Prenatal stress, the maternal microbiome, and the maternal environment: a prospective cohort study during the COVID-19 pandemic

Dissertation

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Abstract

Background: Stress and mood-related disorders are increasingly prevalent in our world, and vulnerability to psychosocial stress and its sequela, depression, is heightened during pregnancy. Psychosocial stress exerts its consequences via several mechanisms, including the gut microbiota-brain axis and its associated signaling pathways. Importantly, the maternal fecal and vaginal microbiomes undergo alterations across pregnancy, however there is little consensus regarding which shifts are adaptive or maladaptive, especially as they relate to prenatal stress and depression. Previous clinical studies interrogating the role of prenatal stress in altering maternal fecal and vaginal microbiota composition have found intriguing but mixed results and have largely been limited to single or two timepoint cross-sectional studies across gestation. Additionally, the COVID-19 pandemic serves as a unique social exposure experienced, in part, through existing societal structures and inequities. Furthermore, persistent racial disparities in maternal health outcomes and emerging interest in the role of the microbiome in the propagation of such health disparities warrants further research.

Methods: We conducted a prospective cohort study of pregnant individuals consisting of repeated administration of psychometrics and collection of fecal and vaginal microbiome samples. Using full-length 16S rRNA sequencing, maternal fecal and vaginal community composition was interrogated. α and β-diversity metrics in addition to taxonomic

abundance were used to compare microbial composition across psychometric responses and pandemic timing. Thematic analysis of semi-structured interviews was used to contextualize a subset of participants' lived experience of the COVID-19 pandemic. Multiple linear regression was used to evaluate racial disparities in fecal and vaginal α-diversity shifts across pregnancy.

Results: Stress and depressive symptoms were associated with increased relative abundance of opportunistic pathogens. Additionally, depressive symptoms, but not stress, were associated with lower relative abundance of butyrate-producing genera in the fecal microbiome. Stress and depressive symptoms were also associated with increased relative abundance of vaginal taxa associated with obstetrical complications and infections. Furthermore, pregnancy during the pandemic was associated with distinct shifts in fecal, but not vaginal, microbiome composition from early to late pregnancy in the absence of significantly different levels of stress and depressive symptoms. Given that the cause(s) of these microbial shifts cannot be determined in the current study, we propose several contributing factors and present qualitative themes which reveal temporal shifts in our participants' experience of the pandemic. Lastly, we found that race was a significant predictor of change in fecal α-diversity, but not vaginal α-diversity, across pregnancy and that the inclusion of additional sociodemographic covariates modified these associations.

Conclusion: These findings underscore previous preclinical and clinical work demonstrating the effects of prenatal stress and depressive symptoms on the maternal microbiome and extend the literature by offering several fecal and vaginal taxa which

may serve a critical role in this relationship. Additionally, the distinct shifts in fecal microbiota related to experience of the COVID-19 pandemic and the emerging racial disparities in fecal α-diversity across pregnancy both demonstrate the influence of these socially mediated processes on the maternal microbiome. Overall, these findings suggest that further interrogation of the role of specific maternal microbial taxa in relation to psychosocial stress and its varied sources is warranted.

Dedication

To my partner and to my parents, whose constant support, encouragement, and love kept me afloat.

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Publications

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Fields of Study

Major Field: Environmental Sciences

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Table of Contents

List of Tables

List of Figures

Chapter 1 Introduction

Stress is the body's physiological response to perceived demand, which can be both adaptive and maladaptive. In clinical research, stress-inducing exposures or experiences are classified as psychosocial stressors. In environmental public health research, these are classified as non-chemical stressors. Regardless of specific name, examples of these stressors include unmet basic needs (i.e. experiencing homelessness, food insecurity, lack of supportive social networks, etc.), the daily hassles of life (i.e. arriving to appointments on time, preparing for exams or meetings, etc.), environmental attributes (i.e., excessively hot climate, noise pollution), and experiencing micro-aggressions or social conflict related to a societal "-ism" (i.e. implicit biases, racism, sexism). These varied experiences are ubiquitous in our modern society, and prolonged or chronic stress exposure can induce psychological and physiological alterations which increase susceptibility to developing affective or mood-related disorders[1]. For this project, the sources of psychosocial stress are conceptualized by 'level': intrapersonal, interpersonal, and structural/institutional. This conceptualization is adapted from the Socioecological Model of Health.

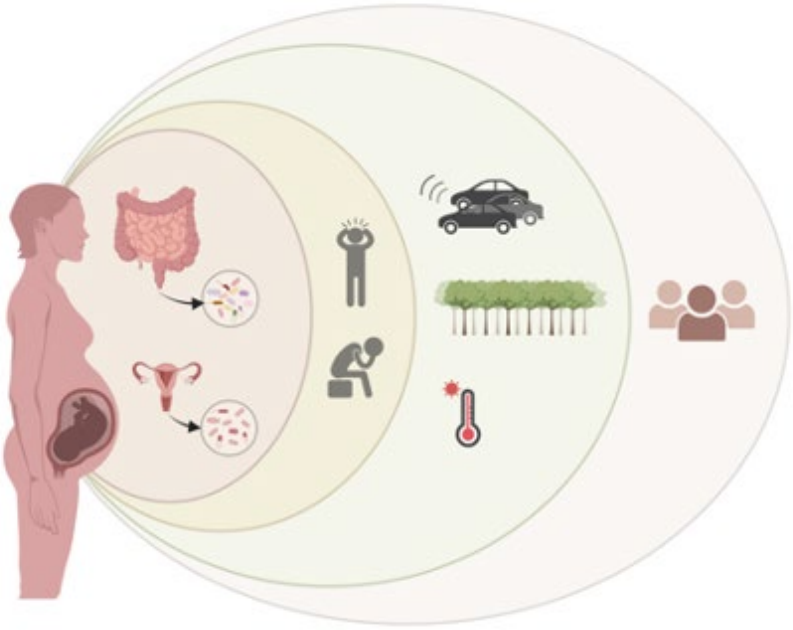


Figure 1.1 Project Framework

Adapted from the Socioecological Model of Health. Figure made in BioRender.

1.1 Pregnancy is a unique and critical window of susceptibility to psychosocial stress and its intergenerational consequences

Prenatal stress, also referred to as *gestational stress*, is defined as the experience of physical, psychosocial, or emotional distress during pregnancy, prior to parturition or delivery. This may also include experiencing mental health issues such as anxiety, depression, and mood-related disorders during pregnancy, when women are more susceptible to these conditions [2]. Preclinical animal studies and human cohort studies conducted over the last several decades have demonstrated prenatal stress, anxiety, and depression to be associated with increased susceptibility to a host of physiological and psychological, cognitive, or behavioral conditions in children[3–5]. These include worse performance on developmental tests, sleep problems, anxiety, difficult temperament,

conduct disorder, emotional problems, childhood attention deficit hyperactivity disorder (ADHD), vulnerability to bullying, and risk of schizophrenia[3]. These transgenerational effects have been documented in offspring gut microbiome composition, immune dysregulation, and behavioral deficits[4,6,7].

Landmark studies conducted by geneticist James V. Neel and epidemiologist David Barker found that maternal malnutrition during pregnancy led to low birth weight and increased susceptibility to heart disease later in the lives of their babies [8–10]. Their body of research birthed the *fetal origins of disease hypothesis*, also known as the *fetal basis of adult disease* and the *thrifty phenotype hypotheses* [8,11,12]. As Plant and colleagues eloquently summarized, these hypotheses make the case for the "biological embedding of gestational psychosocial adversity into vulnerability for future physical and mental illness" [13]. Thus, pregnancy presents itself as a critical time period during which the developing fetus is plastic and therefore sensitive to external influences through the intrauterine environment, the pregnant individual, and by extension, influences from the maternal environment [12]. Specifically, the expectant mother's history of trauma partially mediates fetal susceptibility to later disease [14–16]. In the last several decades, the many mechanisms by which this susceptibility to disease is transmitted have been identified and include a variety of organ systems and pathways [17]. This dissertation focused on alterations in maternal gut and vaginal microbiome composition as mechanisms of transmission.

1.2 Mechanisms by Which Stress is Biologically Embedded

Chronic stress exposure initiates a variety of physiological processes across the body, which can contribute to later susceptibility to disease onset. Stress exposure during development (i.e. infancy and early childhood) can be especially detrimental as the developing brain is plastic and sensitive to change in response to external stimuli, including stress or adversity [17]. Early life stress is thought to increase risk of later psychobiological maladjustment by several pathways, including autonomic nervous system, oxidative stress, cardiovascular system, sleep and circadian rhythms, genetics, structural and functional brain modifications, epigenetic regulation, hypothalamic-pituitary-adrenal axis, peripheral inflammation, and the gut microbiome [17].

1.2.1 Epigenetic Regulation

Exposure to psychosocial stress can be seen as an environmental challenge, which can then influence epigenomic changes. Most of this work has been conducted in animal models examining the effects of maternal care and stress on epigenetic regulation. These changes include modified DNA methylation, histone modification, and non-coding RNAs [18]. For example, a recent systematic review concluded that susceptibility to maternal postnatal depression has been linked to a number of genetic polymorphisms associated with stressful life events and/or maternal history of childhood adversity [19]. If such exposure occurs *in utero*, subsequent changes to the fetal epigenome may lead to altered susceptibility to disease in offspring [20]. The majority of clinical studies of exposure to early life adversity (i.e. postnatal) indicate that it modifies the epigenome at multiple

sites, with subsequent changes to the hypothalamic-pituitary-adrenal HPA axis, serotonin signaling, and immune signaling [21]. These changes occur as a result of epigenetic modifications to genes encoding glucocorticoid receptors, brain-derived neurotrophic factor (BDNF), arginine vasopressin, and corticotropin-releasing factor, among others [22]. Additionally, stress-related epigenetic changes may help explain inter-individual variability in vulnerability and resilience to adversity [17]. These factors, along with the dynamic nature of epigenetic modification, limit the field's current ability to make causal conclusions [21]. Nevertheless, the most easily manipulatable stress-associated epigenetic changes linked to mental health issues have been seen in genes associated with glucocorticoid signaling (i.e. NRC31, FKBP5), serotonergic signaling (i.e. SLC6A4), and neurotrophin (i.e. BDNF), indicating a priming effect on the HPA axis [23].

1.2.2 HPA Axis & Peripheral Inflammation

in utero exposure to prenatal psychosocial stress, is associated with chronically elevated levels of peripheral inflammation (i.e. CRP and IL-6), a pattern which seems to persist in 'healthy' human adults[24–26]. Furthermore, early life stress has been linked to various health conditions with a pro-inflammatory etiology, such as psychiatric disorders[24]. Additionally, in a cohort of patients with major depressive disorder, those with histories of childhood sexual abuse were more likely to have higher levels of the pro-inflammatory cytokines IL-6 and TNF-α in their blood—more evidence of peripheral inflammation[27]. Additionally, histories of childhood maltreatment have been linked to increased levels of

c-reactive protein (CRP), fibrinogen, and pro-inflammatory cytokines paired with blunted cortisol responses in the face of acute social stressors in adulthood[28,29].

As population-level examples, a study of pregnant mothers who were near the World Trade Center attack in September 2001 found that the babies of mothers who subsequently developed post-traumatic stress disorder (PTSD) had lower cortisol levels than babies of mothers who did not experience PTSD (Yehuda et al., 2005). Another study conducted nine years after the initial 1997 Red River Flood Pregnancy Study found that maternal proximity to flooding during pregnancy was significantly associated with maternal prenatal salivary cortisol predicting child hair cortisol, with prenatal trauma (as measured by closer proximity to flooding) altering the association between maternal and offspring cortisol from positive to negative[30].Clinical studies of chronic HPA axis activation in response to early life stress have found evidence for both hyper- and hypo-activation of the vital system. In both cases, it is likely that disrupted glucocorticoid signaling contributes to the pathophysiological effects of early life stress[17]. Thus, such exposure is proposed as an epigenetic embedding of a pro-inflammatory phenotype throughout life[24].

1.2.3 Immune System & Pregnancy

During pregnancy, the maternal immune system across the body adjusts to tolerate and support the growing fetus. Immune markers of a successful pregnancy include modifications in peripheral cytokine production to include fewer Th1-like (pro-

inflammatory) cytokines paired with an increase in Th2-like (anti-inflammatory) cytokines as the pregnancy progresses. While interleukin-6 (IL-6), interleukin-4 (IL-4), interleukin-1β (IL-1 β) and interleukin-10 (IL-10) are the most commonly measured cytokines of interest, IL-6 and IL-10 seem to most consistently demonstrate a positive correlation with susceptibility to mood disorders during pregnancy [2]. Interestingly, a low Th1:Th2 ratio at the time of blastocyst implantation seems to be essential to creating the intrauterine environment necessary for a viable pregnancy, including the ability of innate immune cells to produce specific cytokines[31]. Additionally, this immunomodulation can sometimes result in a period of compromised immunity and increased maternal susceptibility to infection, especially later in gestation[2]. Furthermore, the maternal HPA axis becomes gradually less responsive to stress during pregnancy[3]. These changes seen in a healthy pregnancy can also significantly impact maternal mood and behavior, as increased cortisol levels similar to those seen near birth are associated with transient depressive mood[2].

Of note, converging evidence suggests that expectant mothers diagnosed with depression may exhibit dysregulated cytokine production and, therefore, dysregulation in their immune response[32,33]. Thus, peripheral immune dysregulation (and subsequently altered cytokine production) has been posited as one mediator of susceptibility to peripartum mood-related disorders, although additional clinical research is needed to substantiate this hypothesis[2]. Furthermore, inflammation has been proposed as another mediator of the relationship between maternal prenatal stress and offspring psychiatric risk, although

additional clinical studies must be conducted to further elucidate the mechanisms of this relationship[34].

1.2.4 Prenatal Stress in Mothers with Histories of Childhood Adversity

Childhood adversity can be a moderator of dysregulated inflammatory response, which may then leave the individual more susceptible to sickness behaviors, depressive symptoms, and non-beneficial health behaviors (i.e. sedentary lifestyle)[35]. For instance, pregnant women with histories of ACEs and childhood maltreatment tend to be at greater risk for prenatal symptoms of depression and PTSD, anxiety, and occurrence of stressful life events[36–38]. These women are also more likely to be less resilient in the face of current adversity, including prenatal stress, leaving them more susceptible to its consequences[37]. Of note, these maladaptive effects seemed to diminish with the inclusion of supportive social networks[38]. Lastly, a recently conducted study of Kenyan mother-child dyads found that maternal mental health, including the contributions of prenatal stress, mediated the impact of maternal and familial ACEs on offspring internalizing/externalizing issues[39]. Together, these studies indicate that prenatal stress may have markedly more severe effects on pregnant women with histories of childhood adversity and that prenatal stress may mediate the intergenerational transmission of this trauma.

Linking these findings to the gut microbiota and inflammation, recent studies have also demonstrated that gut microbiota of high-ACE mothers contain a significantly higher

relative abundance of *Prevotella* with trending decreases in abundance of *Eubacterium* and *Phascolarctobacterium*[40]. Furthermore, among other findings, abundance of *Bacteroidetes* was positively associated with interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) levels and negatively associated with acute cortisol response, respectively. Of note, TNF-α levels were also positively associated with relative abundance of *Prevotella*[40]. Although these data and other current evidence indicate that inflammation may be a key mediator of the transmission of maternal stress to offspring psychiatric outcomes, further research is needed to elucidate the mechanisms by which these effects are incurred[34].

1.3 Role of the Microbiome in Health & Disease

"Microbiome" refers to the totality of commensal microbes, including bacteria, archaea, fungi, and viruses, present in or 'colonizing' a given location on or within the human body, contributing to a distinct local community and microenvironment. In the last few decades, the role of these varied microbial communities in maintaining human health and altering susceptibility to disease has been a topic of increasingly active research. For instance, from 2007 to 2014, the National Institutes of Health funded two phases of the Human Microbiome Project which sought to a) sequence microbial communities hosted by healthy humans and those associated with specific disease, and b) integrated bacterial sequencing data with additional data to improve health outcomes of specific conditions such as preterm birth, inflammatory bowel disease, and type II diabetes[41]. This rapid expansion of our understanding of human-associated microbial communities has been

facilitated by the advancement of sequencing technologies allowing for assessment of microbial composition and metabolic activity with greater granularity.

The two most relevant microenvironments for this project are the maternal vaginal and gut microbiome. In this project, 'gut microbiome' specifically refers to the mucosa-associated colonic or fecal microbial community as this is one of the most readily sampled representations of the gastrointestinal (GI) tract in clinical models. Broadly, vaginal microbiome composition does not correlate with genetic variation, unlike fecal microbiome composition. However, fecal microbiome composition is also more susceptible to more transient, non-genetic factors such as diet, environment, and medication, and it has been argued that these influences are more prominent, as compared to heritable factors, especially among generally healthy adults[42,43].

Microbes influence human health and disease through several mechanisms, including biosynthesis and regulation of a number of metabolites and signaling molecules essential for neuroendocrine and neuroimmune communication[44]. Specific microbes may produce these molecules or their precursors as metabolic by-products or influence endogenous steroids which, in turn influencing HPA axis activation[45–47]. Additionally, commensal microbes colonize the healthy gastrointestinal tract, however, both preclinical and clinical paradigms of psychosocial stress exposure demonstrate altered microbial community composition and immunomodulation[48–52]. These consequences are partially the result of the bidirectional communication that exists between the brain and gut microbiota, known

as the gut microbiota-brain axis. This system intricately works in tandem with the central nervous system and immune system, including endocrine, immune, limbic, and metabolic pathways[48].

1.4 Microbiota-Gut-Brain Axis

The bidirectional communication that exists between the brain and various vital organs, including gastrointestinal tract and reproductive tract, is facilitated in part by the peripheral nervous system, specifically autonomic nervous system signaling. Autonomic nervous system is composed of the sympathetic, parasympathetic, and enteric nervous systems. Specifically, the vagus nerve (cranial nerve X), facilitates efferent parasympathetic signaling to the GI tract while afferent sympathetic innervation communicates information from peripheral organs back to the brain. The enteric nervous system refers to neuronal innervation in the lining of the GI tract, also known as the alimentary tract, which facilitates both efferent (brain → organ) and afferent (organ → brain) communication via enabling secretion of enzymes, hormones, and other neuroimmune and neuroendocrine signaling molecules[53]. Of great relevance to psychiatric health, the gut contains and/or produces several of the most essential neurotransmitters and neuroendocrine signaling molecules, such as serotonin, dopamine, and tryptophan, which is produced by intestinal bacteria.

The healthy adult gut microbiome has greater bacterial diversity, as compared to an infant or child's microbiome, and is predominantly colonized by four commensal phyla:

Firmicutes, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*[54,55]. As compared to the small intestine, the large intestine has a higher bacterial load and dominant bacterial families of the small intestine and colon reflect physiological differences along the length of the gut. For example, a gradient of oxygen, antimicrobial peptides (i.e., bile acids) and pH limits the bacterial density in the small intestinal community. In the small intestine, *Lactobacillaceae* and *Enterobacteriaceae* dominate, whereas the colon is characterized by the presence of *Bacteroidaceae, Prevotellaceae*, *Rikenellaceae, Lachnospiraceae* and *Ruminococcacea*[56]. In the last two decades, gut microbiome alterations have been linked to a wide variety of illnesses, including psychiatric disorders, anxiety & stress, neurodegenerative disorders, pain, inflammatory bowel syndrome, obesity, addiction, epilepsy, and stroke[53].

1.5 Vaginal Microbiome

The microbial community in the vaginal canal is overall less diverse than most other microbial sites of the human body, but does tend to exhibit distinct patterns of microbiota composition or phylotypes[55]. The most commonly isolated species are *Lactobacillus (L.) gasseri, L. crispatus, L. jensenii*, and *L. iners*. These lactic acid-producing bacteria help maintain a lower vaginal pH (< 4.5) which is protective against pathogens and secrete metabolites which also help prevent infections[57–59]. Factors that can influence vaginal microbiome composition include age, ethnicity, menarche, menses, pregnancy, infections, birth control, spermicides and antimicrobials, and sexual behaviors[59]. During pregnancy, the vaginal microbiome decreases in richness and relative abundance of

species, but increases in stability of composition[59,60]. Additionally, the prevalence of *Lactobacillus* species generally increases, and other prominent phyla in the vaginal microbiome during pregnancy include *Clostridiales, Bacteroidales*, and *Actinomycetales*[57].

