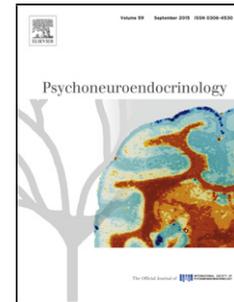# Accepted Manuscript

Title: Emerging literature in the microbiota-brain axis and Perinatal Mood and Anxiety Disorders

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Title: **Emerging Literature in the Microbiota-Brain Axis and Perinatal Mood and Anxiety Disorders**

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Highlights:

- The immune system is a key factor in Perinatal Mood and Anxiety Disorders (PMAD)

- Compositional changes in microbiota during pregnancy interact with immune and stress systems

- Current literature focuses on infant effects and lacks robust maternal measures

- There is a need for research investigating PMAD and the microbiota-gut-brain axis

Abstract:

Perinatal Mood and Anxiety Disorders (PMAD) are common and can cause significant morbidity and mortality for mother and child. A healthy perinatal period requires significant adaptations; however, systems can become imbalanced resulting in depressive and anxiety symptoms. The interface between the microbiome, the immune system, and the stress system may be a model for understanding mechanisms underlying PMAD. Emerging literature from general populations regarding immune, hormone, and HPA axis changes in relation to the microbiome combined with literature on immune, gonadotropin, and stress systems in the perinatal period provides a background. We systematically

investigated literature in the developing field of the microbiome in relation to PMAD. Our inclusion criteria were 1) reporting measure of maternal mood, stress, or anxious or depressed behavior; 2) in the perinatal period, defined as pregnancy through one year postpartum; and 3) reporting measure of maternal microbiome including manipulations of the microbiome through prebiotics, probiotics, or interventions with microbial byproducts. The review identified research studying associations between stress and maternal microbiome; dietary impacts on microbial composition, mood, and stress; and the relationship between the microbiome and the immune system through immunoregulatory mechanisms. Important themes identified include: the importance of studying the maternal microbiome and measures of stress, anxiety, and depression and that multi-hit models will be needed as research strives to determine the effects of multiple mechanisms working in concert.

Keywords: depression; anxiety; microbiome; pregnancy; stress; immunity

1.0 Background

Perinatal Mood and Anxiety Disorders (PMAD), including Antenatal Depression (AND) and Postpartum Depression (PPD), are one of the most common postpartum complications affecting 10-20% of perinatal women.[1–7] In severe cases, these disorders can be crippling,[8,9] and in developed nations, suicide is one of the largest contributors to maternal mortality.[10–13] In addition to their negative impact on maternal health, PMAD can have long-term adverse effects for children, including delayed cognitive and socio-emotional development and poor mental health outcomes later in life.[14,15]

Currently studied risk factors have been unable to account for significant variance in PMAD cases, resulting in limited prevention, diagnosis, and treatment options.[16–21] A biopsychosocial model of PMAD describes how biological (discussed later in this paper), psychological (i.e. coping skills and views of self), and social (i.e. social support or economic status) factors interact to produce depression and anxiety.[16] Normal adaptive changes occurring during the perinatal period would otherwise be considered pathological in nonpregnant women.[22] The suspension of ovulation, development of placenta and fetus, and delivery require alterations of maternal stress systems (e.g., the Hypothalamic-Pituitary-Adrenal axis (HPA axis) and the parasympathetic/sympathetic systems), immune system, and metabolism. For most women, these alterations ensure good health through pregnancy; however, hormones and neurotransmitters altered by pregnancy have important implications for mental health.[22] In the case of PMAD, alterations of these systems have gone awry. Over the last decade, our understanding of the role the microbiome in human health, disease, and development has increased. Emerging evidence suggests that a woman's microbiome may be a biological component influencing the metabolic states of pregnancy.[23] Pregnancy-associated changes in microbial composition may have broader implications than metabolism. Current research suggests the microbiota also participate in the development and regulation of the immune and central nervous systems (CNS), thereby contributing to depressive or anxiety symptoms in the host.[24,2526,23,27–31] The microbiome may be a key biological component missing in current modeling of PMAD. To develop a working hypothesis regarding the microbiome's contribution in PMAD, we conducted a systematic review of the literature examining the microbiome in relation to maternal mood, anxiety, or stress during the perinatal period. This emerging area of research intersects with existing literature regarding the microbiota-gut-brain axis, changes in the microbiome over pregnancy, and changes that occur across pregnancy in immune, gonadotropin, and stress systems.

