glucose and total cholesterol, colonic pH and intestinal permeability, and increased immunoglobulins in colonic epithelial cells and small intestinal absorptive capacity[63].

Dietary effects of both high and low levels of linoleic acid (HLA and LLA) intake and the gut microbiota composition were studied in pregnant and non-pregnant rats by Shrestha et al[64]. The study concluded that consumption of an HLA diet altered gut microbiota composition with significantly lower abundance of the genera *Akkermansia, Peptococcus, Sutterella*, and *Xo2d06* but higher abundance of *Butyricimonas* and *Coprococcus*. Microbiota signatures were also altered in pregnant rats consuming a LLA diet, however, in pregnancy consumption of a HLA diet did not alter gut microbiota composition[64].

Huang et al (65) studied the impact of resveratrol treatment, a natural polyphenolic compound found in grapes and red wine, on the gut microbiota in mice in a comparative study using high-fat diet (HFD). They observed that maternal and postnatal HFD has distinct effects on the gut microbiota metagenome of offspring and that resveratrol treatment; ameliorated the altered plasma propionate level related to maternal and postnatal HFD treatment and improved the altered metabolic dysregulation and dysbiosis seen in HFD exposure[65].

Another prebiotic dietary supplement, mannam oligosaccharide, was studied in pregnant sows and their piglets, showing an improvement in intestinal microbiota with increased *Lactobacillus* and decreased *Escherichia coli*, enhanced intestinal mucosal immune competence, and suppressed intestinal and systematic inflammation in the piglets[66].

Chung et al[67] hypothesised that exercise would positively affect the gut microbiota. Using an exercise and high-fat diet (HFD) mouse model they showed that the relative abundance of five bacterial phyla, Firmicutes, Bacteroidetes, Verrucomicrobia, Deferribacteres, and Actinobacteria, were not significantly altered by diet or exercise during pregnancy. Whilst not being related to alterations in the gut microbial composition, they did however show that murine maternal exercise prevented excess visceral fat accumulation, hyperinsulinaemia, and hyperleptinaemia associated with a HFD[67].

The results of one RCT and a protocol were also published. Given the association with periodontal disease and poor pregnancy outcome, a Peruvian double-blind trial compared xylitol containing and non-xylitol containing toothpaste and their impact on the oral commensal *Streptococcus mutans* in 50 pregnant women. They found no difference in the oral microbiota following 14 days of usage in the mid trimester[68]. An interventional trial protocol was published which plans to randomise administration of the prebiotic, oligosaccharide-sialic acid, from first trimester through to 42 days following delivery, with the aim to explore the intrauterine influence on the long-term health of the offspring[69].

ADDITIONAL MATERNAL MICROBIOME KNOWLEDGE AND MECHANISTIC STUDIES

Dobbler et al[70] published work from a cohort of Brazilian mothers, correlating the vaginal microbiota from women in labour (n=27) and the first pass meconium from their infants, all of whom expect one was delivered vaginally[70]. This study demonstrated three distinct clusters of the vaginal microbiota of women at the time of labour and was significantly correlated with the composition of the infants' gut microbiota as determined by V4 hypervariable region 16S rRNA gene amplicon sequencing.

A series of papers focusing on elucidating mechanistic aspects of gut microbiota changes in association with maternal hormone changes, trace element deficiency, and immune changes during pregnancy were also published during the reporting period. A paper published in Cell Reports looked at the relationship with *Bifidobacterium* and progesterone[71]. This paper demonstrated that *Bifidobacterium* abundance increases in the gut microbiota during pregnancy in both humans and mice, and that progesterone supplementation altered gut microbial composition increasing *Bifidobacterium* abundance, in mice and *in vitro*[71].

Sauer et al[72] developed a mouse model to investigate zinc deficiency in pregnancy acknowledging that 20% of the essential trace element is utilized by intestinal microbiota, and zinc deficiency has been associated with neurological problems of depression, mental lethargy and cognitive impairment, and humans are prone to zinc deficiency during pregnancy. They showed that zinc deficient mice and mice fed with a diet high in zinc uptake antagonists had altered gut microbiota, and that these changes were also accompanied with altered GIT permeability markers, and neuroinflammation with the brain. All of these changes were able to be partially rescued upon zinc amino-acid conjugates[72].

Rothenburg et al[73] investigated the metabolism by methylation/demethylation of methylmercury (MeHg), known to be a neurotoxin in fetal development, by the gut microbiota. They paired the microbiome data with MeHg blood concentrations over the course of pregnancy and concluded that the gut remodeling over the course of pregnancy may change how the body processes dietary methylmercury exposure.

An Australian cross-sectional cohort of 22 pregnant women demonstrated that genus Rosburia is more abundant in the gut microbiota of pregnant women with ketonuria[74], suggesting that butyrate producing bacterium may increase serum ketone levels. The significance of this remains unclear.