In terms of the potential implications of vaginal microbiome composition on health and disease, specific vaginal microbial taxa have been linked to adverse gestational outcomes such as preterm birth (< 37 weeks gestation). In a cohort of majority women of African descent who delivered preterm, lower levels of vaginal *Lactobacillus crispatus* were observed along with higher levels of *Sneathia amnii*, several species of *Prevotella,* and nine other taxa [60]. Another study of primarily 'Caucasian' women found that those who experienced preterm deliveries had greater vaginal microbial richness, alpha diversity, and differential composition, as compared to those who delivered at term. Women who delivered preterm also tended to have decreased abundance of *Lactobacilli* species and increased abundance of *Gardnerella, Atopobium, Sneathia, Gemella, Megasphaera, Dorea, Streptococcus, and Escherichia/Shigella*[61]. This differential vaginal composition may be due to individual genetic and environmental factors or clinical conditions such as bacterial vaginosis, which have been demonstrated to be associated with preterm delivery[62,63].

1.6 The Developing Gut Microbiome

A third relevant microenvironment is the infant or offspring gut microbiome. During vaginal deliveries, newborns are exposed to the maternal vaginal microbiome, which is currently seen as their first and most prominent exposure to microbes and is known as *vertical transmission* of microbiota. Consequently, several clinical studies have demonstrated that colonization of the infant gut microbiome can significantly differ between vaginally delivered infants and those born via cesarean-section, although this remains a contentious topic[64–67]. The gastrointestinal tracts of vaginally delivered infants are first colonized by species of *Prevotella, Sneathia*, and *Lactobacillus*. They are also colonized earlier by *Bacteroides species* and tend to have a greater resemblance to their mother's gut microbiota as compared to infants delivered by cesarean section. In contrast, cesarean section infants' microbiomes are first colonized by microbes indigenous to the maternal skin microbiome such as *Staphylococcus, Corynebacterium,* and *Propionibacterium*[66].

As infants develop, the colonization of their gut microbiome occurs primarily through exposure to microbes via ingestion of breast milk and is also susceptible to influence by exposure to skin-on-skin contact and contact with other people and objects. Previous clinical studies have found a variety of additional factors to be associated with offspring gut microbial composition, including early life stress, maternal race-ethnicity, maternal diet, mode of feeding, maternal marital status, family socioeconomic status, exposure to environmental tobacco smoke, antibiotic use, geographic location, family genetics, and

pet dander[40,64,68–70]. With the addition of solid foods in their diet, the offspring gut microbiome stabilizes around age three and largely resembles an individual's microbial composition in adulthood[71].

1.7 Evidence for Microbiome-Mediated Effects of Prenatal Stress on Offspring

Given evidence that the intrauterine environment may be influenced by maternal characteristics such as individual genetics, clinical conditions, environmental factors, and psychosocial stress, we must next consider the potential effects of this environment on the developing fetus and the offspring's health outcomes[40,72,73]. There are likely multiple mechanisms and pathways by which these intergenerational effects are transmitted, namely via differential HPA axis regulation, altered immune system development, and exposure to maternal microbiota in the offspring.

Of note, the existence of microbes in the intrauterine environment is a controversial topic of ongoing discourse, given that the accepted dogma of the last several decades states that the intrauterine environment is sterile. While some recent studies have found microbes in the placentas and uteri of healthy pregnancies, other studies assert that these findings are likely attributable to contamination [70]. An alternative hypothesis posits that, if the intrauterine environment of healthy pregnancies are sterile, then perhaps stressful conditions (i.e. prenatal stress, childhood adversity) facilitate the translocation of microbes from microbial cavities across the body, such as the gut, vaginal canal, and oral cavity, which would then result in altered gestational outcomes and *in utero* fetal

exposure to microbes[4]. The findings of a recent study support this hypothesis: when fecal matter from pre-eclamptic human mothers was transplanted into mice, the pregnant mice developed a pre-eclamptic phenotype across gestation, demonstrated impaired intestinal barrier function, and exhibited elevated levels of pro-inflammatory placental cytokines and chemokines[74].

Prenatal stress, including general anxiety and cumulative stress experienced during pregnancy, has been demonstrated to be associated with differential maternal vaginal, maternal gut, and offspring gut microbiome composition as well as offspring behavior, in both preclinical and clinical models[72,75–77]. For instance, vaginal microbiota have been demonstrated to mediate the effects of prenatal stress on offspring gut microbiome composition and offspring HPA axis response using a murine model of chronic prenatal stress[78]. Additionally, the gut microbiomes of offspring from human mothers who reported experiencing higher levels of cumulative stress tended to have a lower relative abundance of lactic acid-producing microbes and *Bifidobacteria* paired with a greater relative abundance of *Proteobacteria* known to contain pathogens, which could create conditions for a pro-inflammatory phenotype in the gut[79].

1.8 Gap in the Literature

There is little consensus on which vaginal and fecal microbial shifts during pregnancy are adaptive or maladaptive, especially in response to prenatal stress or depression. Additionally, previous clinical studies have been limited to single or two timepoint cross-

sectional studies across pregnancy. Furthermore, there is a limited understanding of how social experiences or exposures contribute stress and affective symptoms which then may influence gut and vaginal microbiome structure, especially during pregnancy. To address these gaps in the literature, we conducted a prospective cohort study of pregnant individuals consisting of repeated administration of psychometrics, collection of rectal (fecal) and vaginal swabs, and collection of maternal and umbilical cord blood. Broadly, we aimed to identify shifts in maternal fecal and vaginal microbial communities associated with prenatal stress and depressive symptoms across gestation. We also aimed to assess shifts in maternal fecal and vaginal microbiome diversity related to race, a sociodemographic construct capturing social exposures resulting from intrapersonal, interpersonal, and systemic biases.

1.9 Conclusion

The last several decades of research have demonstrated the diffuse, maladaptive consequences that psychosocial stress and its sequelae can induce via epigenetic regulation, immune and hormone modification, and microbiome changes, many of which are mediated by the microbiota-gut-brain axis. A more specific context for these alterations is pregnancy; emerging literature beckons us to consider the implications of *in utero* exposure to prenatal psychosocial stress and its sequelae, including the contributions of the maternal social and built environment.

Chapter 2 Stress and depressive symptom-associated shifts in the maternal gut and vaginal microbiome

2.1 Introduction

Prenatal stress is defined as psychosocial stress experienced by pregnant individuals, also referred to as 'gestational stress' or 'peripartum stress'. Broadly, a 'healthy' pregnancy requires many immunological, hormonal, and metabolic alterations. However, both preclinical and clinical studies have demonstrated that maternal stress and its sequelae, such as depressive disorders can induce alterations to gut microbiome community structure and function in a maladaptive manner[76,79–81].

During pregnancy, the maternal fecal and vaginal microbiomes undergo alterations, however there is little consensus on which changes are adaptive or maladaptive, especially as they relate to prenatal stress and depression. For example, in terms of diversity, it has been demonstrated that pregnant individuals had increased vaginal α-diversity and non-significantly decreased fecal α-diversity during 3rd trimester, as compared to non-pregnant individuals. However, another study aiming to characterize fecal microbial community composition across pregnancy found that, from first to third trimester, fecal microbiome composition featured both reduced α-diversity and increased β-diversity, regardless of 'host' health status (i.e., above 'normal' body mass index). They also reported overall reduced richness and increased evenness. They and more recent groups argue that fecal microbiome composition during pregnancy is largely sensitive to highly individual-specific factors[82,83]. In contrast, a smaller study found that

both fecal and vaginal α-diversity and β-diversity remained largely consistent across gestation, and a more recent study replicated these fecal α- and β-diversity findings[83,84]. Previous clinical studies interrogating the role of prenatal stress in altering maternal fecal and vaginal microbiota composition have found intriguing but mixed results, including the implications of maternal childhood adversity and intergenerational effects on offspring microbiota. These efforts have largely been limited to single or two timepoint cross-sectional studies across gestation[40,72,85,86].

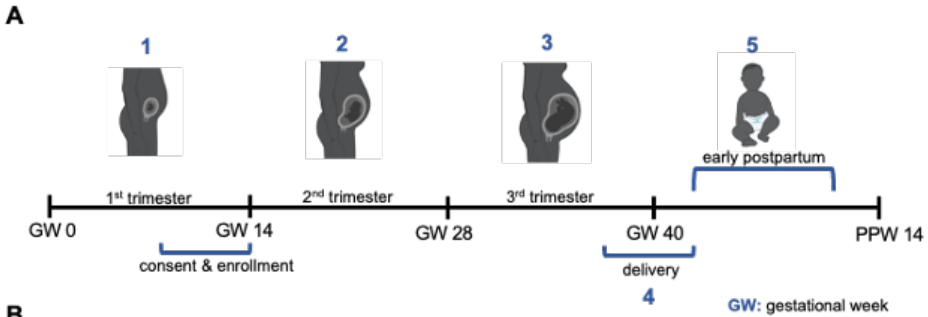
We aimed to test the hypotheses that more severe stress and depressive symptoms would be associated with steeper decreases in α-diversity and increased relative abundance of pathogenicity-associated taxa in both vaginal and fecal microenvironments. Additionally, we hypothesized that umbilical cord inflammation would increase with relative abundance of microbial taxa known to induce inflammation or be otherwise pathogenic.

2.2 Methods

Study design & participants

Pregnant individuals with singleton pregnancies were recruited from 2019-2021 through the Department of Obstetrics and Gynecology at The Ohio State University Wexner Medical Center (OSUMC). Participants were recruited through assessment of electronic medical records at participating clinics. Eligible participants provided verbal and written consent and were enrolled in the study during their first trimester of pregnancy (≤14 weeks gestation). Study participation consisted of five timepoints across pregnancy and

postpartum: once per trimester, within one week of delivery, and 4-8 weeks postpartum. Each timepoint included biospecimen collection and psychometric administration (Figure 2.1). This study was conducted with permission from the Ohio State University Institutional Review Board (2017H0362).



B

Study Visit	Psychometrics	Biospecimens
1	PSS, CES-D	maternal rectal & vaginal swabs (RVS)
2	PSS, CES-D	RVS
3	PSS, CES-D	RVS, maternal blood
4	PSS, CES-D	RVS, umbilical cord blood
5	PSS, CES-D	RVS

Figure 2.1 Study Design

A total of 40 participants enrolled in the study. The current sample (n=35) consists of participants who completed both psychometrics and biospecimen collection during at least one study visit which passed quality filtering after sequencing. Several participants were lost throughout the study timepoints for a few reasons (Appendix A).

Logistical implications of COVID-19 pandemic

Participant enrollment and research activities were ongoing when the COVID-19 pandemic spread to the United States; a state of emergency was declared on March 9, 2020, in Ohio[87]. Stay-at-home and quarantine orders were initially instituted for six

weeks. Per medical center research guidelines, the study paused all in-person research activities from mid-March to June 2020.

Demographic information & psychometric responses

Key participant demographics, including age, race, educational attainment, legal marital status, health insurance status, and health history, including relevant obstetrical history, were abstracted from medical records. Additionally, participants were asked to report self-identified racial/ethnic background, cohabitating status, and educational attainment. The self-report responses were used to verify and supplement medical record data. Previously validated psychometrics were administered across the study to assess participant perceived stress and depressive symptoms (Figure 2.1B). Surveys were administered via tablet at clinic visits until the pandemic, during which surveys were completed via securely emailed link.

The 10-item Perceived Stress Scale (PSS) was used to assess self-reported perception of stress in the past month. It is an adapted version of the original 14-item scale which has been tested for internal and test-retest validity and is widely used in research as a measure of global stress, including studies of maternal stress[88–90]. Participants respond to questions assessing response to stressors using a 5-point Likert scale such that higher scores indicate worse or higher levels of perceived stress in the past month. Scores ranged from 0 to 34, and scale reliability was good across timepoints (Cronbach's α

range: 0.73-0.87, Appendix B). For the sake of brevity, we refer to 'perceived stress' as 'stress' for the duration of this paper.

The Center for Epidemiological Studies Depression Scale (CES-D) was used to assess depressive symptoms [91]. The CES-D has been used in several similar studies of pregnant populations[92,93]. Higher scores indicate more severe depressive symptoms. Scores ranged from 0 to 43, and scale reliability was good (Cronbach's α range: 0.77-0.94, Appendix B). For categorical comparisons, consistent with previous studies, CES-D scores >16 were considered as the clinically relevant cut-off and designated as "high depressive symptoms".

Demographic, health, and psychometric data were examined. Pregnancy timepoint was treated as a categorical variable by trimester, delivery, and postpartum; distributions of gestational age by study visit are presented in Appendix A. Fisher's exact test were calculated for relevant continuous variable comparisons and Wilcoxon rank-sum/Mann-Whitney U tests were used to compare medians across pre-pandemic and pandemic groups using StataBE v.17 (StataCorp LLC, College Station, TX). Correlation between PSS and CES-D scores was calculated using Spearman's Rank order correlation coefficients.

Microbiome sequencing

Rectal and vaginal microbiome samples were collected across the prenatal, delivery, and postpartum periods (Figure 2.1B) using sterile foam-tipped applicators (Puritan Medical Products Co LLC, Guilford, ME). Samples were immediately chilled in a cooler with ice packs until they were transported, exteriors sanitized with 70% ethanol, and stored at -80°C until analysis. Fecal and vaginal RNA extractions and amplifications were performed using the Shoreline Complete StrainID Protocol 1 according to manufacturer's instructions. Next, amplicons were quantified, pooled, and cleaned. SMRT Cell sample libraries then were constructed per manufacturer's instructions (PacBio, Menlo Park, CA). Samples then underwent full-length 16S rRNA sequencing on a PacBio 8M SMRT Cell sequencer at Nationwide Children's Hospital Institute for Genomic Medicine (NCH IGM).

Using full length fastqs provided by NCH IGM, sequences were filtered and demultiplexed using Sbanalyzer. Next, following a workflow established by Shoreline Biome, amplicon sequence variants (ASVs) were inferred using the DADA2 pipeline [94] on R-Studio, and these ASVs were further analyzed in both Quantitative Insights into Microbial Ecology-2 (QIIME2). Rectal samples were rarefied to 5523 sequences per sample and vaginal samples were rarified to 2256 sequences per sample. All samples below this cutoff were removed from the study. Rectal samples were representative of the fecal microbiota community.

To quantify α-diversity (within-sample diversity), Pielou's Evenness, Shannon's diversity index, and Faith's phylogenetic diversity were used. For β-diversity, unweighted Unifrac was used for distance matrices and PERMANOVA and Adonis statistics in QIIME2 were used to measure the effect of specific psychosocial variables on microbiome diversity. Correlational coefficients for continuous variable associations with taxonomic abundances were calculated using the Spearman's Coefficient on SPSS v.27 (IBM, Armonk, NY) given the non-normal distribution of the taxa abundance. Mann-Whitney U tests were used to calculate significance in categorical taxa comparisons. Spearman's rank correlation coefficients were calculated via SPSS.

Lastly, Shannon's diversity index, Pielou's evenness metric, and PcoA plots of unweighted UniFrac distances were used to evaluate whether fecal and vaginal samples from participants who were prescribed antibiotics or psychotropics and experienced adverse obstetrical outcomes were clustering distinctly from the remaining participants. We found no such cases; thus, all samples remained in the subsequent analyses.

Multiplex assays

Approximately 8 mL umbilical cord blood was collected immediately after delivery using 10 mL BD Vacutainer sodium heparin collection tubes (BD Biosciences). Upon collection, tubes were inverted several times then stored at 4°C for up to 24 h before being centrifuged at 1300 rpm for 10 min. The supernatant plasma was then aliquoted into microcentrifuge tubes and stored at -80°C until analysis. Maternal blood was

collected in the same way at around 28 weeks of gestation. These samples were then assayed for several cytokines simultaneously using multiplex electrochemiluminescence immunoassay kits: V-PLEX Pro-Inflammatory Panel 1 (Human) and V-PLEX Human MCP-1 (Meso Scale Diagnostics (MSD), Rockville, MD). Samples were diluted according to manufacturer instructions and assayed in duplicate. Plates were read using the Meso QuickPlex SQ 120 instrument (MSD) and data were analyzed using GraphPad Prism v.9 (GraphPad Software, Boston, MA) for removal of outliers and summary statistics and R for taxonomic associations. Outliers were removed using ROUT method at Q=1% (α=0.01). For these exploratory analyses, we focused on cytokines previously implicated with prenatal stress, anxiety and affective disorders, and maternal-fetal inflammation: CCL2 (MCP-1), interferon-gamma (IFN-λ), interleukin-4 (IL-4), and tumor necrosis factor-alpha (TNF-α)[95–98]. Samples with concentrations below the lower limit of detection of a given analyte were excluded from further analyses. Overall, inter-assay coefficients of variation were less than 10.4% for all analytes of interest.

2.3 Results

Sample characteristics

The present study sample consisted of 35 pregnant individuals of an average age of 30 years, half of whom were experiencing their first pregnancy (50%) and enrolled in the study prior to onset of COVID-19 pandemic-induced public health restrictions in the study's locale (Table 2.1). Briefly, most participants identified as white (60%) and married or cohabitating with a partner (85.7%) with private health insurance (48.6%) and

having earned a bachelor's degree or more (45.7%) (Appendix B). Regarding obstetrical outcomes, the median gestational age at birth was 39.3 weeks and 25.7% of participants delivered via cesarean section (Table 2.1). Additionally, several participants experienced various obstetrical complications (Appendix B) but, for the duration of study participation, none of our participants had documented cases of SARS-CoV-2 infection.

Table 2.1 Brief Sample Characteristics

Demographic background, health and obstetrical history, and psychometric scores are presented for the current sample by timepoint. Additional sociodemographic and obstetrical outcomes are presented in Appendix B.

	Overall	**1st Trimester**	**2nd Trimester**	**3rd Trimester**	**Delivery**	**Postpartum**
	N=35	N=30	N=20	N=27	N=21	N=14
Variable	N (%) or Median (IQR)					
Maternal Age (years)	31.0 (25.0-34.0)	31.0 (25.0-34.0)	32.0 (26.0-34.5)	31.0 (25.0-33.0)	32.0 (24.0-34.0)	32.0 (27.0-34.0)
Enrolled During Pandemic	11 (31.4%)	10 (33.3%)	5 (25.0%)	8 (29.6%)	7 (33.3%)	6 (42.9%)
Pre-pregnancy BMI (kg/m^2)	25.7 (23.3-31.1)	25.5 (23.3-31.1)	25.5 (23.2-29.7)	25.4 (23.2-28.5)	26.6 (24.0-31.1)	25.3 (23.3-28.3)
History of chronic health condition	6 (17.1%)	6 (20.0%)	1 (5.0%)	2 (7.4%)	3 (14.3%)	1 (7.1%)
History of psychiatric condition	5 (14.3%)	4 (13.3%)	2 (10.0%)	4 (14.8%)	3 (14.3%)	3 (21.4%)
Gravidity						
Primigravida	16 (45.7%)	14 (46.7%)	8 (40.0%)	11 (40.7%)	9 (42.9%)	6 (42.9%)
Multigravida	19 (54.3%)	16 (53.3%)	12 (60.0%)	16 (59.3%)	12 (57.1%)	8 (57.1%)
Gestational Age at Birth (weeks)	39.3 (38.9-40.1)	39.3 (38.3-40.1)	39.5 (38.5-40.4)	39.4 (38.3-40.3)	39.6 (39.0-40.4)	39.9 (39.0-40.4)
Cesarean Section Delivery	9 (25.7%)	8 (26.7%)	6 (30.0%)	6 (22.2%)	6 (28.6%)	2 (14.3%)
Number of Adverse OB Outcomes						
0	22 (62.9%)	19 (63.3%)	13 (65.0%)	16 (59.3%)	11 (52.4%)	9 (64.3%)
1	12 (34.3%)	10 (33.3%)	7 (35.0%)	10 (37.0%)	9 (42.9%)	4 (28.6%)
2	1 (2.9%)	1 (3.3%)	0	1 (3.7%)	1 (4.8%)	1 (7.1%)
10-item PSS Score	-	16.0 (8.0-21.0)	16.0 (12.5-20.0)	14.5 (10.0-19.0)	10.0 (7.0-19.5)	13.0 (8.0-18.5)
CES-D Score	-	10.0 (7.0-16.0)	12.5 (9.0-18.5)	11.0 (7.0-17.0)	7.5 (4.5-14.0)	6.5 (5.0-11.0)
Antibiotic Use						
During pregnancy	7 (20.0%)	5 (16.7%)	3 (15.0%)	6 (22.2%)	-	-
During labor	10 (28.6%)	-	-	-	7 (33.3%)	-
Postpartum	2 (5.7%)	-	-	-	-	2 (14.3%)
Psychotropic Use						
During pregnancy	4 (11.4%)	4 (13.3%)	2 (10.0%)	4 (14.8%)	4 (19.0%)	-
During postpartum	2 (14.3%)	-	-	-	-	2 (14.3%)

IQR: interquartile range; BMI: body mass index; OB: obstetrical; PSS: Perceived Stress Scale; CES-D: Center for Epidemiological Studies Depression Scale

Stress, depressive symptoms, and relative abundance of fecal taxa

Given that there were no significant differences in stress and depressive symptoms by pandemic timing (Appendix B), responses were collapsed across timepoints to interrogate stress and depression-associated shifts in fecal and vaginal taxa. During the 2nd trimester, severity of stress symptoms was associated with increased relative abundance of several fecal taxa, including the *Prevotellaceae* family (r=0.534, p=0.015), *Sneathia* (r=0.530, p=0.016), and *Atopobium* (r=0.459, p=0.042). Additionally, depressive symptoms were associated with increased relative abundance of the phylum *Synergistetes* (r=0.461, p=0.041) (Table 2.2). At delivery, depressive symptoms were associated with increased relative abundance of *Lactobacillus* (r=0.541, p=0.068) and *Sneathia* (r=0.710, p=0.001) and decreased relative abundance of *Peptoniphilus* (r=-0.440, p=0.068) (Table 2.3). There were no statistically significant or trending differences in relative abundance of fecal taxa by stress or depressive symptoms during 1st trimester and 3rd trimester.