1.1 The Microbiome, Depression and Anxiety

The gut-brain axis involves bidirectional signaling between the CNS and the gut, which is regulated by the neuroendocrine and neuroimmune systems, the sympathetic and parasympathetic arms of the autonomic nervous system, and the enteric nervous system (ENS).[32] The 'Old Friends' hypothesis states that mammals co-evolved with an array of tolerable organisms, including bacteria, fungus, archaea, and viruses, that helped to develop human immunoregulatory circuits.[33] In a seminal study, Sudo et al. were able to link intestinal microbiota to brain development, namely the hypothalamus, by comparing germ-free mice to conventionally raised mice.[34] Similarly, others have found that the microbiome is necessary for immune development and social behavior.[35] These studies support the symbiotic relationship hypothesis that the host co-evolved with colonizing microorganisms to develop robust immune and neurological systems. Despite the evolutionary history behind this symbiotic relationship, intestinal microflora has only been added to the theoretical model of the gut-brain axis in the past few decades. In an important study by Turnbaugh et al., differences in microbial composition and abundance were observed between obese and lean individuals. These deviations from "normal" are commonly known as a microbial dysbiosis.[36] Subsequent studies have followed, associating a dysbiotic microbiota to psychological disorders, including autism, stress, anxiety, major depressive disorder (MDD), and eating disorders.[35,37] Current research focuses on the the bidirectional communication between the microbiome and brain in order to understand the mechanisms behind these correlations (in part to elucidate cause or consequence of dysbiosis).

Communication between the gut microbiota and brain can involve the vagus nerve, gut-secreted neuropeptides, sensory nerves, and cytokines via direct interactions with the intestinal wall, gut permeability, or production of physiologically relevant microbial metabolites, such as tryptophan and

Short-Chain Fatty Acids (SCFAs).[35,38] Vagal nerve stimulation has been associated with clinical antidepressant benefits and regulation of the HPA axis in patients with treatment refractory depression.[32,39,40] Studies have described a relationship between intestinal microbiota and depression via immune regulation. Experimental manipulation of the gut microbiota has been shown to influence depressive and anxious behaviors.[41] In one study, *Bifidobacterium longum* and *Bifidobacterium breve* were shown to reduce anxiety in an anxious mouse strain in a similar manner as a selective serotonin reuptake inhibitor.[42] Emerging evidence suggests the gut microbiota influences the serotonergic system and regulates 5-HT synthesis and secretion.[43–46] Microbiota influence this system by altering tryptophan availability in the plasma. These mechanisms include 1) direct utilization of tryptophan by bacteria for cellular function or production of indole and, in some species, serotonin; 2) indirectly by inducing the up-regulation of hepatic Indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO) through an inflammatory response, thereby shunting tryptophan into the kynurenic pathway and increasing production of kynurenic acid or quinolinic acid, known to be associated with psychological and neurological disorders;[45] and 3) stimulating serotonin synthesis in the enteroendocrine cells via SCFAs.[45–47] Numerous animal studies demonstrate that manipulation of the gut microbiota increases corticosteroid reactivity and/or glucocorticoid levels.[41] Administration of *Lactobacillus rhamnosus*, considered an anti-inflammatory probiotic, reduced stress-induced corticosterone, as well as anxiety- and depression-related behavior in mice.[48] Two small studies have reported greater amounts of pro-inflammatory gut bacteria and reduced levels of anti-inflammatory gut bacteria from individuals with MDD.[29,30] Additionally, a cohort of chronically depressed patients had greater IgA and IgM antibodies against *Enterobacteriaceae,* which suggests increased translocation of bacteria in depressed patients.[31] The perinatal period provides a unique opportunity to translate findings from current microbiome

research to clinical research of PMAD for three reasons: 1) pregnancy can be accompanied by dramatic changes in the gut microbiota,[23] 2) the perinatal period is a unique time of change in a number of systems implicated in the microbiota-gut-brain axis including the stress system and the immune system, and 3) the phenotypic presentation of PPD includes both anxious and depressive behaviors.[8,9]

1.2 Gonadotropins, Stress, Immune, and Microbiome Changes During the Perinatal Period

While biological mechanisms underlying PMAD remain unclear, research suggests that hormonal sensitivities, the stress system, and the immune system play a role. There is evidence that PPD is associated with sensitivity to fluctuating levels of the gonadal steroids estradiol and progesterone.[49] While similar levels of estradiol and estriol exist across euthymic and depressed women during the perinatal period, expression of estrogen-responsive genes during the third trimester is predictive of PPD and shows increased sensitivity to estrogen signaling, a mechanism for the development of PPD.[50] The gut microbiota is associated with systemic estrogen levels.[51,52] For example, certain gut microbiota are associated with secretion of beta-glucoronidase which deconjugates estrogen into its most bioactive form.[52] In the hypoestrogen postpartum state, deconjugation of estrogen may be a mechanism by which the microbiota participate in the balancing of fluctuating systems. Evidence regarding progesterone's influence on the microbiota is limited. When given vaginally to prevent preterm birth, progesterone did not impact the vaginal microbiome.[53]