Faas et al[75] explored the immune system response to pregnancy, correlating gut microbial composition with splenic and blood immune cells in both germ free and conventional mice. Increases in regulatory T cells and a tendency to increase Th2 cells only in conventional mice, suggests that the microbiota contribute to the adaptation of the maternal immune response to pregnancy[75]. Autoimmunity, in the form of Lupus, has also been explored using a mice model by Mu et al[76]. Whilst reproducing the findings of many previous studies; pregnancy and lactation changed the gut microbiota in their model, but modulation of the same microbiota led to different disease states in post-partum mice. Vancomycin treatment allowed

Lactobacillus animalis to flourish and had opposing and opposite effects on pregnancy naïve mice versus post-partum mice in term of immune cells[76].

Huang demonstrated dramatic remodeling of the gut microbiota at the time of parturition in sows and its' relationship with maternal metabolic changes, detected in serum, driving an environment beneficial to lactation by providing energy from lipid metabolism for milk production[77].

A Japanese study by Tanabe et al[78] also looked at the association with maternal microbial composition and the development of dermatitis in early infancy (DEI) (up to 4 months) or self-reported allergic symptoms at 10 months of infant age. Maternal microbiome analysis occurred at 12 (n=59) and 32 weeks (n=58) gestation. This showed a decreased relative abundance of Proteobacteria at 12 weeks and Actinobacteria at 32 weeks in the group whose infants subsequently developed DEI[78].

DO WE HAVE A FEMALE TRACT/REPRODUCTIVE TRACT MICROBIOTA DURING PREGNANCY?

This has been a question of much debate over the recent years, does the placenta/fetus and uterus harbor a unique microbiota? This review did not miss the opportunity to continue adding to this discussion. Original research by Rackarityte et al[79], used scanning electron microscopy to detect bacteria-like morphological structures in pockets of human fetal meconium in the mid-gestation in 4 subjects, and sparse 16S rRNA sequencing signals in 40 out of 50 fetal intestinal samples[79]. Immune function was also explored and showed that when *Micrococcaceae* was present the fetal intestine exhibited distinct T cell composition and epithelial transcription. Additionally, when *Micrococcus luteus was* isolated in the presence of monocytes, *M. luteus* grew in the presence of placental hormones and remained viable within antigen presenting cells, limiting inflammation *ex vivo* and possessing genomic features linked to the survival of the fetus[79]. Collado and Segata published a subsequent opinion piece emphasizing caution and the need for reproducibility of the above-mentioned paper. Previous research looking for a bacterial presence in the placenta, amniotic fluid and/or meconium required validation amongst other cohorts. To date, there have been unexplained inconsistencies of the bacterial genera detected in these easily contaminated specimens[80].

Kuperman et al[81] also attempted to resolve the controversy regarding the presence of a placental microbiota. Deep microbial analysis using 16S rRNA gene amplification and multiple culture media in 28 human and six mice placentas was performed. Four of the human placentas were further analysed using Gram stain, immunohistochemistry for bacteria, electron microscopy and TaqMan RT-qPCR. They found that none of the placental cultures used for the full analysis, or the environmental cultures, showed bacterial growth[81]. Leoni et al[82] took endometrial biopsies from the uterus of women (n=19) at the time of elective caesarean delivery following the delivery of the newborn, placenta and fetal membranes. They used deep metabarcoding next generation sequencing targeting the V5-V6 hypervariable region of the 16S rRNA gene, identifying *Cutibacterium, Escherichia, Staphylococcus, Acinetobacter, Streptococcus* and *Corynebacterium* as making up the core endometrial microbiota[82].

Weighing in on the existence of a placental microbiota and *in utero* colonization of the fetus was Theis et al[83] who looked at the bacterial profiles of the placenta and fetal brain, lung, liver, and intestine samples in mice though culture, quantitative real-time PCR (q-PCR), and 16S rRNA gene sequencing. These were compared to maternal mouth, lung, liver, uterus, cervix, vagina, intestine and background technical controls. Bacterial loads of all fetal tissue samples were similar to DNA contamination controls and did not yield substantive 16S rRNA gene sequencing libraries. There was no consistent evidence of bacterial communities in the placental or fetal tissues of mice[83].

CONCLUSIONS

This year in review highlights the broad ranging work in the microbiome in pregnancy field and that grappling with the clinical implications of such science is difficult. Preeclampsia was studied and for the first time in several studies, suggesting the gut microbiome has a potential role in preeclampsia aetiology. However further work in multi-ethnic populations is required

to explore these initial findings. Oral and vaginal microbiota have begun to be investigated with regards to GDM associations. Studies on preterm birth and miscarriage highlight the importance of a lactobacillus-dominant vaginal microbiota in reducing these complications, however, as yet, there are not successful human intervention trials that have both successfully manipulated the pregnancy vaginal microbiota and prevented these complications. Regarding pregnancy infection, preliminary work suggests GBS colonization might be decreased with probiotics but requires confirmation. The presence or absence of a placental microbiome (and a fetal microbiome pre-birth) remains a contested space. Twenty-one review papers[84-102] were also published in this last year in attempts to synthesise the current understanding and its clinical implications, with major messages highlighting the confounding factors in microbiome research and small sample sizes. To move the microbiome in pregnancy field forward, the need for both longitudinal and mechanistic studies using metagenomics is now needed.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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