Table 2.2 Stress & depression-associated shifts in relative abundance of fecal taxa

Timepoint	Phylum	Genus	Psychometric	n	Spearman's ρ	p-value
2nd Tri.	Bacteroidetes	Prevotellaceae family	PSS	20	0.534	0.015*
	Synergistetes	-	CESD	20	0.461	0.041*
	Fusobacteriota	Sneathia	PSS	20	0.530	0.016*
	Actinobacteria	Atopobium	PSS	20	0.459	0.042*
Delivery	Firmicutes	Peptoniphilus	CESD	18	-0.440	0.068#
		Lactobacillus	CESD	18	0.541	0.021*
	Fusobacteriota	Sneathia	CESD	18	0.710	0.001**
Postpartum	Fusobacteriota	*F. nucleatum*^	PSS	10	0.564	0.045*

#p<0.10, *p<0.05, **p<0.01; ^species, not genus
N= number of pairs of fecal samples & psychometric responses
PSS: Perceived Stress Scale; CES-D: Center for Epidemiological Studies Depression Scale

Stress, depressive symptoms, and relative abundance of vaginal taxa

Pairwise distance comparisons of two measures of α-diversity revealed that participants with worse depressive symptoms experienced a steeper decrease in vaginal α-diversity as their pregnancy progressed from 1st to 3rd trimester (Shannon's entropy: p= 0.009; Pielou's evenness: p= 0.017) (Figure 2.2). Additionally, 1st trimester depressive symptoms, but not stress, was associated with later adverse obstetrical outcomes; this relationship did not persist into subsequent trimesters. There were no significant differences in vaginal β-diversity between participants by varying levels of stress or depressive symptoms at each timepoint (Appendix B).

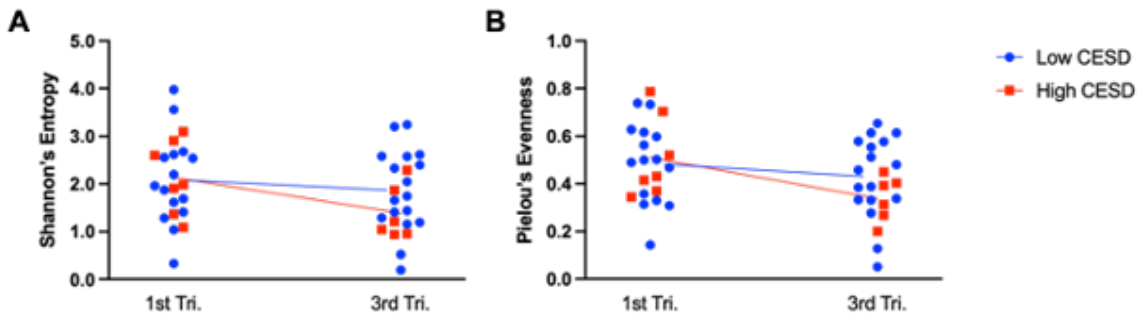


Figure 2.2 Steeper decreases in vaginal alpha diversity from early to late pregnancy among participants reporting more severe depressive symptoms.

Two measures of α-diversity including Shannon's Entropy (**A**) (p= 0.009) and Pielou's Evenness (**B**) (p= 0.017). Red indicates 'high' or more severe depressive symptoms (CES-D score >16); blue indicates 'low' or less severe depressive symptoms (CES-D score < 16).

Additionally, during the 1st and 3rd trimester and postpartum, relative abundance of several vaginal taxa was associated with depressive symptoms (Table 2.3). During the 1st trimester, relative abundance of four genera from the Firmicutes phylum were positively correlated with depressive symptoms, including *Lactobacillus* (ρ=0.441, p=0.045), *Peptoniphilus* (ρ=0.560, p=0.008), *Aerococcus* (ρ=0.454, p=0.039), and *Megasphaera* (ρ=0.481, p=0.027). Also at 1st trimester, relative abundance of two species was positively associated with depressive symptoms: *G. vaginalis* (ρ=0.481, p=0.027) and *S. amnii* (ρ=0.481, p=0.027). During the 3rd trimester, this association with *S. amnii* was found again but less prominent (ρ=0.403, p=0.057), and relative abundance of *Sneathia* was positively correlated with stress (ρ=0.422, p=0.045). There were no statistically significant or trending differences in relative abundance of vaginal taxa during the 2nd trimester or at delivery. During the postpartum period, relative abundance of *Firmicutes*

was positively associated with stress, specifically the genus *Aerococcus* (ρ=0.596, p=0.032) which was also positively associated with depression ((ρ=0.627, p=0.022). Additionally, two genera of *Actinobacteria* were positively associated with both stress and depression: *Gardnerella* (stress: ρ=0.595, p=0.032; depression: ρ=0.580, p=0.038) and *Atopobium* (stress: ρ=0.685, p=0.01; depression: ρ=0.811, p=0.001).

Table 2.3 Stress & depression-associated shifts in relative abundance of vaginal taxa

Timepoint	Phylum	Genus	Species	Psychometric	n	Spearman's ρ	p-value
1st Tri.	Firmicutes	Lactobacillus	-	CESD	22	-0.441	0.045*
		Peptoniphilus	-	CESD	22	0.560	0.008**
		Aerococcus	-	CESD	22	0.454	0.039*
		Megasphaera	-	CESD	22	0.481	0.027*
	Actinobacteria	Gardnerella	*G. vaginalis*	CESD	22	0.481	0.027*
	Fusobacteriota	Sneathia	*S. amnii*	CESD	22	0.481	0.027*
3rd Tri.	Fusobacteriota	Sneathia	-	PSS	18	0.422	0.045*
			S. amnii	CESD	19	0.403	0.057#
Postpartum	Firmicutes	-	-	PSS	10	0.053	0.038*
		Aerococcus	-	PSS	10	0.596	0.032*
				CESD	12	0.627	0.022*
	Actinobacteria	Gardnerella	-	PSS	10	0.595	0.032*

Timepoint	Phylum	Genus	Species	Psychometric	n	Spearman's ρ	p-value
				CESD	12	0.580	0.038*
				PSS	10	0.685	0.01*
		Atopobium	-				
				CESD	12	0.811	0.001**

#p<0.10, *p<0.05, **p<0.01
N= number of pairs of fecal samples & psychometric responses
PSS: Perceived Stress Scale; CES-D: Center for Epidemiological Studies Depression Scale

Maternal & fetal inflammation and relative abundance of fecal taxa

Our analyses focused on cytokines previously associated with prenatal stress, affective disorders, and enteric nervous system signaling, specifically IFN- γ, TNF-α, CCL-2, IL-10, and IL-6 and the 3rd trimester and delivery maternal microbiome. In the fecal microbiome at delivery, relative abundance of Lactobacillus species, a known beneficial commensal genus, was negatively correlated with cord concentration of CCL2 (n=13, r=-0.724, p=0.012). We found no statistically significant associations between the remaining fecal taxa and cord and maternal cytokine concentrations queried (Appendix B).

2.4 Discussion

Stress and depressive symptoms associated with increased relative abundance of opportunistic pathogens and lower relative abundance of butyrate-producing genera in the fecal microbiome.

Overall, we found no significant differences in fecal α-diversity or β-diversity across pregnancy by stress or depressive symptoms (Appendix B), which can be seen as

consistent with the largely non-statistically significant reductions in fecal α-diversity found across several studies of non-pregnant adults with depression, with the exception of Jiang and colleagues (2015)[99]. Contrary to our non-significant β-diversity results, a Dutch population-based cohort found fecal β-diversity to be associated with self-report depression[100]. Additionally, both fecal α- and β-diversity were predictive of depressive symptoms in a recent, ethnically diverse cohort of non-pregnant adults in their mid-40's-50's[101]. During pregnancy, some studies have found significant decreased fecal α-diversity and increased β-diversity across gestation, regardless of health status (i.e., as a feature of pregnancy)[82] while others have reported no significant changes to either metric of fecal diversity across gestation[84]. A recent study found increased fecal α-diversity to be associated with decreased stress and, interestingly, increased ability to cope with adversity[80]. Taken together, these findings suggest that perhaps the extent to which overall metrics of fecal diversity are impacted depends on the severity of the condition experienced (i.e., clinically diagnosed major depressive disorder vs. self-report depressive symptoms), the specific α-metric used (i.e., Shannon's diversity vs. Simpson's), exogenous influences on the host (i.e., murine models vs. clinical studies) and may speak to the limitations of employing α- and β-diversity metrics to interrogate more subtle effects. For instance, a study of adolescents with anxiety and depression found that oral microbiome diversity differed by taxonomic composition but not by overall diversity[102].

During the 2nd trimester, we found that relative abundance of fecal *Prevotellaceae* increased with stress. Consistent with our finding, a study of non-pregnant adult women found that greater abundance of fecal *Prevotellacea*e was highly predictive of major depressive disorder[103]. Interestingly, a study of pregnant women found a greater abundance of fecal *Prevotella* among women reporting two or more adverse childhood experiences[40]. Contrary to our finding, studies of non-pregnant adults have found negative associations between relative abundance of *Prevotellaceae* or *Prevotella* and major depressive disorder and general anxiety disorder[99,104].

Also during the 2nd trimester, relative abundance of fecal *Synergistetes* increased with depressive symptoms. Increasing stress was also positively associated with *Sneathia and Atopobium.* Contrary to our finding, a study of Spanish adults from 2021 to 2022 found decreased relative abundance of *Synergistetes* among those with depressive symptoms [105]. Additionally, fecal *Atopobium* abundance has been shown to be higher among non-pregnant adults with depression, which can be seen as consistent with our finding[99].

At delivery, we found no associations between stress and taxonomic abundance, however, depressive symptoms were positively associated with fecal *Sneathia* and *Lactobacillus*. Because *Sneathia* are generally seen as a pathogenic genus, this finding is not surprising. However, *Lactobacillus* abundance increasing with depressive symptoms is contrary to what we could expect. Additionally, severity of depressive symptoms was negatively associated with relative abundance of fecal *Peptoniphilus*, a genus of butyrate-

producing species. This is consistent with previous literature suggesting that butyrate-producing species may have a protective effect on gut epithelium integrity and lower inflammation and a recent study demonstrating other butyrate-producing genera to be depleted in non-pregnant adults with depression[46,106,107].

During the postpartum period, *Fusobacteria*, and specifically *F. nucleatum*, were positively associated with stress but not with depressive symptoms. *Fusobacteria* species are seen as pathogenic due to their potent lipopolysaccharide membrane which can induce inflammation. They also synthesize indole, whose production requires tryptophan; thus, abundance of *Fusobacteria* may be associated with lower levels of serotonin and, therefore, depressed affect [108]. Supporting this connection, fecal Fusobacteria have been largely reported to be enriched in non-pregnant women with active major depressive disorder [103] and depleted among adults with treated or recovering major depressive disorder (MDD)[104]. Our findings are consistent with these studies.

Stress and depressive symptoms are associated with increased relative abundance of vaginal taxa associated with obstetrical complications and infection.

In the vaginal microbiome, we found that participants reporting worse depressive symptoms experienced a steeper decrease in vaginal α-diversity from early to late pregnancy (1st vs. 3rd trimester). While this finding seems to agree with the broad notion that decreased microbial diversity may be associated with malaise, in contrast to the fecal microbiome, greater diversity, as measured via community state type, in the vaginal

microbiome has been associated with spontaneous preterm birth [84,109]. Thus, our finding contrasts with the vaginal microbiome literature thus far, however few studies have focused on shifts in the vaginal microbiome related to stress and depression.

During the 1st trimester, worse depressive symptoms were negatively associated with relative abundance of *Lactobacillus*; this is consistent with existing literature which suggests that *Lactobacilli* have an anti-inflammatory effect on stress responses and may be less abundant in those with depression[99]. *Lactobacilli* species help maintain a low vaginal pH via production of lactic acid and generally dominate the vaginal microbiome, especially during pregnancy[110].

Also during the 1st trimester, conversely, worse depressive symptoms were positively associated with relative abundance of *G. vaginalis* and *S. amnii*. During the 3rd trimester, the positive association between depressive symptoms and *S. amnii* persisted, and *Sneathia* was also positively associated with worse stress. The positive associations between depressive symptoms and *Gardnerella* reemerged postpartum and were also associated with severity of stress. Generally, *Sneathia* species are thought to be pathogenic; specifically, *S. amnii* is pathogenic in the female urogenital tract and has been associated with several gestational complications and increased intra-amniotic inflammation [111]. Similarly, increased relative abundance of *Gardnerella* species in women with more diverse vaginal microbiomes, specifically in the absence of *Lactobacilli*, were found to be associated with increased risk of preterm birth[84].

Specifically, *G. vaginalis* has been associated with bacterial vaginosis and spontaneous preterm birth[112].

Additionally, during 1st trimester, worse depressive symptoms were also positively associated with relative abundance of *Peptoniphilus, Aerococcus*, and *Megasphaera*. The positive associations between depressive symptoms and *Aerococcus* reemerged postpartum and were also associated with stress. Vaginal *Megasphaera* have previously been associated with bacterial vaginosis, preterm birth, and other pregnancy complications[113,114].

During postpartum, some patterns from the 1st trimester reemerged: both stress and depressive symptoms were again positively associated with relative abundance of *Gardnerella* and *Aerococcus*, as well as with *Atopobium*. Vaginal *Atopobium* has been associated with spontaneous preterm birth, low gestational weight gain, and class III obesity among African American women [109,115]. Additionally, its presence was found to be inversely correlated with that of *Lactobacillus* in late pregnancy[116]. Lastly, abundance of *Gardnerella*, *Sneathia*, and *Atopobium* have previously been positively associated with each other and with bacterial vaginosis[116].

Overall, our most significant findings occurring during the 1st trimester and postpartum period can be seen as consistent with the literature demonstrating that the vaginal microbiome is compositionally distinct and especially stable during pregnancy and, after

delivery, transitions to a different community state type less dominated by *Lactobacilli*[84,110]. The 1st trimester and postpartum period are the closest to a non-pregnant vaginal microbiome that can be observed in our study.

The discordance between stress & depressive symptoms and relative abundance of both fecal and vaginal taxa is intriguing, given that stress and depressive measures were significantly positively correlated across most timepoints (Table 2.2); this is perhaps due to subjective nature of perceived stress' versus the less transient presence or absence of depressive symptoms, and the timing of psychometric administration (following delivery—a significant life event, it is possible that participants feel a relief from stress experienced prior to delivery). Another explanation may be that this discordance hints at the distinct microbe-associated mechanisms underlying stress and depression.

Higher umbilical cord CCL2 concentration associated with lower relative abundance of maternal fecal Lactobacillus species at delivery.

Lastly, at delivery, relative abundance of fecal *Lactobacillus* species was negatively correlated with cord concentration of CCL2. CCL2, also known as MCP-1, is a chemokine which, in tandem with microbes, has been demonstrated to mediate the consequences of prenatal stress on intrauterine inflammation and offspring development[98]. As previously mentioned, *Lactobacilli* are a commensal genus known to inhabit the gastrointestinal tract and generally thought to confer beneficial effects, thus this finding is concordant with existing literature.

Future Directions

Further studies could generate functional/metabolic profiles of fecal microbial communities across pregnancy to further interrogate the role of specific taxa and their metabolites in mediating the relationship between maternal psychological state and microbial activity. Additionally, a larger sample size would facilitate controlling for additional possible covariates such as diet, physical activity, and non-clinical protective factors such as socioeconomic status, social support network, and health behaviors. Lastly, future studies may also consider characterizing sources, types, and duration of perinatal stress to elucidate the unique microbial mechanisms underlying different types of stress exposure.

2.5 Conclusion

In summary, we found stress and depressive symptoms to be associated with increased relative abundance of opportunistic pathogens and depressive symptoms, but not stress, to be associated with lower relative abundance of butyrate-producing genera in the fecal microbiome. Additionally, we found stress and depressive symptoms to be associated with increased relative abundance of vaginal taxa associated with obstetrical complications and infections. Lastly, in concordance with previous literature, we found umbilical CCL2 concentration to be negatively correlated with relative abundance of fecal *Lactobacilli.* Taken together, these findings underscore previous preclinical and clinical work demonstrating the effects of prenatal stress on the maternal microbiome and

extend the literature by offering several taxa which may serve a critical role in this relationship.

Chapter 3 Contributions of the COVID-19 pandemic to psychosocial stress and the fecal microbiome

While the second chapter focused on microbial shifts associated with stress and depressive symptoms without regarding the COVID-19 pandemic, this chapter interrogates the effects of living through the COVID-19 pandemic on the gut microbiome.

3.1 Pandemic experience-associated shifts in fecal taxonomic composition

3.1.1 Introduction

While there is evidence that the maternal microbiome is influenced by psychosocial stress, there is more to uncover about the contributions of broader social exposures and phenomenon on the structure and function of these microenvironments. Simultaneously, the SARS-CoV-2 pandemic, henceforth referred to as 'the COVID-19 pandemic' or 'the pandemic', has been a global source of devastation as it continues to spread since it began in late 2019. Research since then has documented far-reaching consequences of living through a pandemic, including impacts on mental and physical health, employment, and child and adolescent development, in addition to morbidity and mortality[117–120]. Furthermore, the immediate and long-term consequences of the pandemic have been compounded by structural inequities in our social, political, economic, and health care systems[121]. One of the most comparable historical examples to the COVID-19 pandemic is the 1918 influenza pandemic. A recent study argues that the shifts in racial disparities

in influenza morbidity and mortality following the 1918 pandemic may have been partially the result of social determinants such as immigration and behavioral responses, demonstrating interactions between infectious disease outbreaks and extant social processes[122]. Thus, here, we view the COVID-19 pandemic as a social exposure which is experienced through the sieve of existing social structures and inequities. We use this lens to evaluate shifts in maternal gut microbiome composition associated with living through the pandemic, and present qualitative findings from interviews with our participants which contextualize how their lives changed throughout the pandemic.

The COVID-19 pandemic is now a known psychosocial stressor, associated with increased prevalence of anxiety disorders and major depressive disorders, the latter of which disproportionately impacted females[123,124]. Among pregnant and postpartum individuals, increased stress, anxiety and depressive symptoms have been documented in those expressing concern for family, experiencing income disruptions or employment concerns, and balancing multiple personal and professional responsibilities[125–127]. During the first year of the pandemic, pregnant individuals in the first and third trimester of pregnancy were found to be more susceptible to psychological distress and depression, among other mental health challenges, as compared to other pregnant women[128]. Given the increased risk of adverse obstetrical outcomes and psychiatric disorders, it is critical to understand the consequences that living through a global infectious disease outbreak may have on prenatal stress and related psychiatric sequelae based on our knowledge that

stress in the peripartum period increases risk of obstetrical and psychiatric consequences in the next generation[73,129,130].