Estrogen, progesterone, and the HPA axis are strongly linked, and HPA axis abnormalities in susceptible women may be associated with PMAD.[54] In a healthy pregnancy, maternal levels of Corticotrophin-Releasing Hormone (CRH), Adrenocorticotropic Hormone (ACTH), and cortisol increase during pregnancy and peak in the third trimester. During normal parturition, increases in CRH, ACTH, and cortisol are observed, which drop to prepartum levels within the first 1-4 days and slowly return to

prepregnancy levels after several weeks.[55] In non-depressed women, there is a blunting of the response of ACTH to CRH for a few weeks while women with PPD may have continued blunting of ACTH to CRH for 6 to 12 weeks.[54,56] Maternal CRH levels greatly increase during first trimester due to CRH production in the placenta, decidua, and fetal membranes.[20,55] Placental production of CRH leads to further fetal cortisol which in turn causes increased placental CRH secretion. However, while fetal cortisol increases placental CRH secretion, it surpresses maternal CRH secretion, resulting in maternal CRH suppression that has been shown to last at least 6 weeks after delivery while returning to normal by 12 weeks.[20,55] Maternal ACTH levels rise during pregnancy similarly to cortisol level thought they remain within normal limits.[55] Women who have postpartum depressive symptoms exhibit a more blunted ACTH response to CRH than euthymic women in postpartum.[55] Results from Bloch et al. have also shown that women with a history of PPD have HPA axis changes associated with estradiol and progesterone increases mimicking pregnancy levels.[57] While the relationship with the microbiome is yet to be studied in a perinatal population, there is evidence that bacteria play a critical role in HPA axis development and responsiveness.[34,45] Dynamic system changes in the perinatal period may serve as a critical time period for exhibiting these alterations or reconstructing systems.

The HPA axis interfaces with the immune system, which has also been implicated in depression. As discussed by Dantzer et al., the brain monitors peripheral inflammation through several innate immune system pathways including 1) cytokine activation of nerves (i.e. the vagal nerve), 2) increased volume of pro-inflammatory cytokines by stimulation of Toll-like receptors on macrophage-like cells and these cytokines entering the brain through diffusion, 3) systemic circulating of pro-inflammatory cytokines that gain access through cytokine transporters at the blood-brain barrier, and 4) IL-1 receptors located on perivascular macrophages and endothelial cells of brain venules, which produce

prostaglandin E2, which signals the brain.[58] While pregnancy was previously thought to be a period of immune suppression, inflammation fluctuates throughout pregnancy, relying on the maternal immune system to successfully adapt to each developmental stage.[59] Across pregnancy, the maternal innate and adaptive immune systems are involved in local and systemic immunomodulation.[60] Local inflammation occurs during implantation and development of the placenta during the first trimester and early second trimester, followed by reduced inflammation in the second trimester. Inflammation again increases in the third trimester in preparation for birth, and inflammatory homeostasis is regained postpartum.[61] These changes in inflammation along with the interrelated hormonal changes may affect blood flow to mucous membranes and gastric-intestinal (GI) motility, and account for increased nausea in the first trimester with stabilization during the second trimester.[62,63] Macrophages are an important modulator of these systems by switching from a more pro-inflammatory state (with elevated levels of TNF-α, IL-6, and IL-1β) in the first and third trimesters to a more anti-inflammatory state (with increasing levels of IL-10, IL-4, and TGF-β) in the second trimester and postpartum period.[64] The inflammatory response of macrophages to produce proinflammatory cytokines is likely related to changing estrogen and progesterone levels, as well as to changes in serotonin levels, leukemic inhibitory factor, and prostaglandins.[65–69] Edvinsson et al. found that three inflammatory markers were downregulated in patients with AND compared to women without depression and that these women also had a dysregulated HPA axis response.[66] During implantation and delivery, elevated levels of macrophages creating inflammation is necessary, but at other stages, excessive inflammation may result in imbalance and contribute to comorbidity.[64,65] A review by Osborne and Monk identifies the complexity of research to date. Some studies show associations between PMAD with cytokines levels such as IL-6, TNF-α, and

IL-1β, while others have not shown associations that are as clear, reflecting the complexity and careful regulation of the immune system in pregnancy.[6464,70,71]

Throughout the perinatal period, the immune system interacts with microorganisms. The fetal/placental unit is actively involved in these immune changes over the perinatal period and is involved in complex responses to microorganisms.[61] While there is evidence of a placental microbiome, it is controversial whether the uterus and placenta have microbiota in healthy states.[59,72–74] There is evidence that placental microbiota may induce some of the immune changes occurring throughout pregnancy, including tolerance of microorganisms at the maternal-fetal interface.[59,75] Regardless of whether there are microbes associated with the placenta and uterus, maternal microbes such as those found in the vagina and gut may contribute to immunological changes across pregnancy. The vaginal microbiota of pregnant women has a distinctly different compostion compared to non-pregnant women.[76,77] Evidence suggests pregnant women have a more stable microbiota over time than that of non-pregnant women and that bacterial communities are less diverse and dominated by Lactobacillus during normal pregnancy.[76–78] It is proposed that this increased stability promotes resilience against infections, which are risk factors for preterm birth and other obstetric complications.[77] A study by Koren et al. shows that by the third trimester gut microorganisms that induce a pro-inflammatory response increase, and conversely, organisms that support an anti-inflammatory response decrease.[23] In support of these findings, other studies have shown that the gut microbiome can modulate the immune system, including how it develops and operates.[79–81]

Compositional changes in the gut microbial community across pregnancy have been reported, but to date, research is limited. Alterations include increases in abundance of members of the Phylum Actinobacteria and Proteobacteria and decreases in abundance of certain butyrate (a SCFA)-producing

bacteria including *Faecalibacterium, Eubacterium, and Roseburia*.[23] Multiple studies have found a decrease in alpha-diversity (the combination of microbial richness, number of different species, evenness, and abundance of present species within a sample) over the course of pregnancy, and one study noted an increase in beta-diversity (the differences in microbial composition or species between subjects).[23,26,27] This implies that through pregnancy a subject has a reduction in observed microbial species, but the altered microbial communities between different women becomes more divergent. Studies have also shown that microbial composition may be relatively stable between the third trimester and postpartum.[82,23,83] Variation in study sampling and design may account for descrepencies in findings, and their ultimate significance is not yet known.

