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MICROBIOTA AND PREGNANCY

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Abstract – This article reviews the pregnancy microbiota literature published between April 2020 and March 2021. The literature spans exploration of the microbiota during healthy pregnancies, and those with complications such as Gestational Diabetes Mellitus (GDM), preeclampsia (PE) and pre-term birth (PTB). Additionally, oral and vaginal microbial health, hypothyroidism, fertility and pregnancy loss were also studied. The predominant sequencing methodology used was 16S rRNA with only one human study using shotgun metagenomic sequencing. In addition to traditional microbiome papers defining the microbiome, microbial metabolites were also studied and included within this review.
Of the 70 identified papers, 41 articles contained original research: 25 observational human studies, 16 animal gestational mechanistic, associative studies in mice, rats and one porcine study. Twenty-nine review articles were also published during this period and are not included in this review.
The major findings within the past year support the notions of gut, oral and vaginal microbial stability during pregnancy. Variations in the microbiome being associated with different pregnancy complications, including species level stool microbiota differences in women with and without gestational diabetes, which may in future have value for monitoring of gestational diabetes. The presence of individual-specific microbial signatures preserved throughout the pregnancy has also been noted and the focus of the field has shifted to the functional aspects of the microbiome, its metabolites and their function.

Keywords: Microbiota, Pregnancy, Preeclampsia, Gestational Diabetes Mellitus, Pre-term birth.

WHAT CONSTITUTES A HEALTHY MICROBIOME DURING PREGNANCY?

Maternal Microbiome

Six studies, including a total of 142 pregnant women[1-6], reported on the microbiome in uncomplicated pregnancy. Sakurai et al[1] studied the gut microbiota, three groups studied the vaginal microbiota[2-4], and two groups[5,6] weighed into the ongoing controversy of whether the intrauterine environment, fetus or the placenta harbours a unique microbiome.

Sakurai et al[1] studied 45 women and used 16S rRNA sequencing (V1-2 region) on stool samples to longitudinally classify the gut microbiota of trimester one in Japan. The group also used paired serum analysis investigating glucose/lipid metabolism. Their conclusions were that one phylum, TM7, was significantly reduced in late pregnancy but that the overall gut microbial composition did not change over the course of pregnancy.

Regarding the vaginal microbiome of healthy women, Mehta et al[2] became the first group to define this in an Indian cohort using the V3-5 region of the 16s rRNA. Forty otherwise healthy women had a single high vaginal swab taken at less than 19 weeks' gestation.

Their results are consistent with the community state types (CST) previously described[7], with three bacterial genera *Lactobacillus, Halomonas,* and *Achromobacter* being identified in all samples.

Rasmussen et al[3] took this one step further and assessed the vaginal microbiota at three time points in a Danish cohort of 57 women. Samples were collected at 24 weeks, 36 weeks, and at the time of delivery after the rupture of membranes and prior to birth. The vaginal community structure had dramatic changes in the bacterial diversity and taxonomic distribution, with an overall increase in diversity by the end of the pregnancy. This was demonstrated by a stepwise increase of bacterial relative abundance of most bacterial taxa, namely *Moraxella, Streptococcus, Staphylococcus, Enterococcus, Corynebacterium, and Gemella*, in parallel with a gradual decline of *Lactobacillus* longitudinally. They also identified that whilst change occurred, an individual-specific microbial signature was present throughout the pregnancy.

The human vaginal virome in pregnancy was also examined. Tozetto-Mendoza et al[4] undertook a multipronged investigation to see whether the presence of torquetenovirus (TTV) was associated with a change in matrix metalloproteinase (MMP)-8 and lactic acid levels, as well as defining the vaginal microbiota. TTV is the most abundant component of the human virome. It cannot be cultured and is insensitive to current antiviral treatments but is known to be used for identification of anthropogenic pollution and the assessment of functional immune competence in immunopcompromised individuals[8]. The vaginal microbiome was studied using the hypervariable region V1-3 of the 16S rRNA gene. The change in MMP-8, lactic acid levels and the microbiota were studied longitudinally at two or three time points, either 1st trimester and/or 3rd trimester and/or post-partum in 121 women. The group found the presence of TTV during all time points (43.2, 31.5 and 41.1 percent respectively). They also showed presence of TTV in the first trimester was associated with earlier gestational age at delivery and that *Lactobacillus crispatus* dominance (CST 1) was present in the absence of TTV. The authors concluded that while the presence of TTV is associated with normal pregnancy outcomes, the protective effects associated with the *Lactobacillus crispatus* dominance of higher D-lactic acid isomer and lower MMP-8 was associated with reduced vaginal inflammation and the deleterious cervical alterations are minimal (Table I).

FETAL/PLACENTAL MICROBIOME

In recent years, whether the fetus or placenta harbours a unique microbiome has been a subject of controversy. Al Alam et al[5] and Gschwind et al[6] weighed into this discussion. Al Alam et al[5] concluded that there was indeed a detectable microbiome in 18 fetal lung and 10 placental tissue samples[5]. They also stated that there was increasing beta diversity in the fetal lung samples taken between 11-15 weeks of gestation and between 16-20 weeks. It should be noted that 16S rRNA analysis was used, as whole metagenomic sequencing (WMS) yielded no results owing to the low biomass of the samples, and the sample sizes were small. Al Alam et al[5] concluded that the materno-fetal transfer of microbial DNA is a realistic possibility, one that is hypothesised to prime the developing innate immune system[5].