As the COVID-19 pandemic began during our prospective cohort study, we hypothesized that prenatal stress and depressive symptoms would be more severe among our during-pandemic participants, as compared to our pre-pandemic participants. Furthermore, we hypothesized that this worse psychological state would be associated with greater relative abundance of disease associated taxa in the maternal fecal and vaginal microbiome.

3.1.2 Methods

Sample collection, microbiome sequencing, and statistical analyses are as described previously (Chapter 2.2). Briefly, Fisher's exact and Mann-Whitney U tests were used to compare sociodemographic and obstetrical outcomes across pre-pandemic and pandemic groups via Stata. For β-diversity, unweighted Unifrac was used for distance matrices and PERMANOVA and Adonis statistics in QIIME2 were used to measure the effect of specific psychosocial variables on microbiome diversity. Mann-Whitney U tests were also used to calculate significance in categorical taxa comparisons.

3.1.3 Results

Sample characteristics by pregnancy timepoint and stratified by pre-pandemic and pandemic are presented in Table 3.1. Broadly, pre-pandemic and pandemic groups were similar across all timepoints, with three exceptions. At 3rd trimester, the pandemic group

was slightly older ($p<0.05$) and was composed of more white participants ($p<0.05$), as compared to the pre-pandemic group. Additionally, at delivery, the distribution of health insurance source differed between pre-pandemic and pandemic groups ($p<0.05$). Regarding stress and depressive symptoms, contrary to our hypothesis, there were no statistically significant differences in stress and depressive symptoms reported across gestation between pre-pandemic and pandemic groups (Appendix C). Despite the absence of differential stress and depressive symptoms, we found several significant differences in fecal microbiota composition between these groups.

First, there was a significant increase in early pregnancy fecal β-diversity of samples collected during the pandemic, as compared to pre-pandemic (1st tri p=0.021, r^2=0.0707 and 2nd tri p=0.004, r^2=0.0828) (Figure 3.1). There were no statistically significant differences in fecal or vaginal microbiome α-diversity between pre- and during pandemic samples across gestation (Appendix B).

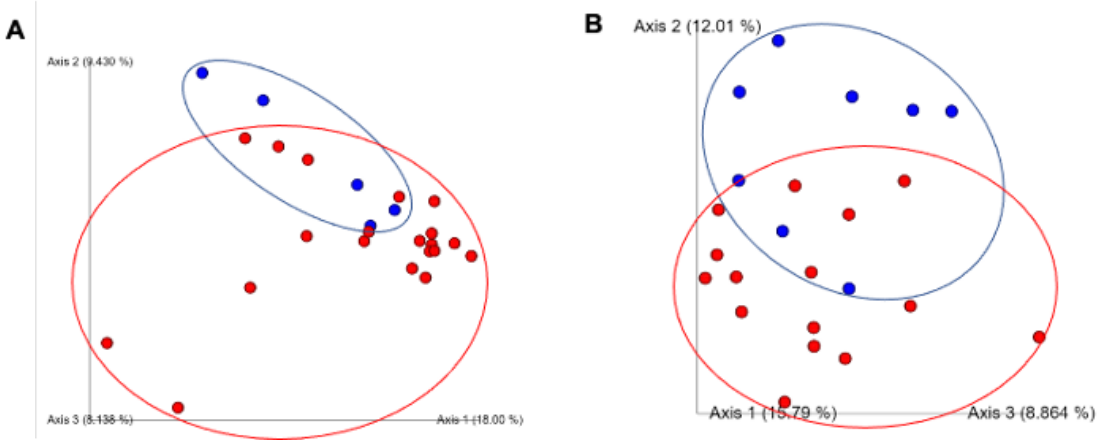


Figure 3.1 Early pregnancy beta-diversity differs between pre-pandemic and during-pandemic group.

Unweighted UniFrac distances were calculated and are plotted on principal coordinate plots, revealing that 1st trimester fecal β-diversity of pre-pandemic pregnant participants differed as compared to those sampled during the pandemic (**A**) (p=0.021, r^2=0.0707) and this distinction became more apparent in the 2nd trimester (**B**) (p=0.004, r^2=0.0828). Red indicates pre-pandemic group; blue indicates during pandemic group.

There were several significant differences in relative abundance of fecal phyla across the prenatal period (Figure 3.2). During the 1st trimester, relative abundance of *Firmicutes* increased while *Bacteroidetes* and *Synergistetes* decreased in the pandemic group, as compared to pre-pandemic (Figure 3.2). This pattern persisted into the 2nd trimester,

where a decreased relative abundance of *Fusobacteriota* was also seen. During the 3rd trimester, relative abundance of *Firmicutes* was again lower in the pandemic group, however *Bacteroidetes*, *Synergistetes*, and *Fusobacteriota* were no longer significantly different.

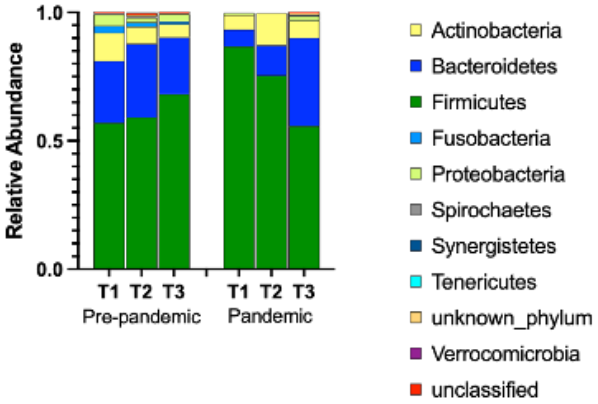


Figure 3.2 Shift in relative abundance of fecal phyla from early to late pregnancy in pre-pandemic group is distinct from that of during pandemic group**.**

Mean relative abundances are graphed above. Among participants sampled during the pandemic, we found increased relative abundance of Firmicutes (1st trimester: $p<0.01$; 2nd trimester: $p<0.05$) and decreased relative abundance of Bacteroidetes (1st trimester: $p<0.01$; 2nd trimester: $p<0.05$). Further differences found in relative abundance by specific genera are reported in Table 3.2.

Within genera, during the 1st and 2nd trimester, relative abundance of *Peptoniphilus* species increased in the pandemic group, as compared to pre-pandemic. There were no significant differences in its relative abundance during 3rd trimester and delivery, then its relative abundance was lower in the pandemic group, as compared to pre-pandemic (Table 3.1).

Table 3.1 Relative abundance of fecal taxa from pre-pandemic to during pandemic

Pregnancy	Phylum	Genus	Pre-pandemic		Pandemic		
			n	*Median*	*n*	*Median*	*p-value*
1st Tri.	Firmicutes	-	20	0.5749	6	0.8504	0.009**
		Peptoniphilus		0.0901		0.1649	0.039*
		Finegoldia		0.0077		0.1555	<0.001**
		Anaerococcus		0.025		0.1303	0.001**
	Bacteroidetes	-		0.2374		0.0638	0.001**
		Prevotella		0.0712		0.0048	0.033*
	Synergistetes	-		4.87E-05		0	0.046*
2nd Tri.	Firmicutes	-	15	0.5761	5	0.7630	0.034*
		Peptoniphilus		0.0746		0.1841	0.019*
		Anaerococcus		0.0364		0.0802	0.013*
	Bacteroidetes	-		0.3191		0.0751	0.047*
	Synergistetes	-		0		0	0.040*
	Fusobacteriota	Sneathia		0.0001		0	0.040*
3rd Tri.	Firmicutes	-	14	0.7236	7	0.4876	0.056#
		Anaerococcus		0.0822		0.0203	0.038*
		Finegoldia		0.0378		0.0054	0.012*
Delivery	Bacteroidetes	Prevotellaceae^	12	0.0148	7	0.0003	0.038*
Postpartum	Firmicutes	Peptoniphilus	5	0.0864	7	0.0305	0.038*

#p<0.10, *p<0.05, **p<0.01
^family, not genus

During the 1st trimester, relative abundance of *Finegoldia* species was significantly higher in the pandemic group. During postpartum, its relative abundance was lower in the pandemic group. There were no significant differences in *Finegoldia* relative abundance during 2nd trimester, 3rd trimester, or delivery. During the 1st and 2nd trimester, relative abundance of *Anaerococcus* species was higher in the pandemic group, as compared to

pre-pandemic. In contrast, during 3rd trimester, its relative abundance was lower in the pandemic group. During the 1st and 2nd trimester, relative abundance of *Synergistetes* species was lower (non-existent) in the pandemic group. During the 1st trimester, relative abundance of *Prevotella* species was lower in the pandemic group. A similar pattern was seen at delivery when relative abundance of Prevotellaceae was lower in the pandemic group. At 2nd trimester, relative abundance of *Sneathia* was lower (non-existent) in the pandemic group.

3.1.4 Discussion

Across the three trimesters, *Firmicutes* and *Bacteroidetes* relative abundance shifted in a complementary manner. This relationship between these phyla is concordant with previous findings[131,132]. Similar patterns have also been demonstrated in preclinical models of stress: both enriched Firmicutes & reduced Bacteroidetes seen in rats experiencing maternal separation and depleted Firmicutes and Bacteroidetes in prenatally stressed mice[76,133].

Similar to our finding, abundance of *Bacteroides* continually declined in frontline healthcare workers (HCWs) in Wuhan during the initial months of the COVID-19 pandemic, as compared to second line HCWs[134]. Contrary to our results, some studies of non-pregnant adults have found *increased* relative abundance of Bacteroidetes in individuals with depression, including among Slovenian adults during pandemic lockdown and trauma-exposed adults[135,136]. Additionally, higher relative abundance of

Bacteroides genera, a prominent genus of the *Bacteroidetes* phylum, has been associated with worse depressive symptoms among those with chronic schizophrenia[137].

Firmicutes are a phylum of gram-positive facultative anaerobes who vary greatly in role and function. *Bacteroidetes* are a phylum of gram-negative obligate anaerobes who are mostly mutualistic, though specific species may be opportunistic pathogens. Furthermore, the *Bacteroidetes* phylum are the main producers of acetate, a vital short chain fatty acid, in the gut[46]. *Bacteroides* species also produce GABA (gamma-aminobutyric acid), a key inhibitory neurotransmitter whose altered response is associated with major depressive disorder. Indeed, increased abundance of fecal Bacteroides was associated with less severe brain signatures of depression (specifically, default mode network and left dorsolateral prefrontal cortex activation) in a small sample of adults with major depressive disorder[138].

Anaerococcus are a genus of strictly anaerobic, mostly commensal microbes, though some species are associated with infections. Specifically, increased abundance of vaginal *Anaerococcus* species have previously been associated with early-onset preeclampsia. One study found fecal *Anaerococcus* to be associated with maternal blood pressure[139]. No other studies report fecal *Anaerococcus* during pregnancy to date. Interestingly, increased relative abundance of fecal *Anaerococcus* has been found among adults with chronic schizophrenia[137].

Peptoniphilus is a genus of butyrate-producing bacteria which is part of the commensal human microbiome, though specific species vary greatly in function. For instance, abundance of vaginal *p. harei* is implicated in preterm birth[140]. Additionally, a recent study found increased *Peptoniphilus* in patients infected with SARS-CoV-2[141]. However, very few studies report fecal *Peptoniphilus* in the context of stress and depression or pregnancy; one recent study reported increased *Peptoniphilus* among elderly adults with constipation but without anxiety or depression[142].

Finegoldia are a genus of anaerobic microbes mostly thought to be opportunistic pathogens colonizing human mucosal membranes. Most previous studies reporting *Finegoldia* focus on the vaginal microbiome or are unrelated to pregnancy, stress, and depression. Interestingly, a recent study reported *lower* abundance of vaginal *Finegoldia*, among other microbes, to be associated with preterm birth in a small cohort [143].

Taken together, *Anaerococcus*, *Peptoniphilus*, and *Finegoldia's* consistent presence in multiple timepoints of our study suggests that its abundance did meaningfully shift, despite the paucity of similar previous findings during pregnancy.

Sneathia are a genus of gram-negative microbes most often associated with bacterial vaginosis and regarded as pathogenic in the female urogenital tract, especially *S. amnii*[111,114]. Again, very few studies report intestinal or fecal *Sneathia* in the context of pregnancy or stress and depression.

Synergistetes are a phylum of gram-negative microbes thought to be commensal to the vaginal microflora, but also may be opportunistic pathogens. The 2nd trimester decrease in relative abundance of fecal *Synergistetes* among pandemic samples is comparable to a recent study of non-pregnant adults which found a decreased abundance of *Synergistetes* among individuals with depressive symptoms [105]. Two studies found fecal *Synergistetes* abundance to be resistant to probiotic administration and oral *Synergistetes* to be increased in periodontitis[144,145]. However, overall, there are very few studies of fecal *Synergistetes* in humans; most work is ecology-focused or based in livestock.

Prevotellaceae are a family of gram-negative microbes very commonly found in the intestinal microflora who can also be pathogenic. Studies of non-pregnant adults have reported it to be increased in abundance among those with depression, while a study of pregnant individuals found its abundance to be increased in those having experienced childhood adversity [40,99]. In our sample, the reduced fecal abundance of *Prevotellaceae* in pandemic samples at delivery could be seen as contrary to these findings, if we assume that the sole psychosocial influence of the pandemic period was inducing more stress.

There were few pandemic-associated shifts in α- and β-diversity metrics in the fecal and vaginal microbiome. The significant increase in 1st and 2nd trimester fecal β-diversity of samples collected during the pandemic, as compared to pre-pandemic indicates that pre-pandemic and pandemic samples were taxonomically distinct from each other. The

strengthening of this association at the 2nd trimester is intriguing as it may suggest an individual-specific effect of pregnancy, as posited by Koren and colleagues[82].

Limitations

It is important to consider these findings with the context of several imitations. Firstly, since the beginning of the study, we prioritized recruiting an ethno-racially and socioeconomically diverse sample, intentionally recruiting participants from two clinics serving generally distinct patient populations. However, the demographic makeup of our sample varied across the study due to several factors including existing patterns in participant attrition and non-clinical barriers to health care access, which were exacerbated by the onset of the COVID-19 pandemic [146–148]. Additionally, due to this being a pilot study and some psychometrics having been validated only in English, our study excluded participants who required the use of an interpreter. Also, we recruited participants in their first trimester of pregnancy; some pregnant individuals do not receive prenatal care until further along in pregnancy owing to a variety of non-clinical factors. Furthermore, we excluded patients with diagnosed and documented chronic health conditions such as diabetes, thyroid conditions, those with documented illicit substance or tobacco use, and those with histories of preterm birth or preeclampsia. These conditions may also be related to maternal psychosocial stress and the maternal microbiome and should be meaningfully included in future studies. Additionally, the study included multiple time points across gestation, but the limited number of participants may lower the study's statistical power while possibly increasing the

likelihood of Type I, false positive, and Type II, false negative, errors. Lastly, participants responded to psychometrics after delivery whereas the rectal and vaginal swabs were collected prior to delivery. It is possible that the psychosocial stressors they felt in the month or week prior to parturition are perceived differently (and therefore, reported differently) following parturition. Lastly, to minimize participant exposure risk and comply with medical center guidelines at the onset of the COVID-19 pandemic, we modified our study design to administer surveys via emailed link and paused maternal blood collection. During the first several months of 2020, some participants aged out of the study or did not respond to attempts to continue study participation.

3.1.5 Conclusion

In summary, we found that pregnancy during the pandemic was associated with distinct shifts in fecal, but not vaginal, microbiome composition from early to late pregnancy in the absence of significantly different levels of stress and depressive symptoms. Overall, far more fecal taxa shifts were seen in early pregnancy (1st and 2nd trimester) when comparing pre- and during pandemic samples. It is possible that these taxonomic shifts persist further into pregnancy and the postpartum period, but that the diminishing sample sizes at later time points limited the statistical power of our analyses. Additionally, while the cause(s) of these microbial shifts cannot be determined, we propose several factors which may contribute to the differences seen. In addition to the psychological stress induced, the first two years of the COVID-19 pandemic saw global shifts in daily lifestyles which likely impacted intrapersonal and interpersonal exposures or experiences.

More specifically, altered daily routines (time spent in indoor/outdoor environments or private/public environments), social interactions, diet, physical activity, and preventive measures such as masks and physical distancing may all have influenced psychosocial stress or directly influenced intestinal microbiome composition. In the next section, these altered experiences are explored further through a qualitative analysis of semi-structured interviews with a sub-sample of our cohort.

3.2 Lived experience of new and expectant mothers during the pandemic

3.2.1 Introduction

Previous studies report that the most common stressors early in the pandemic were loss of employment (and subsequent financial instability and loss of health insurance), inability to provide emotional support for loved ones due to physical distancing, concerns about balancing employment and childcare responsibilities, and lack of cohesive public health messaging from governmental bodies [149]. Among parents, high levels of stress have been attributed to increased economic burden, pandemic-related stress, loss of employment, school and childcare center closures, health, and difficulties using coping strategies[149–151]. Among pregnant and postpartum women, several international studies have demonstrated increased perceived stress, anxiety, depressive symptoms, and postpartum depression during the COVID-19 pandemic, particularly during the initial lockdown phases and among multigravida women[128,152–155].

However, few studies have documented the experiences of new and expectant mothers with a focus on temporal context, especially as we approach the (as of summer 2022) third year of the pandemic. Additionally, the vast variation in public health and policy responses by locality means that the experience of the pandemic may be highly dependent on specific locale and necessitates the study of a variety of geographically and socio-demographically diverse communities. Therefore, this project focuses on the experiences of a subset of new and expectant mothers in Franklin County (FC), Ohio, USA, where a state of emergency in response to the spreading virus was declared on March 9, 2020[87]. We aimed to understand 1) participants' well-being, experiences, and perspectives while pregnant and/or caring for a young child(ren) during the pandemic and 2) how their experiences and perspectives have shifted throughout the pandemic.

3.2.2 Methods

Subject Recruitment: Participants were recruited from a larger prospective cohort study at The Ohio State University Wexner Medical Center. From June 2020 to September 2021, 38 current and past participants of the larger study were approached via both email and phone call to inquire whether they would like to participate in an interview to share their experiences during the COVID-19 pandemic. We assumed that the 20 non-respondents did not wish to participate. The final study sample consisted of 18 participants from the larger study. At the time of phone interviews, participants' obstetrical status (i.e., gestational or postpartum age at the time of interview) ranged from the first trimester to nearly two-years postpartum.

Data Collection: Semi-structured interviews were conducted over the phone during summers and autumns of 2020 and 2021. All interviews were conducted by TR, who had personally met and/or corresponded with all participants prior to the interviews. Participants were informed that our goal was to understand how the COVID-19 pandemic impacted our participants. The interview script, including interview questions, are listed in Supplemental A. Interview questions addressed participants' well-being, reflections on pregnancy/parenthood, lifestyle changes, and perception of the COVID-19 pandemic. Interview questions were written to address these topics and formulated in accordance with published guidance on conducting semi-structured interviews[156].

Thematic Analysis: Interviews were audio-recorded, transcribed verbatim, and analyzed using thematic analysis following Braun & Clarke's phases of thematic analysis in addition to Nowell and colleagues' trustworthiness criteria for thematic analysis[157–159]. Our approach is summarized in Figure 3.3.

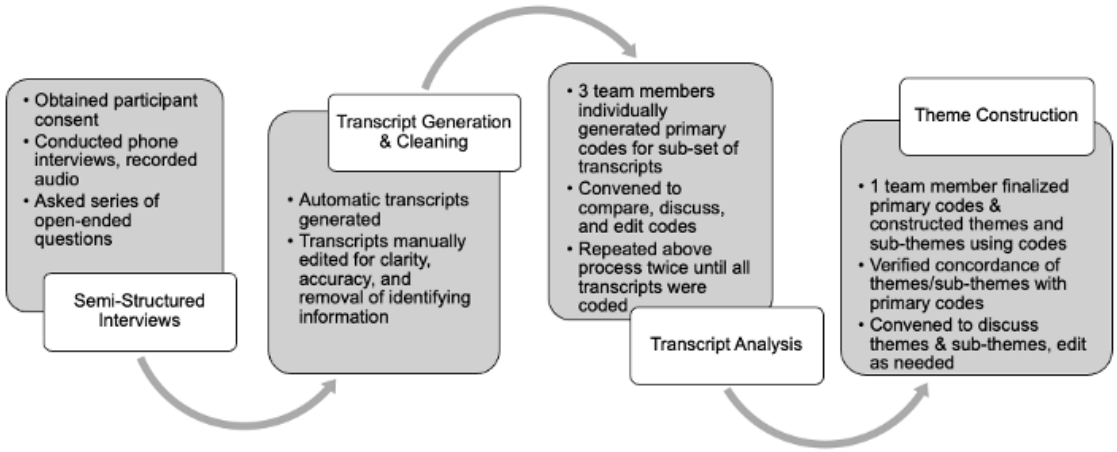


Figure 3.3 Data Collection and Thematic Analysis Approach.