Understanding the influences mediating microbial composition, as well as the consequences of those alterations on the host, is an enormous challenge. In the context of this review, the interface between microorganisms and the immune system offers an initial point of exploration; however, it is important to remember that numerous factors influence microbial composition. In one example, Mor et al. propose a "double-hit hypothesis" with regards to the microbiota associated with preterm labor and suggest that a viral infection changes how the placenta responds to bacterial products, which in turn, alters the response to the "normal" microbiota.[59] In fact, a multi-hit hypothesis may be more accurate, where various biological factors and external influences drive individual microbial composition and response to microbota. Examples of important factors include maternal diet, fluctuating cortisol levels, and antibiotic use.[28,70] Higher use of antibiotics in women with AND suggests that AND is associated with infection during pregnancy.[84] From these investigations, it is difficult to draw conclusions regarding the direction of the relationship between antibiotic-induced microbial dysbiosis and depressive symptoms, but antibiotic exposure has been linked to anxiety and depression in the general population as well.[85]

Ultimately, the relationship between the maternal microbiome and biological systems is bidirectional, whereby the microbial community must adapt to the changes occurring throughout pregnancy which, in turn, impacts pregnancy progression and a woman's adaptation to pregnancy (Figure 1). To understand these associations more completely, a systematic review was undertaken to identify research findings observing the relationship of the maternal microbiome to stress, depression, and anxiety.

2.0 Methods

We conducted a systematic review of peer-reviewed literature to identify sources investigating the relationship between maternal microbiome and maternal mood/behavior in the perinatal period. We searched for studies and reviews in MEDLINE® (via PubMed), Embase, and Scopus from date of database inception through January 8, 2018 using terms for microbiome, perinatal period, and mood disorders. We used either medical subject headings (MeSH) or Embase subject headings (Emtree) where available and keywords when applicable. Searches were supplemented with hand searching of references citing or cited by included articles.

After duplicate references were removed, at team of reviewers independently examined titles and abstracts for inclusion (HR, KW, MK). Next, the full texts of the remaining studies were obtained and each was independently examined by two reviewers for inclusion. At each stage of the review, discrepancies between reviewers were resolved through discussion. Studies were selected based on a set of predetermined inclusion and exclusion criteria. Inclusion criteria were: 1) reporting a measure of maternal mood, stress, or anxious or depressed behavior; 2) maternal population in the perinatal period defined as pregnancy through one year postpartum; and 3) reporting a measure of maternal microbiome, including manipulations of the microbiome through prebiotics or probiotics or

interventions with microbial byproducts. Both animal studies and human subject studies were included. Studies were excluded if they were not available in English. Reviews were excluded. Abstracts meeting criteria, while not explicitly peer reviewed, were included.

3.0 Results

A total of 1276 sources were identified, and after removing duplicates, 735 were evaluated (Supplement 1). The number of articles removed through the screening and exclusion process is displayed in Figure 2. Six hundered fifty-one articles were deemed irrelevant from title and abstract screening, and an additional 66 did not meet inclusion criteria after full text review. Seventeen peer-reviewed publications were identified for inclusion in this review. One additional abstract was also reviewed given this is an emerging area of study. Ten studies were conducted with lab mice, with the remaining eight conducted in human populations.

3.1 Study Findings

3.1.1 Associations between stress and maternal microbiome

Of the studies reviewed, five evaluated the effect of stress on the vaginal microbiome, and three evaluated the effect of stress on the gut microbiome. Stress has been associated with alterations in the vaginal microbiome during the perinatal period. Early research into stress and bacterial vaginosis (BV) showed that those with moderate-high or high chronic stress had significantly higher odds of positive BV status (OR=2.2 and 2.3 respectively).[86] In research investigating racial health disparities, Paul et al. found that while stressful life events were associated with BV infection in both white and African American women, neither perceived stress nor social support was related to BV status, suggesting that objective experience of a stressful event may be more important in impacting microbial composition.[87] In contrast, Uscher-Pines et al. found a higher prevalence of BV among African American women (46% in African

American women, 14% in non-African American women), but when controlling for race, sexual partners,

marital status, and other behavioral risk factors, stress was not associated with BV status or any specific

bacteria.[88] Recent research on stress and the vaginal microbiome has sought to characterize composition

and function in this dynamic. Results from Jasarevic et al. show that vaginal *Lactobacillus* abundance was

decreased in dams exposed to stress relative to control dams (p= 0.0398), and the authors suggest this

is likely associated with an expansion of other microbiota.[89] In a follow-up study, stress-exposed females

showed increases of the Phylum Proteobacteria in early pregnancy, driven predominantly by members

of the *Helicobacter* genus.[90]