In contrast, Gschwind et al[6] concluded that the placenta does not harbour a specific, consistent and functional microbiota. This group studied 38 healthy pregnant women who delivered both vaginally and via caesarean and analysed fetal membranes, umbilical cord and chorionic villi. They showed that isolation of meaningful quantities of viable bacteria or bacterial DNA were only possible outside the placenta. Seven chorionic villi samples were chosen for metagenomic analysis, their conclusion was that the bacterial communities were similar to the negative controls and dependant on the database chosen for the comparative analysis.

ORAL HEALTH

Oral health is essential to systemic health and development, hence early detection of disease-causing pathogens and intervention is critical. Yang et al[9] characterised the subgin-

TABLE 1. MICROBIOME CHANGES IDENTIFIED IN THE LAST YEAR REGARDING PREGNANCY COMPLICATIONS BY ANATOMICAL SITE.

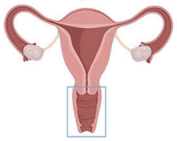
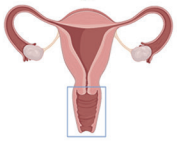
	Microbiome Site			
	Oral 	Gut 	Vaginal 	Placental 
Periodontitis	≈ α diversity ↑ β diversity ↑ *P. intermedia*, *Fretibacterium sp.* HOT360 & *T. denticola* ↓ *R. dentocariosa*			
Gestational diabetes mellitus	↓ α diversity ↑ β diversity ↑ *Selenomonas* & *Bifidobacterium* ↓ *Fusobacteria* & *Leptotrichia*	↓ α diversity ↑ β diversity ↑ ** ↓ ^^		
Pre-eclampsia		↑ microbiota-dependant metabolite TMAO^		
Miscarriage		Collinsella aerofaciens, Roseburia & Arthrobacter ↓ Actinobactera & Verrucomicrobia	↑ α diversity ↑ Proteobacteria, *Pseudomonas*, *U. parvum* & *Mycoplasma hominis*	↑ *Ureaplasma parvum*, *U. urealyticum*,
Preterm birth	↓ *Aggregatibacter actinomycetemcomitans* & *Eubacterium saphenum*		↑ *Gardnerella vaginalis*, *Lactobacillus iners* & *Ureaplasma parvumwas* ↓ Lactobacillus gasseri, Lactobacillus crispatus, or Lactobacillus jensenii	
Fertility			↑ Escherichia coli, Prevotella intermedia, Streptococcus agalactiae & unclassified Shewanella ↓ Lactobacillus iners & Lactobacillus plantarum	

TABLE CONTINUED

gival microbiome of 21 women and their offspring using 16S rRNA gene sequencing in a cross-sectional pilot study, taking maternal oral samples in the third trimester and infant samples between one to three months of age. Significant differences were found in the dominant taxa between maternal and infant microbiome, which remained consistent when accounting for maternal gingival status. Whilst maternal samples were dominated by sever-

TABLE 1 *(CONTINUED)*. MICROBIOME CHANGES IDENTIFIED IN THE LAST YEAR REGARDING PREGNANCY COMPLICATIONS BY ANATOMICAL SITE.

	Microbiome Site			
	Oral 	Gut 	Vaginal 	Placental 
Hypo-thyroidism	↑ Gammaproteobacteria ↓ Firmicutes			
Vaginal infection			≈/↑ α diversity ↑ β diversity ↑ Bacteroidetes, *Actinobacteria*, *Streptococcus sp*p. & Lactobacillus ↓ *Lactobacillus crispatus, Staphylococcus spp., Pseudomonas aeruginosa, Klebsiella pneumoniae, Gardnerella vaginalis, Enterobacter aerogenes, E. cloacae, Candida parapsilosis, C. albicans & Proteus vulgaris*	

**↑ Hemophilus, Gammaproteobacteria, *Nocardiaceae, Saccharibacteria, Fusobacteriaceae, Bacteroides dorei, Bacteroides caccae, Bacteroides massiliensis* & *Bacteroides thetaiotaomicron*;
^^↓ *Christensenellaceae, Clostridiales, Mollicutes, Oxalobacteraceae, Victivallaceae, Rhodospirillaceae, Coriobacteriaceae, Bacteroides vulgatus, Eubacterium eligens, Lactobacillus rogosae* & *Prevotella copri*;
^ TMAO – Trimethylamine N-oxide.

al families such as Streptococcaceae, Fusobacteriaceae, Actinomycetaceae, Veillonellaceae, and Prevotellaceae, the dominant infant taxa were Streptococcaceae. Alpha diversity was significantly higher in the maternal oral microbiome, with more observed features and a higher Shannon index. Within the maternal samples, alpha diversity was inversely related to increasing age, education and income, however when considering maternal gingival status there was no difference in alpha diversity in either maternal or infant groups. There were no associations found between mode of birth, feeding method and the infant oral microbiome. Beta diversity similarly differed significantly between groups, with maternal samples containing multiple caries-associated and commensal organisms. Contrastingly, these were largely absent in the infant group apart from *Megasphaera micronuciformis*, the only periodontal pathogen detected in the infant oral microbiome. These findings provide a preliminary insight into the structure and relationship between maternal and infant oral microbiomes, providing opportunity for future research into factors that influence the developing oral microbiome[9].

Whilst recent studies have linked preterm low birthweight (PLBW) to culturable periodontal pathogens, a significant percentage of oral microbes are unculturable. Xia et al[10] investigated the role of unculturable periodontal pathogens in the context of PLBW in a prospective longitudinal observational study of 90 pregnant women using PCR and ELISA analysis. The women were split into periodontitis/gingivitis (n=70) and healthy (n=20) groups, according to periodontal status. The length of time spent brushing teeth was shorter in the periodontitis/gingivitis group, and saliva levels of pathogens differed between groups. *A. actinomycetemcomitans, T. forsythia, T. denticola, P. gingivalis, Fretibacterium sp.* HOT 360, and *P. intermedia* were significantly enriched whilst levels of *R. dentocariosa* were significantly lower in saliva of the periodontitis/gingivitis group. Twenty-two of the

90 women delivered PLBW infants, with no significant differences found in subject characteristics between groups. *E. Saphenum* was significantly higher in the non-PLBW group, and correlated with gestational week at birth, however there were no other significant differences in pathogens between the groups. Ordinal logistic regression analysis indicated that low levels of *R. dentocariosa* and high levels of *P. intermedia, Fretibacterium sp. HOT360* and *T. denticola* in saliva were significantly correlated with gingival inflammation in pregnancy. Furthermore, serum IgG against *A. actinomycetemcomitans* and *E. saphenum* were negatively correlated with delivery of PLBW infants.

GESTATIONAL DIABETES MELLITUS (GDM)

Gut microbiota has previously been shown to be linked with GDM[11]. In the past year, 4 additional human studies have sought to elucidate the specific species involved in gut dysbiosis, providing potential new non-invasive avenues for the monitoring and management of GDM.

Wu et al[12] performed a case control study in pregnant Chinese women to try and verify potential intestinal microbial markers of GDM using two approaches. First, third trimester stool samples from 23 GDM and 26 non-GDM patients were compared using WMS. Potential biomarkers were then verified using Q-PCR evaluation of the stool samples of another cohort of 37 GDM and 112 non-GDM patients. Alpha diversity at the species level was not significantly lower in women with GDM (p=0.18) compared with healthy controls. Beta diversity however was significantly different, with Bacteroides dorei being positively correlated with GDM (as diagnosed by the Oral Glucose Tolerance Test) in both analyses[12]. As these were third trimester markers, these microbial markers would not be useful in the screening or diagnosis of GDM: however, they have potential applications for GDM monitoring and therapy.

Xu et al[13] conducted a case-control study utilising 16S rRNA sequencing to compare third trimester intestinal and oral microbiota of women with and without GDM. Similar to Wu et al[12], they found not significantly lower alpha diversity in GDM patients, with significantly different beta diversity in comparison to non-GDM controls[12]. GDM patients exhibited increased levels of *Gammaproteobacteria* and *Hemophilus* in intestinal microbiota, and *Selenomonas* and *Bifidobacterium* in oral microbiota. *Fusobacteria* and *Leptotrichia* were decreased in oral microbiota. ROC curve analysis for GDM prediction using intestinal *Hemophilus* and oral *Leptotrichia* were 0.66 and 0.70 respectively[13]. Thus, these may also represent potential microbial markers for monitoring GDM.

Festa et al[14] also compared third trimester intestinal microbiota between 10 women with GDM, and 10 healthy pregnant controls. Whilst microbial diversity was lower in GDM women compared to controls, this result was not statistically significant (p=0.08). However, women with GDM were found to have higher relative abundances of *Bacteroides caccae, Bacteroides massiliensis* and *Bacteroides thetaiotaomicron*, with lower levels of *Bacteroides vulgatus, Eubacterium eligens, Lactobacillus rogosae,* and *Prevotella copri*. This study however was limited by its small sample size, and the analysis of only one stool sample per subject[14].

Meanwhile, Gao et al[15] focused on early pregnancy differences in intestinal microbiota between women with hyperglycaemia and those with normal glucose. 16S rRNA sequencing was used to compare the stool sample microbiome of 22 women with hyperglycaemia (according to the International Association of Diabetes and Pregnancy Study Groups in 2010

Criteria), and 28 age-matched normoglycemic controls at their first prenatal visit <20 weeks' gestation. In concordance with the previous studies, beta diversity was significantly different between the two groups, with lower microbial diversity observed in women with hyperglycaemia. *Nocardiaceae*, *Saccharibacteria_norank*, and *Fusobacteriaceae* were found to be significantly higher in pregnant women with hyperglycaemia, whilst *Christensenellaceae, Clostridiales_vadinBB60_group, Mollicutes_RF9_norank, Oxalobacteraceae*, *Victivallaceae*, *Rhodospirillaceae*, and *Coriobacteriaceae* were higher in controls. Associations with blood glucose metabolism were also demonstrated, with HbA1c positively correlated with *Proteobacteria*, *Fusobacteria*, *Saccharibacteria*, *Bacteroidaceae, Enterobacteriaceae*, and negatively correlated with *Lentisphaerae, Ruminococcaceae, Christensenellaceae, Victivallaceae, Rhodospirillaceae, and Micrococcacea*[15]. The presence of early pregnancy dysbiosis suggests microbial markers may have potential diagnostic value for GDM.

PREECLAMPSIA

Like GDM, preeclampsia is a common complication of pregnancy, and the microbiome is postulated to play a role in its development[11]. Huang et al[16] investigated the potential role of the microbiota-dependant metabolite trimethylamine-N-oxide (TMAO). TMAO was shown to change over the course of the pregnancy, however the role it plays in preeclampsia is harder to elicit. In 264 pregnant subjects; 198 women were healthy controls, 17 had early onset preeclampsia (<34 weeks) and the remaining 49 late onset preeclampsia. Samples were collected in the second trimester and at delivery. TMAO levels were significantly raised at delivery in early onset preeclampsia and patients with severe preeclampsia at the time of delivery whilst controlling for confounding factors. These findings suggest TMAO may have a role in the acceleration of preeclampsia rather than instigating disease[16].

FERTILITY

As a healthy female reproductive tract environment is necessary for successful pregnancy, it therefore follows that the non-pregnant vaginal microbiome may contain biomarkers reflective of a woman's fertility. Using 16s rRNA sequencing, Xu et al[17] analysed the vaginal microbiome of 85 women – 40 with tubal obstruction, and 45 with normal reproductive function. Women with tubal obstruction had higher levels of *Escherichia coli*, *Prevotella intermedia*, and unclassified *Shewanella*. *Escherichia coli* was also more abundant in women with prolonged menstrual cycles and antral follicle count >15. Basal oestradiol level also impacted on vaginal microbiome, with women with higher-than-normal levels of basal E2 (>166 pg/mL) shown to have higher levels of *Streptococcus agalactiae* (also known as Group B *Streptococcus*), and fewer *Lactobacillus iners* and *Lactobacillus plantarum*. Of note, *Streptococcus agalactiae* is often implicated in aerobic vaginitis. Thus, analysing the relative abundances of these bacteria may provide biomarkers of a woman's reproductive health[17].

MISCARRIAGE

Both human and mouse studies have been published in the past 12 months investigating possible associations between the maternal microbiota and miscarriage. Tao et al[18] studied the effects of both pathogenic *E. faecalis* OG1RF and probiotic *E. faecalis* Symbioflor 1 on spontaneous abortion using murine models. In vitro assays demonstrated the antagonistic action of *E. faecalis* Symbioflor 1, which inhibited the translocation of *E. faecalis* OG1RF and alleviated its epithelial damage *in vivo*. *E. faecalis* Symbioflor 1 intervention enhanced the integrity of the uterus, placenta, ileum and colon, and increased the density of placental blood cells. Further investigation of in vivo action revealed *E. faecalis* Symbioflor 1 attenuated the rate of spontaneous abortion in *E. faecalis* OG1RF positive mice from 57±33% to 29±6%18. These promising results indicate the need for further investigation in human trials.

The serious physical and emotional impacts of miscarriage make them an important target for the development of improved methods of early detection and intervention. Fan et al[19] used 16S rRNA sequencing to characterise the vaginal flora of 27 women with unexplained recurrent miscarriage and 31 women with induced abortions (termination of pregnancy) in order to investigate the pathogenesis of recurrent miscarriage in relation to dysbacteriosis. Recurrent miscarriage samples displayed higher alpha diversity and relative abundance of *Proteobacteria*, *Pseudomonas*, *Collinsella aerofaciens*, *Roseburia* and *Arthrobacter,* whereas induced abortion samples had relatively abundant *Actinobacteria* and *Verrucomicrobia*. The expression of chemokines CCL4/CCL8/CCL3/CCL5/CCL2 was also significantly higher in the recurrent miscarriage samples.

Oliveira et al[20] examined the influence of *Mollicutes*, specifically *Ureaplasma parvum,* on miscarriage in a case-control study of 89 women who had first trimester miscarriage and 20 women who had normal pregnancies. PCR was used to analyse samples of placental tissue and cervical mucus. The risk of miscarriage was increased sevenfold if *Mollicutes* were detected in placental tissue. *U. parvum* in placental tissue was also associated with miscarriage and

was detected in the placental tissue of 66% of women who miscarried. Furthermore, microbial load in placental tissue of *U. urealyticum, U. parvum,* and *M. hominis* was significantly higher in women who experienced miscarriage than the control group. These results have important clinical implication, as dysbiosis of vaginal flora may be a potential therapeutic target in recurrent miscarriage[20].

PRETERM BIRTH

Preterm birth is defined as delivery before 37 weeks of gestation and is the worldwide leading cause of death in children under five. Hence, understanding contributing factors and predicting its occurrence is crucial[21].

Volatile organic compound (VOC) analysis is a non-invasive tool utilised to monitor the microbiota's metabolic activity and host response. Recently, VOCs have been established as biomarkers for several diseases[22]. Lacey et al[22] investigated the use of VOC analysis as a tool for predicting preterm birth in an observational cohort study of 216 pregnant subjects between 10-29 weeks' gestation. VOC analysis using vaginal swabs varied depending on when the samples were taken. Mid-trimester vaginal swabs yielded sensitivity of 0.66 (95% CI 0.56-0.75) and specificity 0.89 (95% CI 0.82-0.94), whilst vaginal swabs taken closest to delivery produced sensitivity of 0.73 (95% CI 0.64-0.81) and specificity of 0.90 (95% CI 0.82-0.95)[22]. These results strongly suggest VOC analysis could be used as a predictive tool for preterm birth.

Payne et al examined the relationship between the vaginal bacterial DNA signature and risk of preterm birth in 936 pregnant women in order to develop a microbial DNA test to determine increased risk of spontaneous preterm birth[23]. PCR analysis of vaginal swabs self-collected between 12-23 weeks' gestation revealed no single microbial target predicted risk, however significantly higher levels of *Lactobacillus gasseri, Lactobacillus crispatus,* or *Lactobacillus jensenii* were detected in women who delivered at term (31.2% term *vs.* 13.8% spontaneous preterm birth, $p=0.005$). Most notably, a specific microbial DNA signature consisting of *Gardnerella vaginalis*, *Lactobacillus iners*, and *Ureaplasma parvumwas* (termed GLU) was able to predict spontaneous preterm birth at <37 weeks (sensitivity 37.9%, likelihood ratio 2.22) and ≤34 weeks (sensitivity 44.4%, likelihood ratio 2.52). Additionally, GLU-positive subjects were also more than twice as likely to have preterm premature rupture of membranes[23]. The identification of a unique DNA signature that can predict increased risk of spontaneous preterm birth has clinical potential in guiding antimicrobial therapy.

Parris et al[24] reviewed the current understanding of the disputed placental microbiome in pregnancy, specifically the pathogenesis of placental inflammatory responses associated with spontaneous preterm birth, highlighting potential biomarkers.

THYROID DISORDERS

Whilst both hyperthyroidism and hypothyroidism can influence pregnancy outcome, Wang et al[25] focussed on hypothyroidism, and whether changes in the microbiome may offer insight into its development during pregnancy. Using 16S rRNA sequencing followed by validation with quantitative real-time PCR (qPCR), Wang et al[25] found significant differences in the oral and intestinal microbiome of 30 pregnant women with hypothyroidism, and 31 normal controls. Women with hypothyroidism had a higher relative abundance of *Gammaproteobacteria*, whilst *Firmicutes* were higher in the control group. Hypothyroidism was also associated with higher serum C-reactive protein level, greater weight gain during pregnancy, and increased incidence of fetal distress[25].

INFECTION/AEROBIC VAGINITIS

Aerobic vaginitis (AV) is vaginal dysbiosis dominated by aerobic bacteria associated with inflammation and atrophy of the vaginal epithelium[26]. Due to the potential pregnancy complications, increased risk of contracting STIs and development of pelvic inflammatory disease,

understanding AV risk factors and effective treatment options are essential for the health of women and their offspring. Wang et al[27] set out to characterise the structure of the vaginal microbiota in non-pregnant women with aerobic vaginitis in a case-control study of 80 women with and 160 women without AV, using 16S rRNA gene sequencing. Amongst healthy subjects, *Firmicutes* were the dominant phylum, of which *Lactobacillus crispatus* and *L. iners* were the dominant genera. AV was associated with a more diverse microbiota, enriched with *Bacteroidetes* and *Actinobacteria phyla*, as well as a significant and striking decline in *L. crispatus* and increase in aerobes such as *Streptococcus anginosus* and *S. agalactiae.* Resistance to erythromycin and clindamycin amongst gram-positive bacteria, and to ampicillin amongst gram-negative bacteria was high, highlighting the need for new treatment options. Considering the above results, a potential supplementary agent may be probiotics containing *L. crispatus.*

In another case-control study, Tang et al[28] characterised the vaginal microbiota of 246 women in late pregnancy (>35 weeks) and 204 non-pregnant women with AV using microscopy. Whilst there was no significant difference in the bacterial diversity between the two groups, distribution of isolated pathogens differed significantly. Within the whole cohort, 94% of the isolated bacteria consisted of 32% *E. coli*, 22% *Staphylococcus* spp., 19% *Enterococcus* spp., 19% *Streptococcus* spp., and 8% *Lactobacillus*. Amongst pregnant subjects with AV, relative abundance of *Streptococcus* spp. and *Lactobacillus* were higher whilst in non-pregnant subjects *Staphylococcus* spp. and other bacteria including *Pseudomonas aeruginosa*, *K. pneumoniae*, *G. vaginalis, C. albicans, E. aerogenes, E. cloacae, Candida parapsilosis,* and *Proteus vulgaris* were found in higher abundance. Antibiotic susceptibility tests revealed some (13.4%) multi-drug resistant isolates; however, all isolates were susceptible to nitrofurantoin. All gram-positive cocci were susceptible to linezolid and vancomycin, and *E. coli* was susceptible to meropenem, ertapenem, amikacin, and imipenem. Additionally, when compared to pregnant women without AV, AV-positive subjects had higher rates of premature rupture of membranes, history of vaginitis and neonatal infection[28].

Bacterial vaginosis (BV) is another common vaginal dysbiosis distinct from AV due to lack of inflammation and dyspareunia, as well as characteristic malodourous discharge. BV is also associated with adverse pregnancy outcomes such as preterm birth via ascending infection[26]. In a recent review, Basavabhu, Sonu & Prabha[29] described the mechanism of probiotic intervention against BV as 'colonisation resistance'; including antibiofilm, anti-adhesion, co-aggregation, antibacterial, and host immunomodulation activity to induce an unfavourable environment for BV pathogens. The use of probiotics to reduce incidence of preterm birth both protects against BV and prevents matric metalloprotease (MMP-9) degradation of tissue by binding the extracellular matrix. Probiotics are theorised to stimulate the host inflammatory cascade via interaction between probiotic Microorganisms Associated Molecular Patterns (MAMPs) and host Pattern Recognition Receptors (PRRs) to upregulate systemic anti-inflammatory markers. However, probiotic monotherapy to counter BV and PTB requires further exploration to clarify efficacy.

ANTIBIOTIC RESISTANCE

Antibiotic resistance poses a threat to global public health as it hinders infection control. Antibiotic resistance genes (ARG) in the neonatal gut microbiome suggests maternal transmission of these genes, however the exact mechanism of transmission remains unclear. Sosa-Moreno et al[30] investigated the effects of perinatal exposures and maternal characteristics on ARG and mobile genetic element (MGE) patterns, which transport genes between gut microbes. The resistome, made up of all the ARG in the microbiome, and the mobilome, made up of all the MGE, are responsible for host antibiotic resistance. Of the total 91 participants, 72 were part of mother-infant dyads, 4 were supplementary infants and 15 were supplementary pregnant women. ARG detection numbers ranged from 11-74 in pregnant women and 38-65 in infants, while MGE detection numbers ranged from 1-17 in pregnant women and 9-20 in infants. The most frequently detected genes were transposon related sequences and tetracycline resistance genes. On average, within the mother-infant dyads 24% of MGE and 29% of ARG were shared. Overall structure and composition of ARG and MGE differed sig-

nificantly between infants and pregnant women, with infants having higher alpha diversity. In infants, composition of the resistome was associated with solid food consumption, and in pregnant women, with race.

OFFSPRING IMPACTS FROM PREGNANCY INTERVENTIONS

Offspring – Metabolic Profile

Several animal studies have explored the effects of the maternal microbiome on offspring metabolic profiles. Intrahepatic cholestasis of pregnancy (ICP) is associated with adverse maternal and fetal outcomes. Pataia et al[31] investigated the action of obeticholic acid (OCA), which indirectly decreases bile acid synthesis, on both maternal and fetal lipid and bile acid profiles when administered during hypercholanemic pregnancy in mice. Pregnant C57BL/6J mice were split into control, cholic acid (CA) supplemented, CA and OCA supplemented and OCA supplemented groups and bile acid and lipid levels coecal microbiota, and maternal and fetal morphometry were examined. OCA supplementation decreased bile acid levels in the fetal compartment and had no detrimental effect on fetal or maternal morphometry. However, fetal and maternal dyslipidemia persisted, and maternal bile acid levels were unchanged. Nonetheless, improved fetal hypercholanemia in this study warrants further investigation into the potential use of OCA during cholestatic pregnancy.

He et al[32] examined the effects of late gestation heat stress (LGHS) on maternal microbial transfer and offspring metabolic profile and microbiota in pregnant sows. LGHS significantly altered the maternal intestinal, maternal vaginal and infant intestinal microbial community. LGHS reduced the relative abundance of *Spirochaetes* by 40% and enriched *Eubacterium coprostanoligenes* and *Ruminococcaceae UCG-005* in sow intestinal samples. In vaginal samples, LGHS increased the proportion of *Bacteroidetes* and *Proteobacteria*, and decreased the proportion of *Firmicutes*. Piglets exposed to LGHS in utero had larger populations of opportunistic pathogens and far fewer maternally transmitted commensal bacteria. LGHS reduced maternal intestinal and vaginal microbial transmission from 46% to 12% and 46% to 22% respectively. Furthermore, environmental source transmission increased with LGHS from 17% to 52%. LGHS exposure increased offspring serum cholesterol, adrencorticotrophic hormone, and low-density-lipoproteins, signalling activation of neonate hypothalamic-pituitary-adrenal axis due to maternal LGHS. Considering the potentially adverse neonate and maternal outcomes of LGHS in a context of global warming, this area also warrants further research in human studies.

Offspring – Neurodevelopment

Neonatal morbidities are associated with long term neurological deficits and have also been associated with dysbiosis. Lu et al[33] and Vuong et al[34] both studied maternal gut microbiota impact upon fetal neurodevelopment.

Lu et al[33] performed an interventional mouse study and tested whether manipulation of the maternal gut microbiota *via* probiotic supplementation could optimise the neonate's microbiome and improve their neurodevelopmental outcomes. *Lactobacillus acidophilus* and *Bifidobacterium infantis* was administered to pregnant mice daily from embryonic day 16 to weaning.

Significantly reduced peripheral proinflammatory state postnatally was seen in the dam and offspring. The suppressed neuroinflammation by maternal lactobacillus supplementation was associated with reduced astrocyte/microglia activation and downregulation of the transcriptional regulators CEBPD and IκBα. Furthermore, maternal lactobacillus supplementation promoted neuronal and oligodendrocyte progenitor cell development in the offspring.

Vuong et al[34] investigated how depletion and selective reconstitution of the maternal gut microbiome influences fetal neurodevelopment in mice. Embryos from antibiotic treated mice as well as germ free mice demonstrated reduced brain expression of genes related to neuronal axon development. Metabolomic profiling showed that the maternal microbi-

ome regulated numerous small molecules in the maternal serum and fetal brains. Select microbiota-dependent metabolites promoted axon outgrowth from fetal thalamic explants. Moreover, maternal supplementation with these metabolites abrogated deficiencies in fetal thalamocortical axons. Manipulation of the maternal microbiome and microbial metabolites during pregnancy yielded adult offspring with altered tactile sensitivity in two aversive somatosensory behavioural tasks, but no overt differences in many other sensorimotor behaviours. These findings suggest that the maternal gut microbiome promotes fetal thalamocortical axonogenesis, possibly via signalling by microbially modulated metabolites to neurons in the developing brain during gestation.

Offspring – Myogenesis

Song et al[35] studied both mice and chicken embryos to show the interrelated pathways relating to the regulation of somitogenesis and myogenesis.

Through animal model validation experiments they demonstrated that elevated levels of lipopolysaccharides (LPS) were seen in gut microbiota dysbiosis. They also showed LPS-induced reactive oxygen species contributed to the interference of retinoic acid signalling. This perturbation was primarily responsible for somite formation and differentiation which led to the finding of reduced muscle fibre diameter, inhibited limb development[35].

Offspring complication – Necrotising Enterocolitis

Yu et al[36] tackled the most common gastrointestinal disorder in premature neonates - necrotising enterocolitis. The condition is characterised by gut epithelial necrosis, gut barrier dysfunction and poor mucosal defence development[36]. Prior studies have suggested benefit of probiotic use in premature infants to prevent necrotising enterocolitis[37], however the benefits of maternal probiotics to prevent development of the disease in their infants is unclear. Yu et al[36] administered maternal probiotic supplementation with *Lactobacillus acidophilus* and *Bifidobacterium infantis* from embryonic day 15 to 2 weeks post-natal in mice. This exposure facilitated intestinal epithelial cell differentiation, prevented loss of mucin and preserved the intestinal integrity and barrier function and decreased serum levels of IL-1β, TNF-α and IL-6 in the pre-weaned offspring[36].

Offspring – Immunity

Nyangahu et al[38] used a mouse study to explore whether there were longstanding implications on maternal and offspring gut microbiota and offspring immunity following maternal helminth exposure prior to conception. During gestation dams with previous exposure to *Nippostrongylus brasiliensis* showed significantly altered gut microbiota with increased abundance of Enterococcaceae compared with previously uninfected dams. This difference was also observed in the breastmilk of dams. In relation to the offspring there was also significantly altered gut microbiota with an increased relative abundance of *Coriobacteriaceae* and *Micrococcaceae* in offspring born to previously infected dams. The immune system alteration in offspring of those born to previously uninfected dams revealed increased numbers of activated CD4 T cells in the offspring spleens. Nyangahu et al[38] proposed that preconception helminth infections impact offspring immunity, possibly through alteration of maternal and offspring microbiota.

Supplementation – Resveratrol

Zha et al[39] investigated the protective effects of Resveratrol administration in pregnant mice. Due to the increased energy requirements of reproduction, maternal oxidative stress is significantly increased, reducing quantity of lactation and therefore affecting offspring. Resveratrol

is an antioxidant and anti-inflammatory polyphenolic produced by many plants. Resveratrol was found to modify mammary gland antioxidant capacity and mitophagy, with increased oxidation of fat cells and colloid in the follicles, and increased expressions of transcription factors and proteins related to cell autophagy. Immune effects of resveratrol supplementation in pregnant mice included reduced serum levels of antioxidants and proinflammatory factors, as well as modifying ileal and jejunum surface immune modifying factors. Additionally, Resveratrol remodelled the composition of the intestinal microbiota, resulting in increased alpha diversity and enrichment of *Acidobacteria*, *Bacilales*, *Staphylococcaceae*, and *Staphylococcus*.

TOXIN EXPOSURE DURING PREGNANCY

Pregnancy is a time of great stress and uncertainty for many pregnant women, their partners and care providers when it comes to questions of diet, medications and environmental exposures.

Liu et al[40] sought to investigate the impact of pregnancy exposure to graphine oxide (GO) in mice. They showed that early oral exposure (gestational day 7-16) was associated with dose-dependent pregnancy complications including decreased weight of the dam and live fetus, high rate of embryo resorption and miscarriage or fetal skeletal abnormalities. The gut microbiome analysis showed dramatically decreased α- and β-diversity, and upregulated *Firmicutes*/*Bacteroidetes* ratio associated with GO exposure. The significantly differentiated abundance of *Euryarchaeota* is expected to be a special biomarker for failed pregnancy caused by GO. Liu et al's Spearman correlation analysis[40] suggesting a strong link (correlation coefficient > 0.6) between perturbed gut microbiome, and both abnormally expressed factors of the placental barrier and adverse pregnancy outcomes. With the increasing use of GO in environmental and biomedical materials the result of this study should continue to be explored if GO is to be used in products marketed at women of reproductive age.

Dechlorane Plus (DP), a flame retardant substance used in electrical plastics, is another substance that has been studied in rats during pregnancy. Zhang et al[41] investigated whether DP could be transferred from mother to infant, and if such transfer could influence the composition of the infant gut microbiota. Using 16S rRNA sequencing they found that DP exposure decreased the richness and diversity of the gut microbiota, particularly at genus level. Furthermore, in DP exposure groups, the gut microbiota production of metabolites of short-chain fatty acids was dramatically increased. The influence on metabolic functions, causing long-term impact to offspring, indicates that more attention should be paid to the long-term health effects related to DP exposure[41].

Yan et al published two papers[42,43] following environmental exposure to the neonicotinoid insecticide nitenpyram in mice during pregnancy. The first paper[43] investigated the health effects on female offspring. They found decreased levels of serum triglycerides, total cholesterol, and glucose as well as gut microbiota disturbances with accompanying abnormal faecal metabolic profiles. Pearson correlation analysis found that the harmful effects of nitenpyram appear to be mediated via decreased abundance of *Lactobacillus*. The changes in gut bacterial purine metabolism, branch chain amino acid metabolism, and the Tricarboxylic acid (TCA) cycle are all closely related to the abundance of Lactobacillus. The next paper published[42] aimed to assess the metabolic effects of exposure and their mechanisms. Mice exposed to nitenpyram from gestational day 6 to gestational day 19 showed increases in Desulfovibrio strains and the concentration of hydrogen sulfide, both of are known to destroy colonic mucosa and increase intestinal inflammation and bacterial translocation, leading to non-alcoholic steatohepatitis (NASH)[42].

Yan et al also investigated the role of a metabolite of the pesticide endosulfan, endosulfan sulfate (ES), on the progress of obesity and metabolic disorders in high fat (HFD) and low-fat diets (LFD) in mice during pregnancy[44]. ES was found to alleviate the development of obesity and accumulation of hepatic triglycerides induced by HFD. Following analysis of gene expression, metabolic profiling and the gut microbiome they showed that ES treatment inhibits adipogenesis induced by HFD due to enhanced lipid catabolism, fatty acid oxidation and disturbance of gut microbiota composition. On the other hand, impaired glucose and insulin homeostasis were still conserved in HFD-fed mice exposed to ES. Furthermore, ES treatment impaired glucose tolerance, affected hepatic gene expression, fatty acids composition and se-

rum metabolic profile, as well as disturbed gut microbiota in LFD-fed mice[44]. With the aim of enhancing our understanding of the complex interactions of metabolic disease, environmental pollutants and diet in early life stages. Yan et al[42] showed that at acceptable daily intake levels of ES during pregnancy there was direct impact on glucose metabolism, hepatic lipid metabolism and the gut microbiome dependant on the type of diet consumed.

Similarly, Snow et al[45] observed the influence of maternal HFD on the metabolic response to ozone exposure in the subsequent rat offspring. Offspring from HFD dams had increased body fat and weight relative to control diet (CD). Metabolomic analysis revealed significant sex-, diet-, and exposure-related changes. Maternal HFD was associated with increased free fatty acids and decreased phospholipids (male > female) in air-exposed rats. Microbiome-associated histidine and tyrosine metabolites were increased in both sexes, while 1,5-anhydroglucitol levels decreased in males indicating susceptibility to insulin resistance. Ozone decreased monohydroxy fatty acids and acyl carnitines and increased pyruvate along with TCA cycle intermediates in females (HFD > CD). Ozone increased various amino acids, polyamines, and metabolites of gut microbiota in HFD female offspring indicating gut microbiome alterations. Collectively, Snow et al's data[45] suggests that maternal HFD increases offspring susceptibility to metabolic alterations in a sex-specific manner when challenged with environmental stressors during pregnancy.

Liu et al[46] studied the impact in mice of inhaled fine particulate matter (PM2.5) during pregnancy and its association with adverse pregnancy outcomes. Offspring birth length and weight was significantly lower in the exposed group compared to the control group. In dams, tracheal PM2.5 exposure was associated with changes in the distribution and structure of gut microbiota. At the phyla level, compared to dams in control group, mice in the PM2.5 group had higher ratio of phyla *Proteobacteria*, *Candidatus Saccharibacteria* and *Fusobacteria* and lower ratio of phyla *Acidobacteria*, *Gemmatimonadetes* and *Deferribacteres* in the gut. Compared with control group, the concentration of isobutyric acid was higher in PM2.5 group, but butyric acid concentration was lower in PM2.5 group.

DISCUSSION

This year in review builds on the ever-expanding field of microbiome research in relation to normal pregnancy and changes seen in pregnancy complications. Many physiological adaptations occur during pregnancy, including immunological, metabolic, and hormonal changes. We know that alterations in the maternal microbiota both causes, and results from, physiological and pathological changes during pregnancy. These changes have been examined in both human and animal studies analysing gut, vaginal and oral microbiota. This year revealed publications which demonstrated microbial stability during pregnancy and the presence of individual-specific microbial signatures preserved throughout the pregnancy.

The pregnancy microbiota research space is beginning to investigate the of mechanisms of action, studying the metabolites and/or the volatile organic compounds within the anatomical niches alongside the microbiota. Such as the area of preterm birth, where vaginal samples are being put forward as a means of screening women for individualised preterm birth risk. Similarly, the species level analysis within the GDM studies are also now leading to questions of whether risk profiling in early pregnancy may be able to predict GDM development or whether evolution of the species once GDM is diagnosed can be useful in tracking the disease state.

Consistent with the shift observed in the wider microbiome research community, the mechanisms of microbiome function are entering the pregnancy microbiome research field, with animal studies examining functional metabolites of the gut microbial communities. Whilst this paper focussed on the original research published in the past year there were also 30 review articles published including topics exploring the establishment of the early infant gut microbiome, early life origins of cardiometabolic risk factors and the hygiene hypothesis. Others reviewed pregnancy complications: the hypothesised causes and management options for GDM, PE and perinatal depression, and the placental microbial-metabolite profiles in PTB. Gynaecological management of BV with probiotics to prevent pregnancy complications and the gut microbiome implications of insulin resistance and PCOS development and the subsequent impacts on pregnancy.

CONCLUSIONS

The shift to mechanistic studies is promising within this field of research and the future inclusion of deep metagenomic sequencing to further explore the species level impact of microbial effects on uncomplicated and complicated pregnancies is an exciting area of evolving scientific research.

Conflict of Interest

The authors declare no conflict of interest.

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