Data collection and analysis occurred in the following four steps: semi-structured interviewing, transcript generation & cleaning, transcript analysis, and theme construction.

First, semi-structured interviews were conducted, and audio recorded, and auto-generated transcripts were edited for formatting, clarity, and removal of personally identifying information. Three researchers (TR, JM, JK) then independently familiarized themselves with the data by reading and re-reading the interview transcripts, writing brief notes/reflections, and, afterward, convening as a group to discuss overall impressions. Next, primary codes were generated over the course of three meetings. Prior to the first meeting, TR, JM, and JK independently generated primary codes using Braun and Clark's inductive thematic analysis approach. During the first meeting, TR, JM, and JK compared their primary codes, discussed disagreements, and revised their independently generated primary coding schemes in preparation for the second meeting. For the second and third meetings, this process was repeated on two-thirds and all 18 transcripts, respectively. After the third meeting, the primary coder (TR) drafted a finalized primary

coding scheme, which was discussed with and mutually agreed upon by JM and JK. TR then re-coded each transcript using the finalized coding scheme, developed main themes and corresponding sub-themes, and discussed results with all co-authors who agreed with the final analysis. Microsoft Excel was used to document overall impressions, primary codes, themes, and sub-themes.

3.2.3 Results

Sample Characteristics: Of 18 participants, almost one third reported an annual household income of over $65,000; two thirds earned either a bachelor's degree or graduate degree; and almost three quarters of our participants identified as non-Hispanic white. Three participants (17%) self-identified as non-Hispanic Black and two participants (11%) self-identified as Hispanic/Latina (Table 3.2).

Table 3.2 Sample characteristics of interviewees

Participant	Age	Interview Date	Obstetrical Status*	Parity	Educational Attainment	Annual Household Income	Race/Ethnicity
1	32	Jun. 2020	2 mo. postpartum (pp)	Multiparous (Multi)	Master's	> $65,000	Non-Hispanic white (NHW)
2	32	Jun. 2020	32 wk. GA	Multi	Professional	> $65,000	NHW
3	38	Jun. 2020	35 wk. GA	Multi	Professional	$65,000	NHW
4	35	Jun. 2020	4 mo. pp	Multi	Bachelor's	< $40,000	NH Black (NHB)
5	33	July 2020	32 wk. GA	Multi	Master's	-	NHW
6	31	Aug. 2020	14 wk. GA	Nulliparous (Nulli)	Master's	$65,000	NHW
7	37	Oct. 2020	11 wk. GA	Multi	Some College	> $65,000	NHW
8	25	Oct. 2020	15 wk. GA	Nulli	Associate	-	NHW
9	33	Nov. 2020	12 wk. GA	Nulli	Doctorate	> $65,000	NHW
10	29	Dec. 2020	9 wk. GA	Nulli	Master's	-	NHB
11	34	Jan. 2021	13 wk. GA	Multi	Some College	< $40,000	Hispanic/Latina (HL)
12	27	Jun. 2021	1.5 yr. pp	Multi	Master's	> $65,000	NHW
13	29	Jun. 2021	1.5 yr. pp	Multi	Some College	< $40,000	NHB
14	28	Jun. 2021	10 mo. pp	Multi	Bachelor's	< $40,000	NHW
15	34	Jun. 2021	3 mo. pp	Nulli	Some College	$45,000-54,999	NHW
16	32	Jul. 2021	2 wk. pp	Multi	Doctorate	> $65,000	NHW
17	35	Aug. 2021	5 mo. pp	Multi	Professional	-	NHW
18	33	Sep. 2021	1.75 yr. pp	Multi	Some College	$55,000-64,999	HL

*indicates each participant's gestational age or postpartum age at the time of the interview

Response Context: 15 of 18 participants (83%) reported that they were generally doing well at the time of the interview, and all currently pregnant participants (50%) reported that their pregnancies were progressing well. About one-quarter of our participants reported caregiving for more than one child. Additionally, in mid-2020, most participants reported not knowing anyone infected by SARS-CoV-2; however by late 2020 and early 2021, several participants reported loved ones or friends becoming ill and recovering or dying. Overall, 35% of participants had a loved one with a COVID diagnosis, 12% experienced knowing someone who died of COVID, and 65% did not have any loved ones afflicted with COVID. One participant reported being afflicted with COVID (post study participation); the remaining 17 participants either did not have COVID or did not report it.

Broadly, we noted that participant responses encompassed two levels: their experiences/reflections on a macro-level (e.g., pertaining to greater society or their communities) and on a micro-level (e.g., pertaining to the individual, their daily lives, or immediate social circles). Additionally, because we spoke with participants across the first two years of the pandemic, participant responses varied on a temporal basis. For example, some participants who were interviewed more recently discussed how their experiences changed over time. Considerations of level (macro vs. micro) as well as temporality of participants' experiences influenced the construction of our themes and sub-themes.

Themes: Based on participant responses, we constructed 6 themes and 21 accompanying sub-themes (Figure 3.4). Appendix C lists exemplar quotes from interviewees that convey the essence of each sub-theme.

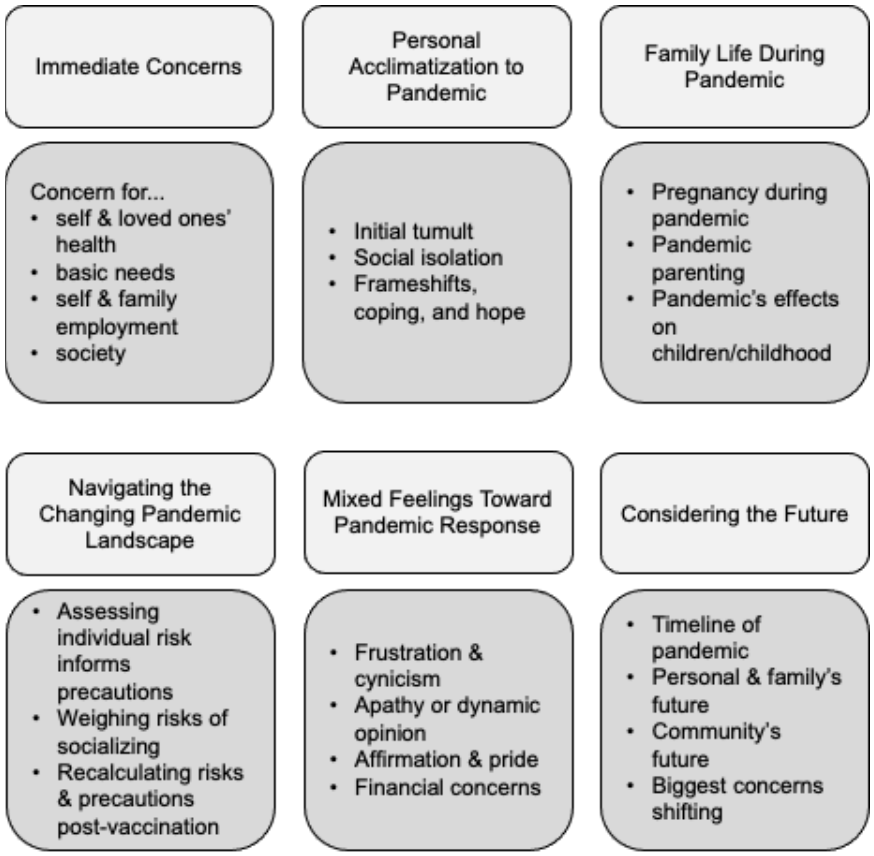


Figure 3.4 Summary of themes and sub-themes constructed

3.2.4 Discussion

Our study brings to light how the shifting pandemic landscape has been differentially experienced and navigated by new and expectant mothers. Our findings also highlight how participants' concerns shifted from short-term, individual-level concerns, such as

their pregnancy or their child's social development, to long-term, community-level concerns, such as the persistence of health inequities and housing instability over the course of the pandemic.

A temporal shift was also seen in participants' perception of the state of Ohio's public health and economic response to the pandemic. Specifically, many participants were initially supportive of the lockdowns and public health prevention measures, stating that they were satisfied with state leadership and the stringent public health measures. However, participants diverged after the first several months with some expressing dissatisfaction with the easing of public health restrictions and others expressing concerns about financial stability because of public health restrictions. Additionally, our study documents how participants feel as they consider the future and what their biggest concerns are, including the polarization of society (some worried about others' health or others' convenience), healthcare, housing, and financial inequalities, and the socialization of their children and what the "new normal" will look like as the pandemic progresses.

Many of our findings echo previously published work on the topic of new and expectant mothers' experience of the pandemic thus far. Regarding health concerns, earlier in the pandemic, all participants were more concerned for their health and the health of older loved ones who were at higher risk for severe illness. Upon rollout of the vaccine, health concerns shifted from themselves and older relatives to younger relatives and children who were unable to get vaccinated (at the time of the interviews). For many participants,

similar to previous findings, individual health risks and comorbid conditions informed their perception of pandemic severity, individual risk tolerance, and precautions taken[160]. Many participants also expressed sadness at the inability to spend time with loved ones, especially those who had planned to lean on loved ones for childcare support and to fully mourn and grieve loved ones who passed during the pandemic[149].

Our findings underscore and extend previously published work indicating that many factors exacerbate parental stress: increased economic burden, pandemic-induced stress, loss of employment, unstable childcare/school schedules, and difficulty coping[150]. Many of these factors were explicitly stated by our participants. Additionally, during the first few months of the pandemic, our participants also underwent a period of fear, uncertainty, and tumult, exemplified by particular concerns about the pandemic disrupting 'normal' experiences of pregnancy[161–163]. As the pandemic continued, many participants described trying to find coping mechanisms while feeling the pressure to keep going despite experiences of burnout[164].

Limitations

This study had several limitations. Firstly, the smaller, socio-demographically homogenous sample size limits the generalizability of our findings. This demographic composition is crucial to note as it reflects the participant population and it contextualizes participant responses within their socioeconomic spheres, which is a strong mediator of individuals' health trajectory and experience of the pandemic[165]. In our cohort, three

participants (17%) self-identified as non-Hispanic Black and two participants (11%) identified as Hispanic/Latina while 25.5% and 6.2% of FC residents identify as non-Hispanic Black and Hispanic, respectively[166]. Thus, our cohort is racially and ethnically comparable to the population in FC. However, regarding educational attainment, about 67% of our cohort has earned a bachelor's degree or higher, which is much higher than the proportion of FC residents with the same educational attainment (40.4%)[166]. Because our sample is skewed toward higher educational attainment, the pandemic parental experiences captured in our study cannot be assumed to be representative of those of mothers and caregivers of lower educational attainment and/or socioeconomic status.

Furthermore, interviews were conducted in Summer and Autumn of 2020 and 2021. Because the nature of the COVID-19 pandemic rapidly changed due to contagion and changing local/federal public health regulations, it is possible and likely that participants' responses differ across time and our findings only capture their thoughts at the time of the interview. Lastly, our semi-structured interview form consisted of themed but open-ended questions. As such, participant responses varied in detail, and we did not always ask them to elaborate or ask follow-up questions to be sensitive to the information they were sharing (i.e., emotionally salient recollections of family members' or their own illness/difficult period) and to continue building rapport for the remainder of the interview.

3.2.5 Conclusion

In summary, our study provides evidence that many expectant and new mothers are struggling during the pandemic, experiencing challenges to their mental health, family life, and career trajectories. The initial months of the pandemic seem to have been the most emotionally tumultuous time given general magnitude of uncertainty about the future during that period. As the pandemic progressed from 2020 to 2021, participants described their experiences and strategies navigating changing life conditions under the pandemic. Participants also described a shift in their concerns from individual-level concerns to community-level concerns over the course of the pandemic. These findings document temporal shifts in expectant and new mothers' experience of a years-long global pandemic and can help improve how social programs and clinical care supports this population.

Chapter 4 Racial Disparities in Microbial α-diversity Across Pregnancy

4.1 Introduction

Given its role in the physiological response to psychosocial stress, the microbiota-gut-brain axis and, more broadly, the gut microbiome, has been a mechanism of interest in the propagation of health disparities in the last several years[167,168]. For instance, several studies have demonstrated that the well-established socioeconomic status-health gradient is also replicated in the fecal microbiome[169–172]. Briefly, one study of twins in the United Kingdom found that a greater discordance in a residential area-based measure of socioeconomic status was associated with a greater difference in fecal microbiome composition between the twins[172]. Another study of generally healthy Chicagoans reported lower fecal α-diversity (within sample diversity) and differential abundance of *Prevotella* and *Bacteroides* associated with lower area-level SES[171].

Simultaneously, the United States (US), has persistent racial disparities in maternal health outcomes, most pronounced in rates of preterm birth, pre-eclampsia, fetal growth restriction, and maternal mortality[173]. Additionally, differential composition of the vaginal microflora is associated with altered susceptibility to preterm birth[114,174]Prenatal stress has also been noted as a potentially significant mediator of racial disparities in risk of adverse obstetrical outcomes, with heterogenous effects across specific outcomes and with unique sources, specifically among non-Hispanic black women[175–177]. Of note, many

of these racial disparities persist regardless of educational attainment, which is regarded as a generally health-protective factor[173].

Previous work focused on US-based samples has demonstrated race-ethnicity-associated differences in vaginal microbiome composition among non-pregnant and pregnant individuals, primarily related to dominance of specific species of *Lactobacilli*: *L. crispatus* versus *L. iners,* by self-report racial-ethnic heritage[178–181]. In the fecal microbiome, differential abundance of specific microbial taxa by self-report ethnicity have been documented in non-pregnant populations[182,183]. However, it remains unclear to what extent these vaginal and fecal microbial patterns are shaped by extant socioeconomic and dietary factors, US-specific social processes, and genetic influences, and few studies have directly interrogated the role of the gut microbiome in health inequities[168].

Thus, we sought to interrogate the extent to which maternal race influenced fecal and vaginal α-diversity in our sample. In contrast to previous studies focused on racial-ethnic heritage, this project sought to evaluate the contributions of societal biases to differential microbial attributes associated with race through the lens of the *weathering hypothesis*. Briefly, the *weathering hypothesis*, conceptualized by Professor Arline Geronimus, posits that cumulative stress experienced because of persistent social, political, and economic exclusion induces physiological consequences which increase vulnerability to disease and accelerate aging in marginalized groups [184,185]. Specifically, we hypothesized that racial

disparities would emerge in the change in vaginal and fecal α-diversity of pregnant individuals in our sample from early to late pregnancy.

4.2 Methods

For these analyses, we used data from our prospective cohort study, focusing on participants at the 3rd trimester with microbiome sequencing data available.

Description of the Measures

α-diversity: Broadly, α-diversity metrics are measures of within-sample diversity. For these models, two measures of α-diversity were chosen: Shannon's entropy, a composite measure of richness and evenness, and Pielou's evenness. Richness refers to the number of distinct microbial taxa present in each community while evenness refers to the relative proportions in which different taxa are present. Vaginal and fecal α-diversity values were calculated using taxonomic identities inferred by full-length 16S rRNA sequencing of vaginal and fecal swabs, as previously described (Chapter 2.2). α-diversity values were examined descriptively and tested for normality using appropriate visualizations in Stata/BE v.17.

Race: To determine participant race, race and ethnicity were abstracted from electronic medical records and verified with self-identified race and ethnicity. Due to low cell counts for participants from minority communities, race was collapsed into two mutually exclusive racial categories: non-white and white.

Other sociodemographic, obstetrical, and health-related variables: To minimize overfitting, covariates and possible confounds were identified based on existing literature linking each covariate to both fecal and/or vaginal α-diversity and race and based on availability in our dataset. All models included the sociodemographic covariates of maternal age, marital or cohabitating status, educational attainment, and health insurance status. For models predicting vaginal α-diversity, parity, number of adverse obstetrical outcomes, antibiotic use during pregnancy, and pre-pregnancy body mass index (BMI) were used as additional covariates. For models predicting fecal α-diversity, antibiotic use during pregnancy, psychotropic use during pregnancy, history of a diagnosed chronic health condition, and pre-pregnancy BMI were used as additional covariates.

Analytic Strategy

Multiple linear regression was used to regress the two measures of α-diversity (Shannon's entropy and Pielou's evenness) in the fecal and vaginal microbiomes at 3rd trimester on the aforementioned covariates in a series of models. α-diversity was the outcome or dependent variable while race was the predictor or independent variable. To evaluate possible racial disparities in change in α-diversity across pregnancy, models also controlled for 1st trimester α-diversity.

4.3 Results

Descriptive Results

Descriptive findings for the vaginal and fecal sub-samples stratified by race are presented in Table 4.1. When controlling for 1st trimester evenness at 3rd trimester, the sub-sample with values for both timepoints was smaller than previously analyzed sub-samples of this cohort. Non-white participants tended to have higher vaginal Shannon's entropy, as compared to white participants, across both timepoints. Vaginal Pielou's evenness shifted across timepoints with non-white participants initially having slightly higher evenness then, by 3rd trimester, having lower evenness, as compared to white participants. Fecal Shannon's entropy was similar between racial groups at 1st trimester and non-white participant's entropy increased while white participants' entropy slightly decreased by 3rd trimester. Fecal Pielou's evenness followed a similar pattern of change across timepoints and was slightly higher in white participants at 1st trimester, as compared to non-white participants.

Table 4.1 Descriptive for vaginal and fecal sub-samples overall and stratified by maternal race

	Vaginal Sub-Sample			Fecal Sub-Sample		
	Total (n=16)	non-white (n=6)	white (n=10)	Total (n=17)	non-white (n=8)	white (n=9)
Outcome Variables - Measures of α-diversity						
Shannon's Entropy						
1st Trimester	1.9 (1.5-2.6)	2.1 (1.9-2.6)	1.8 (1.4-2.6)	6.1 (5.5-6.8)	6.1 (5.3-7.1)	6.2 (5.6-6.6)
3rd Trimester	2.0 (1.4-2.6)	2.1 (1.4-2.4)	1.9 (1.4-2.6)	6.9 (5.8-7.7)	7.6 (6.9-8.4)	5.8 (4.8-6.9)
Pielou's Evenness						
1st Trimester	0.5 (0.4-0.5)	0.5 (0.4-0.5)	0.4 (0.3-0.6)	0.8 (0.7-0.8)	0.7 (0.7-0.8)	0.8 (0.7-0.8)
3rd Trimester	0.5 (0.4-0.6)	0.4 (0.3-0.6)	0.5 (0.4-0.6)	0.8 (0.7-0.9)	0.9 (0.8-0.9)	0.7 (0.6-0.8)
Sociodemographic Background						
Age (years)	32.5 (28.5-34.0)	33.0 (29.0-34.0)	32.0 (28.0-34.0)	32.0 (27.0-33.0)	31.0 (27.0-33.5)	32.0 (27.0-33.0)
Not Married or Cohabitating	2 (12.5%)	2 (33.3%)	0 (0.0%)	4 (23.5%)	3 (37.5%)	1 (11.1%)
Education - bachelor's or more	7 (43.8%)	1 (16.7%)	6 (60.0%)	9 (52.9%)	1 (12.5%)	8 (88.9%)
Health Insurance						
Private	7 (43.8%)	0 (0.0%)	7 (70.0%)	6 (35.3%)	0 (0.0%)	6 (66.7%)
Public/Government	7 (43.8%)	5 (83.3%)	2 (20.0%)	10 (58.8%)	7 (87.5%)	3 (33.3%)
None	2 (12.5%)	1 (16.7%)	1 (10.0%)	1 (5.9%)	1 (12.5%)	0 (0.0%)
Health History & Obstetrical Outcomes						
Multiparous	9 (56.2%)	6 (100.0%)	3 (30.0%)	12 (70.6%)	7 (87.5%)	5 (55.6%)
# Adverse OB Outcomes						
0	10 (62.5%)	4 (66.7%)	6 (60.0%)	9 (52.9%)	5 (62.5%)	4 (44.4%)
1	5 (31.2%)	2 (33.3%)	3 (30.0%)	7 (41.2%)	3 (37.5%)	4 (44.4%)
2	1 (6.2%)	0 (0.0%)	1 (10.0%)	1 (5.9%)	0 (0.0%)	1 (11.1%)
Antibiotics Used in Pregnancy	4 (25.0%)	1 (16.7%)	3 (30.0%)	2 (11.8%)	1 (12.5%)	1 (11.1%)
Pre-Pregnancy BMI (kg/m^2)	25.2 (23.5-27.0)	27.0 (25.7-32.1)	24.3 (22.1-25.3)	25.7 (23.3-28.2)	29.3 (26.1-32.7)	23.8 (22.4-24.6)
Psychotropics Used in Pregnancy	3 (18.8%)	1 (16.7%)	2 (20.0%)	4 (23.5%)	2 (25.0%)	2 (22.2%)
History of Diagnosed Chronic Health Condition	2 (12.5%)	1 (16.7%)	1 (10.0%)	1 (5.9%)	1 (12.5%)	0 (0.0%)

Regression Results

Across all generated models, model 1 controls for only race and 1st trimester α-diversity, models 2 through 4 successively control for three additional sociodemographic factors, and models 5 through 8 control for all covariates included in model 4 in addition to a single additional health history or obstetrical outcome variable.

Table 4.2 Regression results predicting 3rd trimester vaginal α-diversity, controlling for 1st trimester α-diversity

Shannon's Entropy	**Model 1**		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**		**Model 7**		**Model 8**	
	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE
Race (reference = non-white)	0.199	0.312	0.151	0.229	0.282	0.277	0.356	0.340	-0.148	0.414	0.359	0.350	0.306	0.358	0.464	0.369
1st Tri. Shannon's Entropy	0.583**	0.201	0.654***	0.149	0.653***	0.150	0.647***	0.157	0.598***	0.144	0.620***	0.167	0.649***	0.161	0.648***	0.159
Age			-0.087***	0.025	-0.075**	0.028	-0.067	0.037	-0.066*	0.034	-0.0638	0.0386	-0.0626	0.0388	-0.0698*	0.0378
Cohabitating Status (ref = cohabitating)					0.372	0.429	0.400	0.451	0.459	0.408	0.351	0.471	0.532	0.505	0.349	0.463
Education (ref = less than bachelor's)							-0.137	0.332	0.083	0.323	-0.134	0.342	0.00797	0.406	-0.0957	0.341
Parity (ref = nulliparous)									-0.601	0.332						
# Adverse OB Outcomes											-0.137	0.213				
Antibiotic Use (ref = none)													0.261	0.393		
Pre-pregnancy BMI (kg/m^2)															0.0338	0.0410
Constant	0.493	0.707	3.133***	0.917	2.175	1.442	1.801	1.753	2.846	1.684	1.862	1.809	1.461	1.876	0.891	2.098
N	16		16		16		16		16		16		16		16	
R-squared	0.396		0.700		0.720		0.724		0.798		0.737		0.737		0.744	
Pielou's Evenness	**Model 1**		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**		**Model 7**		**Model 8**	
	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE
Race	0.041	0.061	0.033	0.049	0.088	0.053	0.078	0.065	0.007	0.086	0.0779	0.0685	0.0615	0.0652	0.0942	0.0719
1st Tri. Pielou's Evenness	0.528**	0.209	0.635***	0.17	0.601***	0.155	0.600***	0.162	0.562***	0.161	0.604***	0.172	0.618***	0.159	0.596***	0.168
Age			-0.016**	0.005	-0.0111*	0.005	-0.012	0.007	-0.012	0.007	-0.0125	0.00755	-0.0109	0.00707	-0.0126	0.00738
Cohabitating Status					0.157*	0.082	0.154	0.087	0.164*	0.085	0.157	0.0925	0.197*	0.0923	0.146	0.0906
Education							0.017	0.063	0.050	0.067	0.0165	0.0667	0.065	0.0737	0.0234	0.0662
Parity									-0.085	0.069						
# Adverse OB Outcomes											0.00942	0.0405				
Antibiotic Use													0.0867	0.072		
Pre-pregnancy BMI (kg/m^2)															0.00504	0.008
Constant	0.139	0.15	0.593**	0.195	0.196	0.272	0.245	0.336	0.392	0.349	0.242	0.354	0.13	0.343	0.11	0.408
N	16		16		16		16		16		16		16		16	
R-squared	0.336		0.613		0.709		0.711		0.753		0.713		0.751		0.724	

*p<0.05, **p<0.01, ***p<0.001

Vaginal Shannon's entropy

Regression results for vaginal Shannon's entropy are presented in Table 4.2 and predict change in vaginal entropy, from 1st to 3rd trimester. Model 1 predicts that, on average, white participants will have a 0.199-unit greater increase in entropy across pregnancy, as compared to non-white participants, not controlling for sociodemographic or obstetrical factors. We see that race is not a significant predictor across any model ($p>0.05$) but consistently predicts a greater increase in entropy across gestation for white participants, as compared to non-white participants, with the exception of model 5. In model 5, controlling for parity in addition to sociodemographic factors and 1st trimester entropy, white participants are predicted to have, on average, a 0.148-unit smaller change in entropy from 1st to 3rd trimester, as compared to non-white participants. In this model, 1st trimester entropy and age are statistically significant predictors of change in entropy ($p<0.001$ and $p<0.05$, respectively). Age consistently predicts a smaller change in entropy as it increases and is a significant predictor across several models, with its statistical significance reducing with the inclusion of educational attainment, adverse obstetrical outcomes, and antibiotic use (models 4, 6, and 7, respectively). Age remains a significant predictor in models 5 and 8, which control for parity and pre-pregnancy BMI, respectively ($p<0.05$ for both models).

Vaginal Pielou's evenness

Regression results for vaginal Pielou's evenness are presented in Table 4.2 and predict change in vaginal evenness from 1st trimester to 3rd trimester. We see that race is not a

statistically significant predictor in any model ($p>0.05$) but consistently predicts a greater change in evenness among white participants. For example, in model 4, controlling for 1st trimester evenness and all sociodemographic covariates, we see that white participants are predicted to have, on average, a 0.078-unit greater increase in evenness from 1st to 3rd trimester, as compared to non-white participants. Additionally, 1st trimester evenness is a consistently significant predictor of 3rd trimester evenness ($p<0.001$) such that greater 1st trimester evenness is predicted to increase 3rd trimester evenness. In contrast, higher age predicts lower evenness across all models, though it is only a statistically significant predictor in the absence of additional sociodemographic and obstetrical covariates ($p<0.01$ in Model 2; $p<0.05$ in Model 3; $p>0.05$ in Models 3 through 8). Interestingly, cohabitation status predicts higher evenness among those who are not cohabitating. In model 3, controlling for race, 1st trimester evenness, and age, those who are not cohabitating are predicted to have, on average, a 0.157 unit increase in evenness, as compared to those who are cohabitating with a partner. Cohabitation status is also a significant predictor in models 5 and 7, controlling for parity and antibiotic use in pregnancy, respectively ($p<0.05$).

Table 4.3 Regression results predicting 3rd trimester fecal α-diversity, controlling for 1st trimester α-diversity

Shannon's Entropy	**Model 1**		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**		**Model 7**		**Model 8**	
	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE
Race (reference = non-white)	-1.480**	0.587	-1.483**	0.609	-1.224*	0.628	-1.814	1.074	-2.029	1.236	-1.827	1.181	-1.772	1.051	-2.140	1.187
1st Tri. Shannon's Entropy	0.406*	0.213	0.411*	0.223	0.382	0.219	0.367	0.225	0.345	0.240	0.372	0.271	0.390	0.221	0.415	0.239
Age			0.009	0.059	0.033	0.061	0.006	0.073	0.005	0.076	0.005	0.087	-0.010	0.073	0.002	0.075
Cohabitating Status (ref = cohabitating)					1.003	0.783	0.970	0.803	1.102	0.896	0.948	1.022	1.146	0.799	1.090	0.838
Education (ref = less than bachelor's)							0.770	1.123	1.107	1.433	0.779	1.202	1.060	1.125	0.701	1.153
Antibiotic Use (ref = none)									0.535	1.315						
Psychotropic Use (ref = none)											-0.039	1.039				
History of Chronic Illness (ref = none)													1.701	1.393		
Pre-pregnancy BMI (kg/m^2)															-0.078	0.109
Constant	6.512***	1.624	6.229**	2.546	4.040	3.016	5.469	3.724	5.560	3.880	5.535	4.279	5.293	3.646	7.749	4.947
N	17		17		17		17		17		17		17		17	
R-squared	0.416		0.417		0.487		0.508		0.516		0.508		0.572		0.532	

Pielou's Evenness	**Model 1**		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**		**Model 7**		**Model 8**	
	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE	Coef.	SE
Race	-0.125**	0.043	-0.125**	0.044	-0.109**	0.046	-0.123	0.0803	-0.162*	0.087	-0.111	0.088	-0.121	0.081	-0.154	0.088
1st Tri. Pielou's Evenness	0.327	0.211	0.326	0.219	0.319	0.217	0.314	0.227	0.295	0.226	0.269	0.259	0.318	0.228	0.368	0.236
Age			0.001	0.004	0.003	0.004	0.002	0.005	0.002	0.005	0.003	0.007	0.001	0.006	0.001	0.006
Cohabitating Status					0.065	0.057	0.064	0.060	0.088	0.063	0.080	0.073	0.0748	0.0614	0.0765	0.0618
Education							0.019	0.084	0.081	0.101	0.011	0.089	0.0361	0.0868	0.0127	0.0850
Antibiotic Use									0.100	0.092						
Psychotropic Use											0.031	0.074				
History of Chronic Illness													0.098	0.107		
Pre-pregnancy BMI (kg/m^2)															-0.007	0.008
Constant	0.740***	0.166	0.714***	0.211	0.563**	0.247	0.599*	0.304	0.600*	0.301	0.552	0.335	0.595*	0.306	0.801*	0.378
N	17		17		17		17		17		17		17		17	
R-squared	0.418		0.421		0.477		0.479		0.535		0.488		0.520		0.519	

*p<0.05, **p<0.01, ***p<0.001

Fecal Shannon's Entropy

Regression results for fecal Shannon's entropy are presented in Table 4.3 and predict change in fecal entropy from 1st trimester to 3rd trimester. We see that race is initially a significant predictor of change in fecal entropy: in model 1, white participants are predicted to have, on average, a 1.48-unit lower change fecal entropy, as compared to non-white participants ($p<0.01$). The statistical significance of race remains in model 2, with the addition of age, but diminishes with the addition of cohabitation and education covariates in models 3 and 4 ($p<0.05$ and $p>0.05$, respectively). Additionally, 1st trimester entropy is a significant predictor in models 1 and 2 only ($p<0.05$). In model 2, controlling for race and age, a 1 unit increase in 1st trimester entropy is predicted to yield, on average, a 0.41-unit greater change in entropy from 1st to 3rd trimester ($p<0.05$).

Fecal Pielou's Evenness

Regression results for fecal Pielou's evenness are presented in Table 4.3 and predict change in fecal evenness from 1st trimester to 3rd trimester. We see that race is a statistically significant predictor in models 1 through 3 and in model 5. In model 1, white participants are predicted to have, on average, a 0.125-unit smaller change in fecal evenness across pregnancy, as compared to non-white participants ($p<0.01$). The statistical significance of race is greatly diminished with the addition of educational attainment as a covariate: from model 3 to model 4, race's p-value increases from <0.01 to >0.05. Additionally, in contrast to models of Shannon's entropy, 1st trimester evenness

is not a significant predictor of change in evenness across gestation in any model, though it consistently predicts greater change in evenness with higher 1st trimester evenness.

4.4 Discussion

Comparing models of vaginal entropy and evenness, in contrast to our hypothesis, there were no racial disparities in the variation of vaginal α-diversity across pregnancy. However, we see that 1st trimester α-diversity is a statistically significant predictor across all models and that age is sometimes a significant predictor of smaller change in both entropy and evenness among white participants, as compared to non-white participants. Interestingly, cohabitating status is only a significant predictor in the evenness models. Relatedly, a recent study noted that intra-household sharing of specific microbial taxa was a significant contributor to individual the individual gut microbiome, however no studies have focused on the influence of cohabitation on the vaginal microbiome[186].

Comparing models of fecal entropy and evenness, in accordance with our hypothesis, we see that race is initially a statistically significant predictor of the change in fecal α-diversity across gestation. Both entropy and evenness are predicted to change less among white participants, as compared to non-white participants. Additionally, the statistical significance of race is greatly diminished with the addition of educational attainment as a covariate (model 4 for both entropy and evenness). This may indicate an interaction between race and socioeconomic status (for which educational attainment is a proxy in the US) in our sample. This can be seen as concordant with previous work demonstrating

that, during pregnancy, race and socioeconomic status are stronger predictors of adverse pregnancy outcomes when considered together[187].

Lastly, higher 1st trimester fecal entropy and evenness consistently predict a greater change in each metric across gestation. This finding considered in combination with our finding that 1st trimester vaginal α-diversity is highly predictive of the change in vaginal α-diversity across pregnancy suggest that the microbial shifts occurring across pregnancy may be highly individual-specific, an idea supported by previous studies[82].

Limitations

The findings of these regression models should be interpreted with limitations in mind. First, due to participant attrition and microbiome sample quality, sample size was greatly reduced in the models controlling for 1st trimester α-diversity at 3rd trimester. Future iterations of these models can be improved using several approaches. First, by transforming α-diversity values using other transformations, such as cubing or taking the square-root, which may yield more normally distributed values for the outcome, thereby more closely meeting the normal distribution assumption of ordinary least-squares linear regression. Secondly, a series of partial F-tests could be conducted to test whether inclusion of specific sociodemographic or health covariates meaningfully improves model fit. Thirdly, these models assume a linear relationship between α-diversity and included continuous/integral covariates (i.e., age, pre-pregnancy BMI). Future iterations could improve model fit by verifying this assumption and transforming covariates as

appropriate. Similarly, these models do not include any interaction terms, which could capture possible multicollinearity between related covariates, such as age and parity or educational attainment and health insurance status.

4.5 Conclusion

Given the persistent racial disparities in maternal health outcomes in the US and growing interest in the microbiome as a mediator of health disparities, we used a sub-set of our prospective cohort study of pregnant individuals to assess racial disparities in the variation of vaginal and fecal α-diversity from early to late pregnancy. Using multiple linear regression models, we found no significant racial disparities in vaginal α-diversity but did find racial disparities in fecal α-diversity which diminish when controlling for additional covariates. Emergence of racial disparities in a study of this size indicate that the gut microbiome may be implicated in the biological embedding of cumulative psychosocial stress induced by social processes. Future studies should continue to meaningfully interrogate this relationship in the context of pregnancy.

Chapter 5 Conclusion

5.1 Summary of Major Findings

In summary, we found stress and depressive symptoms to be associated with increased relative abundance of opportunistic pathogens. Additionally, depressive symptoms, but not stress, were associated with lower relative abundance of butyrate-producing genera in the fecal microbiome. Stress and depressive symptoms were also associated with increased relative abundance of vaginal taxa associated with obstetrical complications and infections. Lastly, in concordance with previous literature, umbilical CCL2 concentration was negatively correlated with relative abundance of fecal *Lactobacilli.* Together, these findings underscore previous preclinical and clinical work demonstrating the effects of prenatal stress on the maternal microbiome and extend the literature by offering several fecal and vaginal taxa which may serve a critical role in this relationship.

Secondly, we found that pregnancy during the pandemic was associated with distinct shifts in fecal, but not vaginal, microbiome composition from early to late pregnancy in the absence of significantly different levels of stress and depressive symptoms. Overall, far more of these fecal taxa shifts were seen in early pregnancy as compared to late pregnancy, delivery, and postpartum, possibly due to diminishing sample sizes at later time. The cause(s) of these microbial shifts cannot be determined in the current study, thus we propose several contributing factors: the first two years of the COVID-19

pandemic saw global shifts in daily lifestyles, likely altering intrapersonal and interpersonal exposures or experiences such as time spent in indoor/outdoor environments or private/public environments, social interactions, diet, physical activity, and preventive measures employed. These factors may all have influenced psychosocial stress or directly influenced intestinal microbiome composition.

We characterized these altered experiences in a sub-sample of our cohort using semi-structured interviews from which we constructed themes and sub-themes speaking to the scope and temporality of changes in experience of the pandemic as it continued. We found that many expectant and new mothers struggled during the pandemic, experiencing challenges to their mental health, family life, and career trajectories, with the initial months of the pandemic having been the most emotionally tumultuous time. As the pandemic progressed from 2020 to 2021, we documented participants' experiences and strategies navigating changing life conditions and shifts in their concerns from individual-level to community-level. These findings document temporal shifts in expectant and new mothers' experience of a years-long global infectious disease pandemic.

Thirdly, we used multiple linear regression to test for racial disparities in changes in fecal and vaginal α-diversity across pregnancy. We found that race was a significant predictor of change in fecal α-diversity, but not vaginal α-diversity, and that the inclusion of additional sociodemographic covariates modified these associations.

5.2 Summary of Major Contributions

This dissertation presents the findings of a prospective cohort study of pregnant individuals containing multiple timepoints with repeated measures of both psychometrics and biospecimen collection, including full-length 16S rRNA sequencing of fecal and vaginal microbial samples. Additionally, we report distinct shifts in relative abundance of both fecal and vaginal taxa associated with symptoms of stress and depression across multiple timepoints during pregnancy and postpartum. We also demonstrate that the COVID-19 pandemic, which has been a disruptive force globally, is associated with distinct shifts in fecal, but not vaginal, taxa during pregnancy and postpartum. Lastly, we present an interrogation of racial disparities in microbial α-diversity change across pregnancy, finding evidence for racial disparities in fecal, but not vaginal, α-diversity. The emergence of racial disparities in a study of this size provide additional evidence for the gut microbiome as a mediator of the biological embedding of cumulative psychosocial stress induced by social processes.

5.3 Future Directions

Future studies might consider the use of metabolomics to better understand the role of each microbial group in its community. Additionally, future studies could focus on a more specific clinical population such as pregnant individuals with active major depressive disorder to interrogate its specific microbe-associated mechanisms. In terms of additional covariates, future work should endeavor to control for the role of maternal diet,

perhaps using self-report food recall information to calculate established indices of diet demonstrated to influence intestinal microbiome community structure and function, such as the Healthy Eating Index[188]. Additionally, inclusion of lifestyle/health behavior factors such as physical activity, sleep quality, cohabitation with domestic pets would strengthen future work in this area.

We may also consider the sources of psychosocial stress (i.e., measures of pregnancy-specific stress, stressful life events, shared community-level traumatic events) in place of a global measure of perceived stress[189,190].

Greater racial-ethnic and sociodemographic representation in future studies will allow for a more comprehensive consideration of the specific contributions of environmental and social exposures, and health behaviors on psychosocial stress and the maternal microbiome. Additionally, future analyses assessing racial disparities could probe to what extent maternal race moderate the relationship between perceived stress and vaginal or fecal α-diversity.

Future work can also consider the contributions of the built environment to stress and the maternal gut microbiome. In the past few decades, much sociological, environmental, and public health research has focused on the consequences of the physical environment on human health. More specifically, several environmental exposures have been associated with mental health outcomes and/or gut microbial alterations. The Public Health

Exposome Framework and Analytics are a novel, comprehensive approach to characterizing the totality of exposures across time and calculating risk trajectories for specific populations and health outcomes[191,192]. It conceptualizes the environment into four domains: natural, built, social, and policy, and combines these with additional individual-level health information. The built environment can be broadly defined as the collection of places in which humans spend their time.

Future studies will use this prospective cohort study to assess several attributes of the built environment which have previously been demonstrated to affect psychosocial stress and its sequelae: traffic-related noise pollution, ambient $PM_{2.5}$ exposure, proximity to public greenspace, and excessive ambient heat exposure[193–198]. Additionally, the burden of these exposures and their consequences is often disproportionately experienced by communities of marginalized individuals—sometimes referred to as *environmental justice communities*[199].

We will assess questions such as: *Is increased proximity to public greenspace associated with lower symptoms of stress and depression? Is greater exposure to heat associated with more severe symptoms of stress and depression? Is greater proximity to traffic associated with more severe symptoms of stress and depression? To what extent does heat exposure moderate the relationship between stress and fecal α-diversity at 1st trimester? To what extent does proximity to public greenspace moderate the relationship*

between stress and fecal α-diversity at 1st trimester? And to what extent does proximity to traffic moderate the relationship between stress and fecal α-diversity at 1st trimester?

To evaluate these questions, we will use multiple linear regression, focusing on the 1st trimester of pregnancy and using residential zip code-based weighted averages of each environmental variable. These variables will be derived from publicly available datasets such as EJScreen[200].

5.4 Clinical Considerations

To reduce the negative impacts of prenatal stress and its negative sequelae on perinatal mental health, gestational outcomes, and offspring health, greater emphasis must be placed at multiple levels, including access to comprehensive prenatal and postnatal care[201]. Here, emerging therapeutic interventions related to the microbiome are summarized:

The gut microbiome has been a target of interest for the most recent efforts to mitigate or prevent the adverse effects of prenatal psychosocial stress and depression. Some of the most prevalently researched interventions include orally administered pre- and probiotics and fecal microbiota transplantation. Prebiotics are defined as substrates which are selectively utilized by host microorganisms (i.e. dietary fiber and inulin found in some fruits, vegetables, and grains) while probiotics are live microorganisms which can be found, for example, in a variety of foods such as some yogurts, kombucha, sauerkraut, and sourdough bread. Both prebiotics and probiotics are anticipated to confer a

'beneficial' effect on gut flora via a variety of mechanisms to yield a corresponding plethora of improved health outcomes[202,203]. The vast majority of research on these interventions has been conducted with preclinical models which demonstrate that a) specific cocktails of prebiotics can help reduce chronic stress, anxiety, and depressive-like behavior and b) distinct strains of probiotics can effect a number of neuronal processes, including behavioral symptoms of affective disorders[53]. Clinical studies conducted within the last decade have mixed results due to strain-specific effects, study design, sample demographics, variable dosage, confounding covariates (i.e. physical environment, diet, lifestyle or health behaviors, history of adversity), and individual genetic differences[48,49,53]. Still, interest in the use of pre- and probiotics to improve psychiatric conditions via intervention of the microbiota-gut-brain axis—referred to as *psychobiotics*—continues to grow[53].

To date, studies of pregnant women have found little evidence that oral probiotic administration during pregnancy changes risk of a variety of outcomes, including preterm birth, bacterial vaginosis, and gestational diabetes[202,204]. Additionally, orally-administered probiotics demonstrate potential anxiolytic and stress-relieving effects, specifically prenatal supplementation of *Lactobacillus rhamnosus* HN001[48,205]. Thus, future studies of pre- and probiotics should include studies of pregnant women and explore the potential effects of these microorganisms on maternal gestational mental health, birth outcomes, and offspring microbiomes and behavior.

A third potential therapeutic intervention in reducing the effect of maternal stress on offspring outcomes is fecal microbiota transplantation (FMT). FMT generally consist of the administration of a donor fecal sample to a recipient to transfer intestinal microbiota from donor to recipient. For instance, FMT can be conducted across two humans via colonoscopy, across two mice (i.e. wild-type donor and gnotobiotic recipient) via oral administration, and across a donor human and recipient mouse—referred to as the "humanization" of the murine microbiome[53]. Preclinical applications of FMT demonstrate intestinal microbiota's ability to mediate anxiety-like and depression-like behavior through several potential pathways[206,207]. Similar significant findings were reported in a recently conducted clinical study of patients with inflammatory bowel disease whose psychiatric symptoms decreased after one month of FMT treatment[208]. While these findings are encouraging, further research must be conducted to elucidate the role of microbes in psychological symptom alleviation and frame FMT's full therapeutic limitations and potential.

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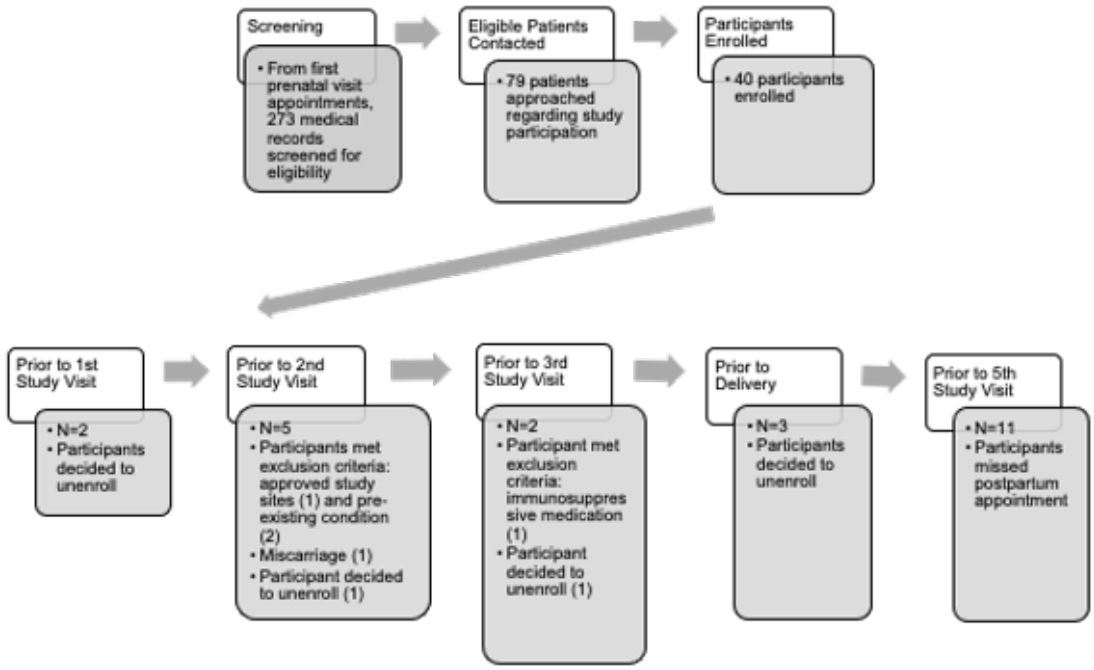
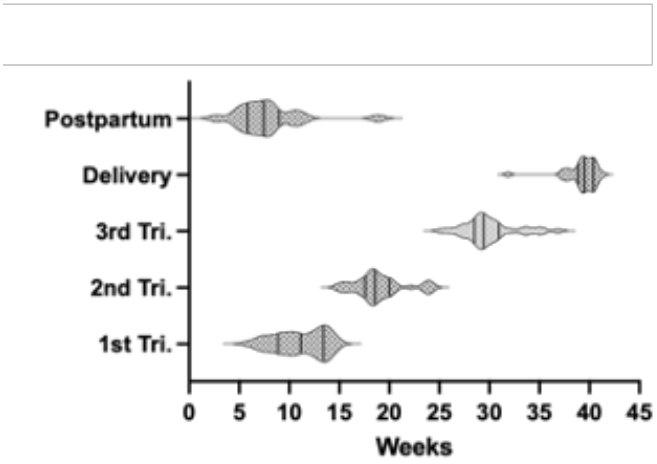


Figure A.1 Participant Recruitment and Loss



Study Visit	n	Median (IQR)
Postpartum	18	7.43 (8.86-13.43)
Delivery	28	39.5 (17.57-20)
3rd Tri.	29	29.43 (28.5-30.93)
2nd Tri.	31	18.57 (38.9-40.4)
1st Tri.	35	11.14 (5.823-9)

Figure A.2 Distribution of Gestational Age by Study Visit

Gestational age and weeks postpartum with minimum and maximum values are displayed via violin plot and interquartile ranges and sample sizes are listed by time point. At 1st trimester timepoint, n=3 values, all under 14 weeks GA, are missing due to participant exclusion from study following first visit.

Appendix B. Additional Sample Characteristics & Comparisons

Table B.1 Sample Characteristics

	Overall N=35	**1st Trimester** N=30	**2nd Trimester** N=20	**3rd Trimester** N=27	**Delivery** N=21	**Postpartum** N=14
Variable	N (%) or Median (IQR)					
Racial Background						
non-white	14 (40.0%)	11 (36.7%)	8 (40.0%)	11 (40.7%)	11 (52.4%)	4 (28.6%)
white	21 (60.0%)	19 (63.3%)	12 (60.0%)	16 (59.3%)	10 (47.6%)	10 (71.4%)
Educational Attainment						
Less than bachelor's	19 (54.3%)	16 (53.3%)	10 (50.0%)	14 (51.9%)	14 (66.7%)	7 (50.0%)
Bachelor's or more	16 (45.7%)	14 (46.7%)	10 (50.0%)	13 (48.1%)	7 (33.3%)	7 (50.0%)
Marital or Cohabitating Status						
Married or cohabitating	30 (85.7%)	26 (86.7%)	16 (80.0%)	22 (81.5%)	19 (90.5%)	13 (92.9%)
Not married, not cohabitating	5 (14.3%)	4 (13.3%)	4 (20.0%)	5 (18.5%)	2 (9.5%)	1 (7.1%)
Health Insurance						
Private	17 (48.6%)	15 (50.0%)	10 (50.0%)	11 (40.7%)	10 (47.6%)	9 (64.3%)
Public	16 (45.7%)	13 (43.3%)	10 (50.0%)	14 (51.9%)	10 (47.6%)	5 (35.7%)
None	2 (5.7%)	2 (6.7%)	0	2 (7.4%)	1 (4.8%)	0
Obstetrical History & Outcomes						
Parity						
nulliparous	17 (48.6%)	15 (50.0%)	8 (40.0%)	11 (40.7%)	9 (42.9%)	6 (42.9%)
multiparous	18 (51.4%)	15 (50.0%)	12 (60.0%)	16 (59.3%)	12 (57.1%)	8 (57.1%)
Adverse OB Outcomes						

	Overall	1st Trimester	2nd Trimester	3rd Trimester	Delivery	Postpartum
	N=35	N=30	N=20	N=27	N=21	N=14
Low Birth Weight (<2494.76g)	2 (5.7%)	2 (6.7%)	1 (5.0%)	1 (3.7%)	2 (9.5%)	0 (0.0%)
Preterm Birth (<36 wk GA)	1 (2.9%)	1 (3.3%)	1 (5.0%)	1 (3.7%)	0 (0.0%)	0 (0.0%)
Postpartum Pre-Eclampsia	1 (2.9%)	1 (3.3%)	0	1 (3.7%)	1 (4.8%)	1 (7.1%)
Gestational Diabetes	2 (5.7%)	2 (6.7%)	0	2 (7.4%)	2 (9.5%)	0 (0.0%)
GBS Positive	5 (14.3%)	3 (10.0%)	3 (15.0%)	4 (14.8%)	4 (19.0%)	3 (21.4%)
Chorioamnionitis	2 (5.7%)	2 (6.7%)	2 (10.0%)	2 (7.4%)	1 (4.8%)	1 (7.1%)
Psychometrics - Cronbach's α						
10-item PSS Score	-	0.81	0.82	0.73	0.87	0.80
CES-D Score	-	0.77	0.80	0.78	0.94	0.78
Psychometrics - Spearman's ρ (p-value)						
PSS vs. CES-D for Fecal sub-sample	-	0.47 (0.02*)	0.55 (0.01*)	0.84 (0.00***)	0.65 (0.003***)	0.71 (0.02*)
PSS vs. CES-D for Vaginal sub-sample		0.49 (0.02*)	0.55 (0.01*)	0.81 (0.00***)	0.24 (0.45)	0.64 (0.05)

OB: obstetrical; GA: gestational age; GBS: group B streptococcus; *p<0.05, **p<0.01

Table B.2 Alpha and beta-diversity metrics across groups

.	Fecal α-diversity	Fecal β-diversity	Vaginal α-diversity	Vaginal β-diversity
PSS & CES-D				
1st Trimester	NS	NS	NS	NS
2nd Trimester	NS	NS	NS	NS
3rd Trimester	NS	NS	NS	NS
Delivery	NS	NS	NS	NS
Postpartum	NS	NS	NS	NS
Pre-Pandemic vs. Pandemic				
1st Trimester	NS	Figure 3.1	NS	-
2nd Trimester	NS		NS	-
3rd Trimester	NS	NS	NS	-
Delivery	NS	NS	NS	-
Postpartum	NS	NS	NS	-

NS: not statistically significant, $p>0.05$

Table B.3 Correlations between relative abundance of fecal genera and 3rd trimester maternal and umbilical cord cytokines

	3rd Trimester			Delivery							
	Fusobacterium			**Fusobacterium**		**Peptoniphilus**		**Lactobacillus**		**Sneathia**	
	N	Spearman's ρ	p-value	N	ρ	p-value	Pearson's r	p-value	ρ	p-value	ρ	p-value
Maternal Blood												
IFN-γ	-	-	-	8	-0.374	0.408	-0.415	0.355	0.071	0.879	0.49	0.264
TNF-a	-	-	-	8	0.433	0.331	-0.349	0.442	0.036	0.939	0.535	0.216
CCL2	-	-	-	8	0.611	0.145	-0.361	0.426	-0.464	0.294	0.223	0.631
IL-6	-	-	-	8	0.118	0.801	0.187	0.688	0	1	0.134	0.775
Umbilical Cord Blood												
IFN-γ	11	0.456	0.217	13	-0.048	0.889	0.161	0.636	0.018	0.958	0.2	0.555
TNF-a	11	0.525	0.147	13	0.105	0.759	-	-	-	-	-	-
CCL2	10	-	-	13	0.439	0.177	-0.361	0.276	-0.724	0.012*	0.1	0.77
IL-10	11	0.479	0.192	12	0.123	0.735	-0.03	0.935	-0.236	0.511	-0.406	0.244
IL-6	9	-	-	12	-0.124	0.717	-0.237	0.483	-0.023	0.947	-0.4	0.223

Appendix C. Sample Characteristics Stratified by Pandemic Group

Table C.1 Characteristics Stratified by Pandemic Group

Demographic, obstetrical, and psychometric outcomes are presented for each timepoint by pre-pandemic and during pandemic sub-groups. Wilcoxon rank-sum and Fisher's exact tests were used to compare distributions across sub-groups. Frequency and percentage or median and interquartile range are presented.

	1st Trimester				**2nd Trimester**				**3rd Trimester**				**Delivery**				**Postpartum**			
	Total	Pre	During	*p*	T	Pre	During	*p*	T	Pre	During	*p*	T	Pre	During	*p*	T	Pre	During	*p*
Variable	N=26	N=20	N=6		N=20	N=15	N=5		N=21	N=14	N=7		N=19	N=12	N=7		N=12	N=5	N=7	
Demographics																				
Maternal Age (years)	31.5 (25.0-34.0)	29.0 (24.5-33.0)	33.0 (31.0-35.0)	0.2	32.0 (26.0-34.5)	29.0 (24.0-35.0)	33.0 (32.0-34.0)	0.29	32.0 (25.0-34.0)	28.0 (24.0-33.0)	34.0 (32.0-35.0)	0.04*	29.0 (20.0-33.0)	25.5 (19.5-33.0)	33.0 (28.0-34.0)	0.14	31.5 (25.5-35.5)	24.0 (20.0-27.0)	34.0 (31.0-37.0)	0.088
Pre-pregnancy BMI (kg/m2)	26.1 (23.3-31.1)	27.0 (23.2-29.7)	25.3 (24.6-33.2)	0.95	25.5 (23.2-29.7)	26.6 (23.3-31.1)	25.0 (23.1-25.2)	0.46	26.6 (23.3-31.1)	27.8 (24.0-32.1)	23.8 (20.9-27.8)	0.1	27.5 (24.0-32.5)	27.9 (24.9-31.8)	25.0 (23.1-33.2)	0.55	25.3 (23.5-30.4)	28.3 (25.7-32.5)	25.0 (22.1-25.4)	0.17
Race - white participants	16 (61.5%)	11 (55.0%)	5 (83.3%)	0.35	12 (60.0%)	8 (53.3%)	4 (80.0%)	0.6	10 (47.6%)	4 (28.6%)	6 (85.7%)	0.02*	8 (42.1%)	3 (25.0%)	5 (71.4%)	0.07	8 (66.7%)	2 (40.0%)	6 (85.7%)	0.22
Education - bachelor's or more	12 (46.2%)	10 (50.0%)	2 (33.3%)	0.65	10 (50.0%)	8 (53.3%)	2 (40.0%)	1	10 (47.6%)	5 (35.7%)	5 (71.4%)	0.18	7 (36.8%)	3 (25.0%)	4 (57.1%)	0.33	5 (41.7%)	2 (40.0%)	3 (42.9%)	1
Not married, not cohabitating	4 (15.4%)	4 (20.0%)	0 (0.0%)	0.54	4 (20.0%)	4 (26.7%)	0 (0.0%)	0.53	5 (23.8%)	5 (35.7%)	0 (0.0%)	0.12	2 (10.5%)	2 (16.7%)	0 (0.0%)	0.51	1 (8.3%)	1 (20.0%)	0 (0.0%)	0.42
Health Insurance				0.25				0.3				0.056				0.01*				0.56
Private	12 (46.2%)	8 (40.0%)	4 (66.7%)		10 (50.0%)	6 (40.0%)	4 (80.0%)		8 (38.1%)	3 (21.4%)	5 (71.4%)		8 (42.1%)	2 (16.7%)	6 (85.7%)		7 (58.3%)	2 (40.0%)	5 (71.4%)	
Public	12 (46.2%)	11 (55.0%)	1 (16.7%)		10 (50.0%)	9 (60.0%)	1 (20.0%)		12 (57.1%)	10 (71.4%)	2 (28.6%)		10 (52.6%)	9 (75.0%)	1 (14.3%)		5 (41.7%)	3 (60.0%)	2 (28.6%)	
None	2 (7.7%)	1 (5.0%)	1 (16.7%)						1 (4.8%)	1 (7.1%)	0 (0.0%)		1 (5.3%)	1 (8.3%)	0 (0.0%)		0	0	0	
Stress & Depressive Symptoms																				

	1st Trimester				2nd Trimester				3rd Trimester				Delivery				Postpartum			
	Total	Pre	During	*p*	T	Pre	During	*p*	T	Pre	During	*p*	T	Pre	During	*p*	T	Pre	During	*p*
10-item PSS Score	15.0 (8.0-20.0)	14.0 (8.0-23.5)	18.0 (12.0-20.0)	0.86	16.0 (12.5-20.0)	16.0 (13.0-20.0)	16.0 (9.0-18.0)	0.43	15.0 (11.0-20.0)	15.0 (12.0-20.0)	17.0 (9.0-23.0)	0.91	9.0 (7.0-18.0)	9.0 (6.0-15.0)	18.0 (7.0-25.0)	0.28	16.5 (9.0-20.0)	9.0 (7.0-22.0)	17.0 (9.0-20.0)	0.73
CES-D Score	9.5 (7.0-16.0)	9.5 (7.0-14.5)	11.5 (8.0-17.0)	0.71	12.5 (9.0-18.5)	13.0 (9.0-27.0)	10.0 (9.0-16.0)	0.57	11.0 (7.0-17.0)	10.0 (7.0-17.0)	11.0 (5.0-18.0)	0.97	7.5 (5.0-14.0)	7.0 (4.0-14.0)	8.0 (5.0-14.0)	1	6.5 (5.5-10.5)	6.0 (5.0-6.0)	8.0 (6.0-11.0)	0.37
Health History & Obstetrical Outcomes																				
History of Chronic Health Condition	5 (19.2%)	3 (15.0%)	2 (33.3%)	0.56	1 (5.0%)	0 (0.0%)	1 (20.0%)	0.25	1 (4.8%)	1 (7.1%)	0 (0.0%)	1.00	2 (10.5%)	1 (8.3%)	1 (14.3%)	1	1 (8.3%)	0 (0.0%)	1 (14.3%)	1.00
History of Psychiatric Condition	4 (15.4%)	4 (20.0%)	0 (0.0%)	0.54	2 (10.0%)	2 (13.3%)	0 (0.0%)	1.00	4 (19.0%)	2 (14.3%)	2 (28.6%)	0.57	3 (15.8%)	2 (16.7%)	1 (14.3%)	1	1 (8.3%)	1 (20.0%)	0 (0.0%)	0.42
Multigravida	16 (61.5%)	11 (55.0%)	5 (83.3%)	0.35	12 (60.0%)	9 (60.0%)	3 (60.0%)	1	14 (66.7%)	10 (71.4%)	4 (57.1%)	0.64	11 (57.9%)	8 (66.7%)	3 (42.9%)	0.38	7 (58.3%)	4 (80.0%)	3 (42.9%)	0.29
Multiparous	15 (57.7%)	11 (55.0%)	4 (66.7%)	1	12 (60.0%)	9 (60.0%)	3 (60.0%)	1	14 (66.7%)	10 (71.4%)	4 (57.1%)	0.64	11 (57.9%)	8 (66.7%)	3 (42.9%)	0.38	7 (58.3%)	4 (80.0%)	3 (42.9%)	0.29
Gestational Age at Birth (weeks)	39.3 (38.9-40.3)	39.3 (38.2-40.2)	39.9 (39.0-40.4)	0.3	39.5 (38.5-40.4)	39.4 (37.4-40.4)	40.4 (39.0-40.7)	0.22	39.6 (38.3-40.1)	39.4 (37.4-40.0)	39.9 (38.3-40.6)	0.25	39.9 (39.3-40.4)	39.5 (39.1-40.2)	40.1 (39.3-40.7)	0.22	39.6 (38.9-40.4)	40.0 (39.4-40.4)	39.0 (37.9-40.4)	0.29
Cesarean Section Delivery	8 (30.8%)	7 (35.0%)	1 (16.7%)	0.63	6 (30.0%)	4 (26.7%)	2 (40.0%)	0.61	5 (23.8%)	4 (28.6%)	1 (14.3%)	0.62	6 (31.6%)	3 (25.0%)	3 (42.9%)	0.62	2 (16.7%)	1 (20.0%)	1 (14.3%)	1
Adverse OB Outcomes																				
Low Birth Weight (<2494.76g)	2 (7.7%)	2 (10.0%)	0 (0.0%)	1	1 (5.0%)	1 (6.7%)	0 (0.0%)	1	1 (4.8%)	1 (7.1%)	0 (0.0%)	1	2 (10.5%)	1 (8.3%)	1 (14.3%)	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Preterm Birth (<36 wk GA)	1 (3.8%)	1 (5.0%)	0 (0.0%)	1	1 (5.0%)	1 (6.7%)	0 (0.0%)	1	1 (4.8%)	1 (7.1%)	0 (0.0%)	1	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	
Postpartum Pre-Eclampsia	1 (3.8%)	1 (5.0%)	0 (0.0%)	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	1 (4.8%)	0 (0.0%)	1 (14.3%)	0.33	1 (5.3%)	0 (0.0%)	1 (14.3%)	0.37	0 (0.0%)	0 (0.0%)	0 (0.0%)	