Stressors also impact intestinal microbial composition during pregnancy. Gur et al. found dams

exposed to prenatal stress had significantly different fecal microbial community composition than

samples from non-stressed control dams.[91] In addition, microbial composition differed in the placentas

from stressed versus non-stressed dams, but the difference did not remain statistically significant after

controlling for multiple comparisons. Gur et al. suggest this may be due to low bacterial load within the

placenta and difficulty measuring given the relative abundance of host DNA in the placental samples.[91]

Researchers stressed dams between embryonic day (E) 10-E16. On E17, dams were tested in an elevated

plus maze to examine anxiety-like behavior. The association between stress exposure and higher

anxiety-like behavior approached significance (t (14) = 2.010, p = 0.0641).[91] Similarly to their vaginal

microbiome findings, Jasarevic et al. found that the gut microbial communities differed between the

control and stressed dams.[90] In control females, variation between individuals (Beta-diversity) was

significantly greater in early pregnancy compared to late pregnancy, while for stressed dams less inter-

individual variation was observed in both early and late stages of pregnancy. Taxa differences were found

in stress-exposed females such as increase of *Rikenellaceae*, *Odoribacte*r, and *Mucispirillum* and

decrease of Bacteroides seen in early pregnancy through parturition. Stress increased the Desulfovibrionaceae family within the Proteobacteria phylum found late in pregnancy. Stress-exposed females had some significant associations of certain bacteria not found in control dams.[90] This work indicates maternal stress during the first week of pregnancy induced a lasting gut and vaginal dysbiosis. In an abstract from human subjects research, Togher et al. reported reduced microbial diversity in gut and vaginal samples associated with higher levels of depression in the second trimester. However, this association disappeared in the third trimenster.[92] This is preliminary work but may indicate the importance of the timing of factors in pregnancy. These studies provide a small but unified body of evidence exhibiting the effect of stress on the maternal microbiome, but are insufficient for drawing conclusions regarding the impact of microbial composition on experiences of stress, behavior or mood.

3.1.2 Dietary Impacts on Microbial Composition, Mood, and Stress

Due to the impact of diet on host microbial composition,[93] factors such as prebiotic and probiotic consumption have the potential to effect mood with host microbiota on the causal pathway. Of the studies reviewed, six evaluated the relationship between dietary factors, microbial composition and mood or stress. There is evidence that prebiotic consumption may impact the effect of stressors on maternal outcomes. In open field mouse experiments, dams exposed to Acrylamide (ACR, a known neurotoxin) displayed increased anxiety-like behavior compared to control dams, while supplementation with prebiotics (fructo- and xylo-oligosaccharide, non-digestible carbohydrates that are easily fermented by intestinal organisms) restored exploratory behavior in ACR-exposed dams, indicative of reduced anxiety.[94] ACR-exposed dams also supplemented with prebiotics showed a reduction in markers of oxidative stress and a recovery of antioxidant enzymes in the maternal brain to control levels. Glutathione S transferase levels in the maternal brain increased in both ACR-exposed dams and ACR-

exposed dams supplemented with probiotics.[94] Enumerations of *Bifidobacteria* and *Lactobacilli* (commensal intestinal organisms that selectively ferment xylo-oligosaccharide and fructo-oligosaccharide, respectively) on selective media were significantly higher in ACR-exposed dams supplemented with prebiotics compared to ACR only-exposed dams and control dams. Postive correlations between *Bifidobacteria* and *Lactobacilli* abundance and maternal brain dopamine and GABA levels were also significant. This study only enumerated *Bifidobacteria* and *Lactobacilli* on selective media and did not explore other possible contributing species.[94] A second study by this same group found similar findings with the oligosaccharide inulin, another non-digestible carbohydrate, and exposure to ACR.[95] These studies suggest that fermentation of non-digestible carbohydrates by intestinal microorganisms mitigate the neurotoxic affects in the brain, and suggest a beneficial link between diet, microbes and the brain.

In a human randomized control trial of 423 women, Slykerman et al. investigated the effects of perinatal supplementation of the probiotic *Lactobacillus rhamnosus* HN001 had on postpartum depression and anxiety.[96] Women were randomized to receive HN001 at a dose of 6×10^9 colony-forming units (CFU) or placebo to be taken daily from enrollment (14-16 weeks) through six months postpartum. Mothers in the probiotic treatment group reported significantly lower depression scores (effect size, -1.2 points, Edinburgh Postnatal Depression Scale; EPDS) and anxiety scores (effect size -1.1, State Trait Anxiety Inventory 6; STAI-6) than those in the placebo group. When using a clinically significant cutoff for depression and anxiety levels and correcting for infant colic and time since delivery due to retrospective measurement of symptoms, probiotic supplementation remained significantly associated with reduced anxiety, but not depression.[96] Cowan et al. found that maternal separation, with or without probiotic treatment, did not alter dams' anxiety-like behavior or locomotor activity or responsiveness to

their pups.[97] The systematic search also identified a study by Freeman et al. that found 2.6% of psychiatrically-ill women reported consumption of a probiotic supplement.[98] However this paper did not compare results to any other pregnant population, and it is difficult to know if probiotic consumption differs in the general perinatal population.