	1st Trimester				2nd Trimester				3rd Trimester				Delivery				Postpartum			
	Total	Pre	During	*p*	T	Pre	During	*p*	T	Pre	During	*p*	T	Pre	During	*p*	T	Pre	During	*p*
Gestational Diabetes	2 (7.7%)	1 (5.0%)	1 (16.7%)	0.42	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	2 (9.5%)	1 (7.1%)	1 (14.3%)	1	2 (10.5%)	1 (8.3%)	1 (14.3%)	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	
GBS Positive	3 (11.5%)	2 (10.0%)	1 (16.7%)	1	3 (15.0%)	2 (13.3%)	1 (20.0%)	1	4 (19.0%)	3 (21.4%)	1 (14.3%)	1	3 (15.8%)	3 (25.0%)	0 (0.0%)	0.26	2 (16.7%)	1 (20.0%)	1 (14.3%)	1
Chorioamnionitis	1 (3.8%)	1 (5.0%)	0 (0.0%)	1	2 (10.0%)	1 (6.7%)	1 (20.0%)	0.45	1 (4.8%)	1 (7.1%)	0 (0.0%)	1	1 (5.3%)	0 (0.0%)	1 (14.3%)	0.37	1 (8.3%)	0 (0.0%)	1 (14.3%)	1
Antibiotic Use																				
During pregnancy	4 (15.4%)	3 (15.0%)	1 (16.7%)	1	3 (15.0%)	3 (20.0%)	0 (0.0%)	0.54	5 (23.8%)	4 (28.6%)	1 (14.3%)	0.62	-	-	-	-	-	-	-	-
During labor	-	-	-	-	-	-	-	-	-	-	-	-	6 (31.6%)	5 (41.7%)	1 (14.3%)	0.33	-	-	-	-
Postpartum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 (16.7%)	0 (0.0%)	2 (28.6%)	0.47
Psychotropic Use																				
During pregnancy	4 (15.4%)	3 (15.0%)	1 (16.7%)	1.00	2 (10.0%)	2 (13.3%)	0 (0.0%)	1.00	4 (19.0%)	3 (21.4%)	1 (14.3%)	1	4 (21.1%)	3 (25.0%)	1 (14.3%)	1	-	-	-	-
Postpartum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (8.3%)	0 (0.0%)	1 (14.3%)	1

*p<0.05

Appendix D. Constructed Themes and Sub-Themes

Table D.1 Constructed Themes & Sub-themes.

Each sub-theme is represented by exemplar quotes from participant interviews.

Theme	Sub-Theme	Exemplar Quote(s)
Immediate Concerns	Concern for Self & Loved Ones' Health	*Summer (SU) 2020: Definitely [concerned for] our parents and grandparents because [they're] more susceptible...we do have other members that have additional health concerns that could make them even more susceptible to severe infections.* *SU 2020: ... [concerned for] the baby...so we are thinking when things are back to normal, should we take her to daycare or have a babysitter so that we can make sure we can keep her safe?* *SU 2021: I [was] very concerned during the latter half of my pregnancy, making sure I didn't get COVID...there was miscommunication and [I'd been] hearing that if my husband got COVID, he couldn't see the baby, so making sure he stayed healthy.* *SU 2021: At the beginning -- the first [several] months -- I was genuinely concerned that myself or husband or kids were going to contract COVID and have a bad response and potentially die.* *SU 2021: My parents, my husband's parents, I think, would be examples of family members that are...more at risk...they're all vaccinated now [so] it feels safer, but...it's not 100%...the concern is turning...toward, like nieces and nephews that aren't able to be vaccinated [yet]...they would be the most at-risk population.*
Immediate Concerns	Concern for Basic Needs	*SU 2021: I've been unemployed since March [2020], and I've been going through programs to get help to pay rent.* *SU 2021: My husband was out of work for two months...it was temporary, and he did collect unemployment for a couple of weeks at that time.*

Theme	Sub-Theme	Exemplar Quote(s)
Immediate Concerns	Concern for Self & Family Employment	*SU 2021: I was just coming off maternity leave and then everything fell apart...a year-and-a-half before I was scheduled to graduate, I lost funding...Coupled with that, my husband is going back to school...so our whole family's timing was thrown off...that changed my career trajectory.* *SU 2021: I'm only part time. When [pandemic hit Ohio], I got furloughed for three months along with...my other co-workers...My husband [and] I made sure that we had a savings account that could account for these types of issues...So luckily, we [didn't] have to get into anything.*
Immediate Concerns	Concern for Society	*SU 2020: We took a lot of precautions...and you know when I go shopping, I see people with no masks...And one time... was a guy standing behind me, close to me, like was coughing, and I told him to stand back, and we have to unite together...Even if you don't care about yourself, you have to care about others.*
Personal Acclimatization to Pandemic	Initial Tumult	*The first year of my daughter's life was super stressful and COVID changed everything. I was breastfeeding the whole time...and every month there was a new life-altering realization, and it was really hard.* *...[well] it's just been kind of wild and so we haven't really been able to mourn...*
Personal Acclimatization to Pandemic	Social Isolation	*We had all kind of things lined up like my family would visit and then my husband's family would visit. COVID really put a lot of that on hold.* *I have noticed, like [my] mood decline a little bit when being kind of stuck inside...I've been able to handle it better because it's winter. And we weren't going anywhere anyway. But over the summer, when there's like things to do...it was more of a bummer.* *I had cabin fever big time, and I've gotten to the point now I can get out. I'm not as much of a people person as I once was, just because the big group things were where COVID really hit people hard. Yeah, I can do with small gatherings [so] -- kind of conditioning myself to tone down.*

Theme	Sub-Theme	Exemplar Quote(s)
Personal Acclimatization to Pandemic	Frameshifts, Coping, & Hope	*SU 2020: We just keep praying and hope for the best.* *SU 2020: I'm the type of person during a stressful situation to just do what I have to do...I wonder what that will mean for me once it is over and I have to sit and process it, but for now, I'm just kind of in 'get shit done' mode...I feel like I'm burnout, but we just have to keep on keeping on, there's nothing else to do.* *[The pandemic lockdown] gave me better connection to family...We started weekly [video chats] where we would play a board game, so I feel like we developed a closer relationship over the pandemic...in some ways it allowed to be connect with people I hadn't before.* *If I didn't run, I would feel [the isolation] a lot more...but I feel comfortable exercising outside, and in some ways, it's nice not having a lot of [local] friends so I don't feel like I'm missing out on a lot, so cabin fever levels [were and continue to be] pretty low.* *We try multiple times a day to get out and go on a walk...and we have a garden...we just make the best of it. I've kind of just planned on--basically made all the things I was planning on for winter just during the summer...my daughter isn't old enough; she doesn't know the difference.* *Summer 2021: Through the Fall and as vaccines are starting to kind of come through, that felt like there was the light at the end of the tunnel...with a change in [government] administration, [I hoped that] our government is going to work toward...addressing it and that the vaccines should help.*
Family Life During Pandemic	Pregnancy During a Pandemic	*SU 2020: My biggest concern is being pregnant and worrying what will happen if [the pandemic] keeps happening. If I weren't pregnant, I wouldn't be as worried about it.* *SU 2021: My biggest concern in the beginning for me was honestly just being able to enjoy the pregnancy...I did not get to experience a typical first pregnancy [because of the pandemic].*

Theme	Sub-Theme	Exemplar Quote(s)
Family Life During Pandemic	Pandemic Parenting	*SU 2020: We were going to have a lot of extra help watching the baby so without that, it has been hard on us and our family members, no one knew when they could see us and meet the baby.* *SU 2021: For three months [in 2020], I was home...and my husband was home the first two months. Um, he...was able to go in, so I was working from home...During that time, I was nursing a baby and taking care of a toddler going through the terrible-two's, so it was like a circus and hard to get work done...I was only able to work 5 hours [a week].* *SU 2021: Last Fall [2020] was a time when I had also taken time off to spend time with my child before starting my job, so... COVID kept people at home, but I was planning to be home anyways. So, I don't feel like it impacted me as negatively because I planned for [staying home more] already.*
Family Life During Pandemic	Pandemic's Effects on Children/Childhood	*SU 2021: Autumn (AU) 2020: It does kind of suck because my daughter is now 17 months old, and she can't play with other kids [or] go to the park. Really, the fun things that I want to do with her.* *SU 2021: It makes it hard to enjoy anything because COVID is still real, and our kids aren't vaccinated.*
Navigating the Changing Pandemic Landscape	Assessing Individual Risk Informs Precautions	*AU 2020: Before I found out I was pregnant...at the hospital, our unit was a COVID unit...and in August we found out we were pregnant, so now at work they just [have me] avoid any COVID patients or any admissions that haven't been swabbed.* *Winter 2020: So I actually haven't been back to the office regularly...since COVID really picked up in March, and now being pregnant, I don't know when...it will feel safe to go back into the office...I have a [pre-existing condition] ...and so that's why I was working from home...I have a few other coworkers who also have some [pre-existing conditions]. We are all working from home, so we don't know when we will be going back into the office.*

Theme	Sub-Theme	Exemplar Quote(s)
		SU 2021: I mean...we took this pretty seriously...like grocery shopping once a week...we weren't really seeing friends at all...When I found out I was pregnant... we really cut back...so really our exposure was sending our daughter to daycare, grocery shopping once a week, and then getting take-out.
Navigating the Changing Pandemic Landscape	Weighing Risks of Socializing	*Winter 2020: I really wanted to see my family during the holiday[s], and it's very hard to, you know, decide like okay, do you want to risk it and go home? [Or] not get that opportunity or have to like [video chat] your family?* *SU 2021: Our family did come visit us a couple times, but that made us nervous because they were a lot more lax than we were. And there are a lot of families in our townhouses who had different ideas about COVID protocols, and then overtime just paying attention to CDC guidelines...and even though the mask mandate is lifted, I still wear a mask.*
Navigating the Changing Pandemic Landscape	Recalculating Risks & Precautions Post-Vaccination	*SU 2021: I've been going to office... one to two days a week. And that's probably been happening since...really, since I was fully vaccinated which was in March...life has certainly changed, we haven't seen a lot of people. Simply [being] vaccinated, we're starting to see some people now.* *SU 2021: Since vaccinations, which would be like April timeframe, we've started to see family that has been fully vaccinated...We started with outdoor seating, we started to do some indoor seating...to a restaurant, like twice.*
Mixed Feelings Toward Pandemic Response	Frustration & Cynicism	*SU 2020: I think I've taken it extra hard that there are people that choose not to wear masks as I have been directly affected, and my family's been directly impacted by it...It's also been difficult [watching] people not really pay attention to it and what it is capable of. We know firsthand that it's real. And that it's [very] serious.*
Mixed Feelings Toward Pandemic Response	Apathy or Dynamic Opinion	*AU 2020: I think that they actually handled it really well and as best that it could be handled for something that we didn't expect to happen. And I have had different feelings kind of from the beginning to now, where there were points in time when our numbers really jumped...and I thought [we'd] need to shut down again...I was working through the whole time, my*

Theme	Sub-Theme	Exemplar Quote(s)
		income wasn't affected...But then for people whose income was affected, we can't just shut down their economy so I've had different feelings...about if we should shut down again. *SU 2021: I've been grateful to live in a state that did recognize it and has put some measures in place. That being said, like,...we weren't the best state in terms of cases and deaths...so I wouldn't put us at the best.*
Mixed Feelings Toward Pandemic Response	Affirmation & Pride	*SU 2020: We were really happy with what [the governor] did and we think he was really proactive with shutting down aspects of the economy. I think we did good with keeping things contained to avoid the exponential increase.* *SU 2020: I am pretty proud to be in Ohio and I think there was good response at first...overall I think we did a good job and am pleased there hasn't been a large resurgence.*
Mixed Feelings Toward Pandemic Response	Financial Concerns	*SU 2020: It was very destructive to the economy and all sectors of business. It's kind of everything is shut down, what do you do, so yeah it was just a massive destruction to the economy.* *AU 2020: I have a lot of clients who deal with a lot of financial… like they've been hit really hard, they're business owners... and financially like they don't know how they're going to survive over the next few months.* *SU 2021: I kind of fall into the category of people that believe that [the initial quarantine period] was necessary. And we are starting to bounce back from that. I also recognize that, as [I] stated earlier, personally, I was not disrupted. So, I think it's a little unfair of me to say, like [there was] no financial disruption.*
Considering the Future	Timeline of Pandemic	*AU 2020: I feel like it's something that just going to stick around kind of like the flu or the chicken pox, it's just going to stick around and be here, but I think like, once we kind of get...a better grasp of how to handle it, like a vaccine...then everything will kind of calm down a little bit.* *Winter 2021: I think it's just a day-to-day thing to take care of yourself and kids it's not easy to know what's going to happen.*

Theme	Sub-Theme	Exemplar Quote(s)
Considering the Future	Personal & Family's Future	*AU 2020: It's like, we're not getting any [younger] so how long can we wait to have another baby?...We can't wait for another baby cause like I'm already at advanced maternal age. I can't wait any longer. Okay, this [pandemic] could be years.* *SU 2021: My concerns are about like, what does the new normal look like? And thinking about my toddler and the second child, what does their future look like post-COVID? Because they didn't really have a pre-COVID, and I just don't know what that will look like.* *SU 2021: [The pandemic] just changed the way I think about the health of my kids and their space.*
Considering the Future	Community's Future	*SU 2020: One thing that I've struggled with the most is that I going into this I really thought that if you gave people the chance to do the right thing and protect others based on scientific evidence that they would do it. Personally, and emotionally. I'm having a hard time reconciling the fact that there is there a large swath of the population that are more concerned about their lack of convenience, and they are about their countrymen and like what does that say about us in this society?...And what that's going to mean for our kids. And you know how we can address that and also...just kind of like where we go from here?* *SU 2021: Super stressed out about...homelessness and eviction and people just not having homes and there's been like an inadequate response both federal and state guidelines in terms of the support there.* *SU 2021: Just have large concerns about our country moving forward...this [pandemic] highlighted a lot of the drastic inequalities in our society, and I guess I was hoping that having all of these exposed would lead to people to want to make changes in our world. But it seems that people are actually doubling down on the systems and policies that are reinforcing [inequalities]. Now my concerns are why aren't people waking up, learning, and being more compassionate with what we just went through -- about how to care for one another and sacrifice to help other people?*

Theme	Sub-Theme	Exemplar Quote(s)
Considering the Future	Biggest Concerns	*SU 2020: I'm really scared that things won't have normalized by the time I give birth and that...I will have to be alone in the birthing room.* *SU 2020: I'm worried about my family's health and the overall economy of not only the US but the world and I know a lot of people have been greatly affected and hurt. I'm worried if we are going to face a recession and hopefully things aren't as bad as what they are predicting.* *AU 2020: Honestly, the people who don't follow social distancing or guidelines, or they believe it's a hoax overall, and they're putting others at risk by not following [guidelines].* *SU 2021: My main concern is my kids getting sick and finding a job and not having contacts with a lot of people [increasing risk of COVID exposure].* *SU 2021: I think early [on in the pandemic] it was the unknown--how is [COVID] being spread? And mid-way through, "is it going to end?". Are people with their own occupations going to survive? What is the status of the economy going to be?*

Appendix E. Semi-Structured Phone Interview Script

These questions are to be administered to study participants over the phone, with follow-up questions as needed (i.e., *can you tell me more about that experience? What do you mean by the phrase "____"? What factors helped you come to that conclusion?*):

Interviewer: Hello, this is [interviewer name] from the Maternal Stress & Microbiome Study at Ohio State. I'm calling to speak with ____.

Hi [participant name], do you have a few minutes to chat? I'd like to understand how you may have been affected by the ongoing epidemic.

Great, thanks for your time. So, I will ask you a series of questions and I'm just looking to understand your experience, so there is no right or wrong answer. This is not quite a conversation, so if I seem guarded or like I'm not sharing my experience with you, it's because I'm trying to make sure I don't bias your answers with my own.

Lastly, if it's alright with you, I will record this phone call so that we can make sure to capture your full response, but I can focus on being present with you during our interview.

Any questions before we get started?

Great, here we go.

How is your pregnancy going? OR How are you and your baby doing?

Has your life changed since we last saw each other? If so, how?

Has your life changed directly or indirectly due to COVID-19? If so, how?

If 'yes' to the last question…

- Are/were any of your loved ones afflicted by or diagnosed with COVID-19?
- Are you/your loved ones unemployed because of COVID-19?
- Are you/your loved ones having a hard time finding necessities? (i.e., food, toiletries)
- Are you/your loved ones concerned about your financial situation?
- Are you/your loved ones concerned about your housing?
- Are you concerned about the health of a loved one? (adult)
- Are you concerned about the health of your child(ren)?

Are you able to/have you been staying home as much as possible?

How intensely do you feel cabin fever?

When you leave your home, do you take precautions (i.e. mask, gloves, hand sanitizer, avoiding touching public/shared surfaces)?

- What type of mask do you wear?
- Does everyone you live with follow the same precautions?

- On a weekly basis, how frequently do you leave your home?
- When you leave your home, do you use public transportation?

How frequently have you been getting sustained physical activity (avg. per week)?
- What type of activity?

Has your diet changed since COVID-19?

Since COVID-19, has your cleaning routine changed at home? Do you use different disinfectants or use cleaners more/less frequently?

What is your perception of the state of Ohio's response to COVID-19 on a scale of 1 to 10 regarding public health?

10 = minimal deaths, good overall population health, efficient response
1 = catastrophic numbers of deaths, bad overall population health, inefficient response

What is your perception of the state of Ohio's response to COVID-19 on a scale of 1 to 10 regarding financial health?

10 = stable local economies, minimal disruption to workers/businesses
1 = unstable local economies, massive disruption to workers/businesses

What is/are your biggest concern(s) during this pandemic?

For how long are you mentally prepared to have to deal with COVID-19?

When do you think we will actually no longer have to worry about COVID-19?

Is there anything else you'd like to share that we have not covered yet?

Thank you so much for your time, we really appreciate your participation.

We will shortly email you several surveys, these are the same ones you've taken before on the tablet when we met in person. We will work to make sure that no one sees your survey responses without approval. But, because we are using the Internet, there is a chance that someone could access your online responses without permission. In some cases, this information could be used to identify you. Your data will be protected with a code to reduce the risk that other people can view the responses.

For your participation in the interview and in the surveys, we will mail a $25 Target gift card to you. This will happen once during each trimester and once in the postpartum period.

Can you tell me a good email and mailing address for you? *OR* Could you confirm that the following email and mailing address are still good ways to reach you, please? ____

Again, thanks for your time, [participant name]. Please let me know if there's anything our study can help with at this time. Take care.