Bruce-Keller et al. manipulated the microbiome of dams through depletion and repopulation with fecal transplant from males fed with a high fat diet (HFD).[99] Dams experienced a temporary 5% weight loss, but overall there was no difference in weight outcomes between dams repopulated with the HFD-fed male fecal samples (HFD dams) and dams repopulated with the control diet-fed male fecal samples (CD dams). For all three time points (before mating, during pregnancy, during lactation), statistically significant differences in microbial community composition were detected between CD and HFD dams (beta-diversity); however, no significant differences in diversity of communities within each subject (alpha-diversity) were observed at any time. Pre-pregnancy group-based differences were statistically significant, and both pregnancy and lactation introduced shifts in community composition of both CD- and HFD-manipulated dams while maintaining group differences. The taxonomical distribution within groups at Phylum levels for the maternal fecal samples revealed that before pregnancy, HFD-reconstituted dams show a statistically significantly elevated level of Bacteroidetes compared to CD dams, which had higher Verrucomicrobia. Bacteroidetes occupied a higher proportion of community composition postnatally in both groups. No other phylum-level changes could be observed.[99] Bruce-Keller et al. utilized time spent with pups as a measure for maternal behavior, and despite microbial composition differences, no significant differences in time spent with pups between CD and HFD dams were noted.[99] Notably, analyses of types of interactions between mother and pups, such as time spent grooming or feeding the pups or measures of maternal depressive or anxious behaviors, were not

included. Evidence from these studies exhibit the complexity of dietary, prebiotic, and probiotic interventions, which encompass a variety of factors that may have dissimilar impacts on microbial composition, and subsequently, on maternal mood and behavior. Targeted prebiotics may promote growth among specific genera that appear to decrease inflammation and improve anxiety, while other dietary components, such as fat consumption, impact different genera. New evidence suggests probiotics have the potential to improve maternal mood and anxiety symptoms, but it is limited. It is unclear from existing research which dietary components are most important to consider in relation to mood, anxiety, or stress during the perinatal period.

3.1.1 Immunoregulation, PMAD, and the Microbiome

The microbiota-gut-brain axis is highly regulated and influenced by immunological status and immune responses to microbial dysbiosis and translocation of microbial products. Of the studies reviewed, six articles assess the relationship between host immunoregulation, stress, and the microbiome during the perinatal period: one studying prenatal stress in relation to microbial communities in a transgenerational mouse model; two indentifying inflammatory responses to specific bacteria and have studied in relation to perinatal depression and anxiety in a humans; and three studying lipolysaccharide (LPS), a bacterial endotoxin, as a marker of dysbiosis and an effector of immunoregulation in the perinatal period.

To relate stess induced microbial dybiosis to inflammation in utero, Gur et al. analyzed cytokine expression. They found that IL-1β was significantly elevated in the placentas associated with female offspring and fetal brains from stressed dams when compared to non-stressed dams.[91] Furthermore, Brain Derived Neurotrophic Factors (BDNF) was decreased in the placenta and in the brains of adult female offspring, which is attributed to changes in IL-1β levels. Immune alterations associated with

maternal bacterial dysbiosis are also transmitted to female offspring, indicating that microbiota-gut-brain signaling related to stress may be transgenerational.

In humans, Roomruangwong et al. found lowered IgM-mediated autoimmune responses to oxidative specific epitopes (OSEs), molecules expressed on outer and inner cell membranes, during pregnancy, indicating a suppression of immune regulation toward cellular debris, and resulting in the enhancement of oxidative stress. This IgM-mediated response was significantly associated with gram-negative bacteria and suggests there is a reduction in bacterial translocation leading to the reduced IgM-mediated response found in all pregnancies, including mothers with prenatal depression. Alcohol use was associated with greater IgM-mediated responses and therefore possibly greater bacterial translocation. IgM-mediated responses were inversely associated with the tryptophan catabolite (TRYCAT) pathway (also known as the kynurenine pathway). Elevated TRYCAT pathway activity was strongly predictive of women with a lifetime history of depression and premenstrual syndrome (PMS), possibly indicating a chronic activation of the TRYCAT pathway in this group. Though an association was not detected with perinatal depression, this study points to complex immune regulation buffering the response to bacteria that warrants further investigation.[100] Subsequently, Roomruangwong et al. found that while perinatal anxiety was not associated with IgM responses, postnatal anxiety was significantly associated with IgA responses to *Pseudomonas aeroguinosa*, a common environmental bacteria and opportunistic pathogen.[101] Anxiety may have unique associations independent from depression, especially as related to immunity.

Criteria for review also included microbial byproducts that may be associated with stress, anxiety or depression during the perinatal period. Microbial byproducts may provide insight into mechanisms by which the microbiota participates in the gut-brain axis. Solati et al. found that acute exposure of

pregnant dams during gestation to LPS is associated with increased anxiety as indicated by decreased time and number of entries in the open arms of the elevated plus maze (EPM).[102] Increased serum corticosterone levels and concentrations of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, 1.5 hours post-injection were also observed in LPS exposed dams.[102] Male offspring of dams injected with LPS displayed opposite results from their mothers using these same behavioural tests and cytokine assays.[102]

Confirming the transgenerational findings by Gur et al., Walker et al. also found that elevated stress and anxiety induced in neonates via an inflammatory response to LPS persists into adulthood where treated rats exhibited more anxiety, reduced maternal behaviours with their own offspring, and spent more time in non-maternal behaviors. Second generation females then displayed greater anxiety and altered corticosterone reactivity. Second generation males had increased anxiety levels, with no changes in corticosterone reactivity. Cross-fostering with untreated mothers reversed these effects.[103]

In research investigating stress, LPS, and fetal loss, Friebe et al. found that LPS injections or acute sound stress in mice led to increase in fetal loss via similar mechansims.[104] Blocking the Toll-like Receptor-4 (TLR-4) via TLR-4 antibody or pretreating before the stressor with bacteriocidal permeability increasing protein (BPI) that binds to LPS kept fetal loss from occurring with the stressor present, but only BPI prevented fetal loss in LPS-injected mice. Blocking TLR-4 also prevented stress-triggered immune responses, increased T regulatory cells, and reduced migration of dendritic cells to the lymph nodes, but TLR-4 blockage only partially decreased the HPA axis response and did not impact progesterone release.[104] Stress often induces intestinal permeability and thereby increases translocation of LPS into the blood and contributes to stress-induced fetal loss through the initiation of the inflammatory response via TLR-4 and proliferation of dendritic cells. This research provides additional

evidence connecting psychological stress to systems that respond to bacterial LPS during pregnancy and supporting a multi-hit hypothesis, where stress alone may not initiate fetal loss without a "bacterial danger signal."

From these studies, we see that stressed animals have microbial dysbiosis associated with greater pro-inflammatory responses and that certain bacteria are associated with greater inflammation and anxiety. Because dysbiosis leads to increased permeability and increased translocation of bacterial byproducts, LPS may be a mechanism by which microbes alter mood as well as an indicator of dysbiosis. Certain factors, such as alcohol, may increase translatocation. Immunological responses to microbes during the perinatal period are an important factor in etiology of the maternal psychological status and warrant further investigation.

4.0 Discussion

Multiple findings from this review demonstrate a relationship between stress, the microbiome, and health and behavior outcomes during the perinatal period. Our first finding is that maternal stress results in changes in the vaginal and gut microbiota and that specific bacterical abundance and community structure are associated with inflammation and psychological status.[86–92] Interestingly, bacteria identified in this review in association with stress exposure have been identified in other studies relating to psychological outcomes. For example, differences in relative abundance of *Rikenellaceae* as found by Gur et al. were also associated with fear as a component of child temperament among 18-36 month-olds.[105] Similarly, Jasarevic found an increase in Desulfovibrionaceae families within the Proteobacteria phylum, and while *Rikenellaceae* increased, Bacteroidetes decreased overall.[89,90] Proteobacteria is thought to be more pro-inflammatory and has been found in greater amounts in a sample of healthy women by Koren et al.[23] Conversely decreased abundance of certain bacteria are

associated with inflammation. Highlighted in this review, Gur et al. found decreases in *Bifidobacteria* in stressed female offspring while Krishna et al. showed prebiotic supplementation increased *Bifidobacteria* and decreased inflammation.[91,94]

Another primary finding of this review is that a majority of studies providing direct intervention in maternal microbiome (i.e. high-fat diet, prebotics, or probiotics) or mimicking microbial effects (i.e. LPS injection) detect significant changes in anxious or depressive behaviors or symptoms.[94–96,102,103] Notably, all of these studies evaluating maternal behavior or symptoms antenatally detected an effect, while only half of the studies detected an effect in postpartum evaluation.[94–97,99,102,103] Evidence for intervening through the microbiota-brain axis is promising, though there are limitations to these studies. The majority of these studies are animal models.[94,95,97,99,102,103] The only human study regarding probiotics and PMAD retrospectively assessed mood.[96] Inconsistencies in probiotic study findings may be due to different bacteria included, different impacts on commensal bacteria, the makeup of commensal bacteria prior to probiotic intervention, or host factors.[96,97] Additionally, while Roomruangwong et al. did not find perinatal anxiety associated with IgM responses, they did find postnatal anxiety was significantly associated with IgA responses to *Pseudomonas aeroguinosa*. Roomruangwong et al. also found history of depression and history of sensitivity to hormonal change were also important factors, therefore an account of historical systems along with current symptoms needs to be considered.[100,101] For instance, Walker et al. showed a multigenerational transmission of altered maternal behavior that was initiated with a neonatal inflammatory stress response in the first generation and differing maternal care.[103] Therefore, maternal behavior and mother-baby interactions should be considered in further research. Animal studies tended to examine anxious behaviors in relation to bacterial exposure and inflammation, while human PMAD studies focused on depressive

symptoms. Future human and animal research would benefit from consideration of both anxiety and depressive symptoms. In light of findings that suggest a change in microbial composition in conjunction with other physiological changes over the course of the perinatal period, longitudinal studies provide a unique investigative opportunity.[23,26,27,99]

A number of studies found in our search addressed transgenerational transmission and only focused on offspring measures, not maternal measures, and were therefore excluded. More specifically, many studies stress the mothers but do not assess its impact on the mother before assessing the impact on the offspring. Other studies, while describing offspring microbiota, did not determine maternal microbial composition, which makes it difficult to understand whether the microbiota is associated with symptoms identified in the mother, associated with offspring measures, or if the microbial composition is transferred. One excluded article by Zijlmans et al. found that infants of mothers with high stress in the third trimester had greater abundance at that phylum-level of Proteobacterial groups, considered more inflammatory, and lower relative abundances of lactic acid bacteria and *Bifidobacteria* species, considered anti-inflammatory; however, this study did not evaluate maternal microbiome profiles.[106] The emerging literature indicates that the microbiota-brain axis is an important mechanism of transmission of stress from mother to baby. Paul et al. who found stressful events, and not perceived stress, were associated with differences in vaginal microbiota. Stress, particularly for human studies, is a broad term, and these findings suggest further study should include multiple stress measures and dimensions.[87] Furthermore, the immune system is a key component of this communication and developmental factors such as BDNF are important endpoints of communication. The microbiota, immune system, HPA axis, hormones (i.e. estradiol, progesterone, allopregnanalone, serotonin) and the CNS all communicate simultaneously during their development; therefore, it must be remembered that

a mother's system is greatly influenced by her mother, and both will be transmitted in the development of the next generation.[23,45,54,79,107,108]

This review highlights possible mechanisms of stress communication utilizing the microbiota-brain axis and its relationships to the development of anxiety and depressive symptoms during the perinatal period (Figure 3). The critical role of the HPA axis is one important pathway hypothesized and supported by evidence from this review. LPS provides a model of how certain bacterial byproducts lead to inflammation and activation of the HPA axis. Similar to findings in the general population, those who experience stress during pregnancy may have an increased risk of PMAD due to changes in their microbiome shifting toward proinflammatory composition and increased translocation of bacterial products, such as LPS.[31,102,103] However, Roomruangwong et al. found that, while immune responses to gut bacteria may influence onset of prenatal immune response and postnatal anxiety, pregnancy itself may be protective against translocation.[100,101] Roomruangwong et al. emphasize that the TRYCAT pathway is highly regulated over the course of the perinatal period, which makes it difficult to study.[100,101,109] Due to the decreases in gonadotropins after delivery, translocation may be more likely during the postpartum period. Sex-based differences noted in results suggest that estrogen and progesterone may be important components of this relationship.[102,103] Other research shows activation of the TLR-4 pathway occurs with stress and LPS exposure. Results from these studies, therefore, support the multi-hit model.[104] The microbiome provides opportunities to understand how the immune system develops, acts, and adapts over the perinatal period. Future research in this area is needed to study how gonadotropins, the HPA axis, and the immune system work together.

Current research in this area is natally focused, but we need research that strives to better understand maternal outcomes and mechanisms. Future work should combine maternal psychological

data with biological measures, including the microbiome and immune and stress system indicators, and factors that may mediate or moderate these effects such as social or environmental variables. Sample sizes must be large enough to evaluate multiple factors and to account for multiple comparisons. Designs should utilize nuanced phenotyping with the goal of understanding symptomatic components and trajectories through the perinatal period in order to appropriately define outcomes. While individual species and families may be associated with outcomes, future research should continue to evaluate the impact of microbial community interactions and composition in relation to host facotrs. Research that translates animal model findings to human populations and the human studies that produce mechanistic hypotheses which can be tested in animal models for proof of principle are important future directions.

4.1 Conclusions

Emerging literature suggests that the microbiota-brain axis in relation to PMAD is an important area of study that has the potential to provide us with better identification and treatment for women, ultimately improving outcomes for mother and child. An individual's microbial signature may serve as both a complex biomarker that can identify differing personalized mixes of etiologies (e.g., of HPA axis changes and of immune system changes) and also serve as an important symbiont and communicator between the host and the environment, responding to transitions as well as driving transitions (i.e., via metabolites, vagus nerve, immune system modulator). Dietary components including prebiotic and probiotic consumption may be mediating factors and future points of intervention. Important themes identified include: the importance of studying the maternal microbiome and measures of stress, anxiety, and depression; and that multi-hit models will be needed as research strives to determine the effects of multiple mechanisms working in concert.

Conflicts of Interest: None

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Figures:

Figure 1. Factors that influence microbial composition and the host systems that communicate with the microbiota during pregnancy.

Figure 2. Records Excluded During Screening Process.

Figure 3. The microbiota-gut-brain axis in pregnancy without elevated symptoms of depression and anxiety and in perinatal depression and anxiety highlighting some hypothesized changes in the gut microbiota, immune system, and metabolism. Tryptophan metabolism is an example of a metabolic pathway impacted by microbial dybiosis that possibly impacts the brain.

Figures: