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Abstract

Background/Purpose: Current standards of care for decreasing the incidence of preterm labor and preterm birth are not decreasing the public health burden of spontaneous preterm birth. Preterm labor and birth contribute to the high infant mortality rate in the United States and around the world. The purpose of this paper is to review and examine research articles regarding the vaginal microbiomes and their association with preterm birth and preterm labor. A better understanding of the microbiome and how it influences labor may be a key component to reducing preterm labor and birth and ultimately decrease the infant mortality rate in the US.

Theoretical Framework: The Life Perspective Rhythm Model is used in the delivery of nursing care. The primary purpose of nursing is the promotion and maintenance of an optimal level of wellness and health for the patient. There are four main constructs to the model which include person, health, wellness, and metaparadigm of nursing.

Methods: Twenty-three research articles were thoroughly studied to answer the questions, "How do the vaginal microbiome community states relate to preterm labor and birth?" and " If the microbiome is implicated, are there any interventions that can alter the microbiome to reduce preterm birth?"

Results/Findings: Several microbiome environments were found to be associated with preterm labor and preterm birth. These primarily were those associated with microbiomes dominated by *Lactobacillus iners* and *Lactobacillus jensenii* as well as other pathogens and *Lactobacillus spp.* depleted environments. Microbiome environments protective against preterm labor and preterm birth were dominated by *Lactobacillus crispatus* and *Lactobacillus gasseri*. Differences were noted among various racial and ethnic groups with higher incidence of negative microbial composition and preterm birth in the African American and Hispanic population. Risk factors in

all ethnic groups included high BMI, poor diet, negative health behaviors, and stress. Potential interventions were discussed among these as well as the potential for the use of oral probiotics to decrease the incidence of preterm labor and preterm birth.

Implications for Research and Practice: Long term, further understanding of the vaginal microbiome and the role it plays in preterm labor and preterm birth can help to define what is a healthy microbiome during pregnancy and help to develop strategies that can promote normal microbiota during pregnancy.

Keywords: *"Bacteria"; "Lactobacillus crispatus (L. iners)"; Lactobacillus iners (L. iners) " ; "Lactobacillus gasseri (L. gasseri)"; "Lactobacillus jensenii (L. jensenii)"; "Lactobacillus species (L. spp.)"; "microbiomes"; "Preterm birth (PTB)"; "Preterm labor (PTL)"; "Preterm premature rupture of membranes (PPROM)"; "Spontaneous preterm birth (sPTB)"; "Vaginal microbiota"; "Viruses".*

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Chapter I: Introduction

Preterm birth is one of the primary causes of perinatal mortality and morbidity throughout the world (Kindinger et al., 2017). Preterm labor and birth are considered the world's primary cause of premature death in children under the age of five. Annually, approximately 11% of all births in the world are premature, with one million out of six million reported child deaths resulting from complications of prematurity (Garcia-Basterio et al., 2017). The United States has a 5.8% infant mortality rate, compared to the average of all other countries in the world at 3.5% (CDC, 2020). Preterm rupture of membranes prior to the onset of labor (PPROM) occurs in approximately 30% of all spontaneous preterm births (sPTB) and is correlated with an increase in vaginal bacterial diversity that occurs prior to the onset of membrane rupture (Brown et al., 2019).

In 2019, preterm birth occurred in 1 out of every 10 births within the United States. The various types of bacterial communities that are present within the lower female genital tract play a significant role in maternal/fetal health (Freitas et al., 2018). The state of pregnancy alone contributes to changes in the vaginal microbiome. These changes occur throughout pregnancy. The underlying physiology present in pregnancy has the ability to impact bacterial and viral communities within the vaginal microbiome. When increased diversity is present, the incidence of preterm birth increases significantly. Changes in the diversity of the vaginal virome are similar to changes that occur within the vaginal microbiome during pregnancy, indicating the underlying physiologic changes that occur in pregnancy possibly regulates both bacterial and viral communities (Wylie et al., 2018). Because microbiology terms may be a challenge to the reader a glossary has been created and can be found as Appendix A.

Physiology

The human body is not a sterile organism. It is the domain for millions of different microorganisms. Bacterial flora that can be found on the human body are considered to be the human microbiome with each individual having their own unique types of microbes. Many bacteria can contribute positively to human health. These bacteria provide resistance to infection, break down nutrients, and train the immune system (Walther-Antonio et al., 2014). Pregnancy is associated with significant physiological changes, and maternal niches of the microbial community structure have the potential to change and shift. Many of these changes are not harmful, but dysbiosis within the maternal vaginal microbiota may be correlated with increased incidence of adverse pregnancy outcomes such as preterm birth (Walther-Antonio et al., 2014). Vaginal dysbiosis is a shift from the most favorable vaginal microflora to bacterial diversity which is associated with adverse health outcomes. (VandeWijgert, 2017).

Within the vaginal tract, more than 50 microbial species are noted to be nonpathogenic (Kindinger et al., 2017). During reproductive years, the vaginal microbiome varies quite significantly among women. Healthy vaginal microbiomes have been found to have some protective aspects against bacterial vaginosis, sexually transmitted diseases (STD's), urinary tract infections (UTI's) and human immunodeficiency virus (HIV) (Zheng et al., 2019). This protection is associated with the presence of lactic acid-producing bacteria primarily of multiple *Lactobacillus* species (spp.) which, through competition, decrease the presence or growth of more pathologic microbes. For example, in the incidence of the presence of bacterial vaginosis there is a significant decrease in the presence of *Lactobacillus* spp. (Kindinger et al., 2017).

A number of community state types (CST) are influenced during pregnancy (Kindinger et al., 2017). These include four primary *Lactobacillus* spp. including L. *crispatus* (CST I), L. *gasseri* (CST II), L. *iners* (CST III), and L. *jensenii* (CST V). (CST IV) includes all other microbial communities that are significantly lacking *Lactobacillus* spp. but are full of primarily anaerobic bacteria. The physiologic premise is that increased amounts of circulating estrogens control accumulation of glycogen within the vaginal epithelium. Glycogen is separated by host amylase into complex sugar substances of maltotetraose and maltotriose. These develop carbon sources that serve as nutrition for the *Lactobacillus* spp. Interaction between hormone and metabolic signaling within the vaginal mucosa act in a protective manner, preventing exposure of pathogenic bacteria. This leads to greater stability of the microbiome and the community state types as gestation advances and a decrease in the incidence of individuals who have a microbial community that lacks *Lactobacillus* spp. (Kindinger et al., 2017). Within the Lactobacillus genus there are species that appear more protective and some that are concerning.

Statement of Purpose

The purpose of this paper is to review and examine research articles regarding vaginal microbiomes and their association with preterm labor and preterm birth. The questions for the literature review are, "How do the vaginal microbiome community states relate to preterm labor or birth?" and "If the microbiome is implicated, are there interventions that can alter the microbiome to reduce preterm birth?"

Evidence demonstrating a need

Worldwide, approximately 11% of babies are born prematurely prior to 37 weeks gestation. African American women are 1.5 times more likely to have preterm birth than Caucasian women and have twice the risk of very early preterm birth, prior to 32 weeks gestation (Elovitz et al.,

2019). There has been limited success in developing strategies that prevent the incidence of preterm birth. Emerging research is discovering that the maternal microbiome is associated with vital functions of normal maternal health. Alterations in the microbiome may play a role in the etiology and increased incidence of preterm labor and preterm birth. Preterm birth within the United States occurs in approximately 500,000 or 1 in every 9 births. It is noted to be the primary reason for infant mortality, responsible for 35% of all infant deaths. Elovitz et al. (2019) concluded that the vaginal microbiota plays a significant part in preterm birth.

Current efforts that are being used to target women with a previous sPTB are not decreasing public health burdens associated with sPTB (Elovitz et al., 2019). Targeting the microbiome may be a new approach.

Significance to Nurse-Midwifery

Certified nurse-midwives (CNM's) are licensed and independent providers of health care with authority in all 50 states. They are required to follow standards for education and certification set by the Accreditation Commission for Midwifery Education. In August 2017, the number of licensed CNMs was 11,826 with 101 licensed certified midwifes (CMs) who are not nurses. They attend births, work in primary care and reproductive care of women. Approximately 33% of nurse-midwives work in primary care providing access to annual exams, providing medications, nutrition counseling, patient/parenting education, and reproductive healthcare. The American College of Nurse Midwives (2020) reports that approximately 53.3% of nurse-midwives attend labor and deliveries. In 2014 approximately 94.2% of nurse-midwives attended births in the hospital setting with 3% in birth centers and 2.7% in patient homes. Midwives are involved in 8.3% of all births within the United States (American College of Nurse-Midwives, 2020).

Women who receive prenatal, intrapartum, and postnatal care from a CNM are less likely to have incidence of sPTB prior to 24 weeks and require fewer medical interventions in comparison to women under the care of an obstetrician or family physician (Poltera, 2013). Compared to genetic factors, the presence of a hostile vaginal microbiota is considered a risk factor that is modifiable in prevention of PPROM (Brown et al., 2019). Identification of women at risk followed by influencing the bacterial community either through prebiotic or probiotic therapies are thought to be promising strategies in the prevention of PPROM and PTB (Brown et al., 2019).

Being able to understand and characterize the vaginal microbiota during pregnancy will help provide information that allows for prognostic, diagnostic, and therapeutic values (Romero, Nikita et al., 2014). There is potential functional and clinical significance for the nurse-midwife in understanding microbial community state types and metabolic profiles that are associated with sPTB to increase comprehension of the processes of inflammation that are correlated with sPTB (Stafford et al., 2017). To enable the midwife to create strategies that prevent negative reproductive outcomes, it is important to have knowledge and understanding of microbiome community state types and their role in the incidence of sPTB and PPROM.

Some of the hallmarks of Midwifery that would pertain to this topic are “Incorporation of evidence-based care into clinical practice”, “Utilization of health promotion, disease prevention, and health education”, and “Incorporation of evidence-based integrative therapies’ (American College of Nurse-Midwives, n.d.). Applicable pearls of midwifery would be the use of appropriate interventions that are based on the best available clinical evidence, interprofessional education, and collaboration to enhance optimal patient outcomes. Most important in relation to this topic would be to practice according to the most up to date evidence and to continue with interprofessional learning activities to expand knowledge base. Participating in research

opportunities is also essential in improving patient outcomes and for shared learning (American College of Nurse-Midwives, n.d.).

Many routine practices in prenatal care and in labor and birth environments are associated with the potential to influence the microbiome. Ensuring safe progression of labor often includes multiple cervical examinations, internal fetal heart rate monitoring, and uterine contraction monitoring. Many of these tasks are important but need to be used with clinical judgment to ensure that the disturbance to the microbiome is minimized as much as possible (Cahill et al., 2012).

One significant issue is cervical examinations that have the potential to introduce or facilitate infection. Patient education is a primary role of the nurse-midwife. Patients should be offered education about the prenatal influences associated with the microbiome, birth route associated factors, and antibiotic use during pregnancy. Even though the relationships between the microbiome and health outcomes are not completely understood, midwives are in a position to provide evidence-based care and education in regard to the adoption of healthy behaviors that have the potential to promote a healthy microbiome (Cahill et al., 2018).

Theoretical Framework

The Life Perspective Rhythm Model is a model developed by theorist Dr. Joyce Fitzpatrick in 1989 that was based on the model of practice developed by Martha Rogers of the Theory of Unitary Human Beings. Martha Rogers' Science of Unitary Human Beings describes global concepts of human being, environment, health, and nursing. The theory is focused on unitary human environment mutual processes. Instead of health and illness, Rogers defines health as a statement of the life process. She describes four aspects to this process. Energy field, openness, pattern, and pan dimensionality. Human and environment in conjunction influences processes of change that improve health. When addressing the health and treatment of a patient, the patient

cannot be divided from their environment. Martha Rogers theory included eight separate areas. Fitzpatrick further refined this theory building her theory on four subdivisions (Nursing Theory, 2020).

The Life Perspective Rhythm Model consists of four constructs that involve the delivery of nursing care. The focus of the model is to view the differing components in life experiences as rhythms. Human development is centered along varying rhythms allowing humans to learn communication and interaction with those around them. Nursing uses these rhythms to achieve optimal wellness for patients through the use of the concepts of person, health, wellness, and metaparadigm. The medical field is often consulted when any rhythm reaches a high point or a low point in patient health status. Nursing is responsible to understand and to help the patient with putting their rhythms back together in order to support all of the other rhythms of the body. The goal is to return to a state of health. Rhythm refers to the regular and recurrent quantitative changes that occur in a variable biological process. They can be described, are measured, and will repeat. The stages of life and illness are equated to rhythms. The principal purpose of nursing is promoting and maintaining optimal levels of wellness and health for patients (Nursing Theory, 2020).

This is a useful model for studying different methods for identification of modifiable factors within the vaginal microbiome that may lead to preterm labor/birth. The four main constructs to this model include person, health, wellness, and metaparadigm of nursing. Understanding all of these areas are significant to ensure successful outcomes for patients. The person is not just the patient but all those who interact with the person in their environment. The human being is considered an open system with specific rhythmic behaviors. These behaviors are drawn from several areas including the culture of the patient and their surrounding society (Nursing Theory, 2020).

Health focuses on the health of the patient and their environment. Health also includes all those around the patient who have an effect on the patient. To ensure optimal health, patients need the assistance of healthcare providers, a willingness to change and the ability to follow recommendations for a healthy life. The support of family and friends is an essential component of the environment. Optimal health is the goal and nursing professionals work to improve health through all interactions with the patient (Nursing Theory, 2020).

Wellness refers to being in a state of optimal health. It is important that providers learn techniques to promote wellness through encouraging healthy lifestyle changes and habits. In understanding the whole patient and their environment and how these interactions affect them, the midwife is able to make a complete assessment of the patient. Determination can be made of any factors that may be modified to ensure a healthy vaginal microbiome. This allows for a healthy pregnancy with the goal to decrease preterm labor/birth. The patient's willingness to provide information and follow any recommendations is essential (Nursing Theory, 2020). The midwife role of educator is used to decrease any negative maternal or neonatal outcomes that may occur related to vaginal dysbiosis during pregnancy.

The metaparadigm of nursing recommends how the profession should function. It begins with the nurse having a wholistic understanding of the patient, their life, and environment. The nurse includes the patient's social situation as part of the assessment of wellness. Finally, the responsibility of the nurse to the patient is defined by expectations that nursing will incorporate theory, practice, and procedures that become standardized to help support each individual patient in each situation. The use of this model helps to integrate a complete professional action plan for each patient. Each individual needs to have their own specific care plan that is formulated for them.

Using this theory that includes the concepts of life cycle, wellness, as well as maintenance, can be used in any culture or setting (Nursing Theory, 2020).

Summary

Preterm labor and birth contribute to the high infant mortality rate in the United States and around the world. A better understanding of the microbiome and how it influences labor may be an essential component in reducing preterm labor and birth and ultimately decrease the infant mortality rate in the US. Nurse-midwives are significant providers of primary care for women and their reproductive health. They are in a position to ensure optimal pregnancy outcomes for their patients through knowledge and evidenced-based practice. By using Fitzpatrick's Life Perspective Rhythm Model, the patient is assessed as a whole and determination can be made of any modifiable factors that the patient may incorporate. It is a model that helps the midwife to fully know and understand each patient which then allows for meeting the goal of a healthy pregnancy and positive maternal/neonatal outcomes.

Chapter II: Methods

Chapter two outlines the methods used to identify and select the research articles that are discussed in the literature review. Each of these studies related to vaginal microbiomes and the role these play in the incidence of preterm labor and preterm birth. This chapter will discuss the search strategies used as well as the inclusion and exclusion criteria applied to those searches, and the number and type of studies found. The Johns Hopkins Nursing Evidence-Based Practice: Model and Guidelines (Dearholt & Dang, 2012) critiquing criteria are reviewed and will be used to evaluate the articles.

Search Strategies

The purpose of this critical appraisal of the literature was to determine the role that vaginal microbiomes may play in the incidence of preterm labor and preterm birth. An initial search utilizing the database CINAHL and limiting the results to peer-reviewed articles, written in English, using the terms “microbiome preterm birth” or “microbiome pregnancy” or “microbiome preterm labor” or “microbiome women’s health pregnancy” yielded 419 articles, 416 of which were from the years 2010-2021. The same search terms were applied to the database PubMed, which yielded 2,999 results. After limiting the results to the years 2010-2021, 2,932 results remained. The initial search was limited to studies that were published in the last 10 years to include the most recent and relevant research on this subject.

In order to ensure a complete and thorough search, data mining was also used to allow for formulation, analyses, and basic induction processes that help in determining the most relevant information and knowledge needed for the review. Most of the articles published in regard to

vaginal microbiomes and preterm labor or preterm birth were published within the past ten years. Duplicates were removed, and inclusion and exclusion criteria listed below were applied to the remaining articles.

Inclusion and Exclusion Criteria

The following inclusion criteria were utilized: 1) Research studies; 2) Studies with pregnant women aged 18-40; 3) Healthy viable singleton pregnancy; 4) Studies with pregnant woman willing and able to give informed consent; 5) Studies discussing the role of vaginal microbiomes and effects in pregnancy; 6) Studies with reference to the correlation of vaginal microbiomes and preterm birth; 7) Studies with reference to the correlation of vaginal microbiomes and preterm labor. Exclusion criteria were: 1) Non-research studies; 2) Studies on non-humans; 3) Systematic reviews; 4) Literature reviews; 5) Studies in non-pregnant females; 6) Emergency obstetric care required; 7) Pregnant women with medical or obstetrics complications that would make it difficult to comply with study requirements.

After applying inclusion and exclusion criteria, 160 articles remained. Remaining articles were evaluated to determine the degree of relevance to the topic of vaginal microbiomes and preterm labor/birth. Following evaluation and review of titles and abstracts, the level of evidence of each study was determined as well as the overall quality of the studies. Classifications of quality were determined with the use of the John Hopkins Nursing Evidenced-Based Practice: Model and guidelines (Dearholt & Dang, 2012). This helped in determination of low, good, or high-quality research. This tool allowed for the ranking of the evidence sources to determine the strength that the evidence provided.

Criteria for Evaluating Research Studies

Some research designs are more powerful in being able to answer specific research questions on the effects that may be present or interventions that may be used. This has helped to develop the hierarchy of evidence that allows a framework for ranking the evidence and any applicable interventions. It also helps in determining which studies should be given the most weight when evaluating the same question from differing types of studies (Akobeng, 2005).

The Johns Hopkins Nursing Evidence-Based Practice: Model and Guidelines (Dearholt & Dang, 2012) classifies research articles according to their level and quality. Level I studies include experimental studies, randomized controlled trials (RCT), and systematic reviews of RCTs.

Randomized control trials are considered to be the most rigorous scientific method to help in determining the effectiveness of recommended interventions. Bias can occur when there are flaws in the design and management of a trial. It is significant for individuals who are reading medical reports to be able to develop the skills to critically appraise randomized controlled trials. Also significant is the ability to assess the trial methodology and the validity, magnitude as well as precision of the treatment effect and how applicable the results may be for use. (Akobeng, 2005).

Level II studies include quasi-experimental studies and systematic reviews of a combination of RCTs and quasi-experimental studies. Level III studies can include both non-experimental studies and qualitative studies as well as systematic reviews of 1) a combination of RCT's, quasi-experimental, and non-experimental studies; 2) non-experimental studies only; and 3) qualitative studies with or without a meta-synthesis. Level IV studies are non-experimental but include opinions of respected authorities, nationally recognized expert committees, or panels based on scientific evidence.

The quality of each article is ranked as high, good, or low based on the consistency of the results, sample size, design, level of control, and definitive conclusion (Dearholt & Dang, 2012). Research quality is divided into three sections categorized as A, B, and C (Dearholt & Dang, 2012). High quality (A) has consistent generalizable results. There is sufficient sample size with adequate control with definitive conclusions and consistent recommendations gathered from comprehensive literature review and reference to scientific evidence (Dearholt & Dang, 2012). Good quality (B) research contain reasonably consistent results with sufficient sample size, some control, fairly definitive conclusions and consistent recommendations (Dearholt & Dang, 2012). The lowest level is low quality (C) which often has inconsistent results, insufficient sample size or study design used, and no conclusions can be drawn (Dearholt & Dang, 2012).

Articles were evaluated for strength and quality using the Johns Hopkins Research Evidence Appraisal Tool (Dearholt & Dang, 2012). For the purpose of this literature review only studies at a level of III or higher were accepted. All systematic reviews were excluded. Following determination of level of evidence, the literature was reviewed for quality. All literature used in this study was; of high and good quality except for one study which had a small sample size and used an observational nature of study but did have supporting and similar results as all other literature chosen.

Numbers and Types of Studies Selected

Twenty-three research articles were part of this literature review, eight articles are classified as level I evidence, six articles are classified as level II evidence, and nine articles are classified as level III evidence; eleven articles met criteria for being high quality, eleven articles met the criteria for being good quality, and one article met the criteria for being low quality. The 23 articles selected for the literature review can be found in Appendix B. These articles include

two cross-sectional cohort studies, two longitudinal cohort studies, one nested case-control study, one nested case-control study in 3D cohort, two nested case-control studies within a prospective cohort study, two nested case-control studies within a prospective longitudinal study, seven prospective cohort studies, one prospective pilot study, two randomized control studies, one randomized double-blind placebo controlled trial, one retrospective cohort study, one retrospective case-control longitudinal study. Nested sampling is "an approach to sampling in mixed method studies in which some, but not all, of the participants from one strand are included in the sample for the other strand" (Polit & Beck, 2012, p. 735). The search yielded research from all over the world. The number of times a country was included in the selected research articles is as follows: United States (7), Canada (3), Japan (1), Belgium (1), Austria (1), Brazil (1), China (1), Norway (1), United Kingdom (4).

Higher incidence of studies completed on the subject of vaginal microbiomes and the association with preterm labor and birth are found within the United States. Not all countries that completed the studies were of similar economic development. No religious identification interfered with studies completed.

Summary

Identifying individuals at risk of preterm labor or birth related to the differing vaginal microbiomes present is important in early identification of risk to introduce prevention strategies to decrease the incidence of preterm labor and preterm birth. The majority of research completed on this subject has been completed within the past ten years. A thorough search of the literature was completed, and 23 articles were selected and included in the final matrix. This chapter outlined the search strategies, inclusion and exclusion criteria, the number and type of articles chosen, and the criteria by which the articles were evaluated.

Chapter III: Literature Review and Analysis

Introduction

The matrix includes purpose of the study, descriptions of the samples/settings, design methods, relevant findings, quality of each article, strengths, limitations, and recommendations for practice. It is arranged alphabetically. The purpose, design, and relevant findings of the studies were evaluated, and the data synthesis is presented in chapter three. Microbiology terms may be a challenge to the reader, so definitions are placed in a glossary at the end of the chapter to assist in understanding and having a place for reference.

Synthesis of major findings

The 23 scholarly articles appraised in this review identified vaginal microbiome composition that is correlated with increasing risk for PTL and PTB. Several themes emerged, highlighting common bacterial associations with PTL and PTB as well as associations with healthy vaginal environments. The synthesis of the major findings will address the following topics: Protective microbiome, non-protective microbiome, variations by BMI, race/ethnicity/genetics, viruses, stress, interventions of antibiotics, nutrition, probiotic foods and supplements, and progesterone that affect the microbiome.

Protective microbiome

Ten studies evaluated the microbiome of women who delivered at term (Brown et al., 2019; Kindinger et al., 2017; Petricevic et al., 2014; Romero, Nikita et al., 2014; Romero, Biede et al., 2014; Stafford et al., 2017; Tabatabaei et al., 2018; Verstraelen et al, 2009; Walther-Antonio et al., 2014; Zheng et al., 2019).

Lactobacillus spp. are the primary dominant species associated with a healthy vaginal microbiota. This genus contains key metabolites including lactic acid which maintains the acidic

and anaerobic environment that is needed to protect against pathogenic infection (Kindinger et al., 2017). The lower female genital tract primarily contains a microflora dominant with lactobacilli. Lactobacilli provide protection for the vagina against entry of ascending and systemic infectious disease (Kindinger et al., 2017).

Romero, Nikita et al. (2014) examined 32 nonpregnant women and 22 women who delivered at term between 38-42 wks. gestation to determine the healthy microbiota present in pregnancy. In this retrospective, case-control, longitudinal study, observation was made of stability patterns within the vaginal microbiota during pregnancy that could be used for a fixed point of reference. Fifteen taxa were found to change in pregnancy. Taxa are a group of one or more populations of an organism. Four dominant *Lactobacillus* spp. were found to increase and be protective with the other eleven taxa decreasing in a normal healthy pregnancy. The composition of the vaginal microbiome in healthy pregnancy changed as a function of continuing gestational age, with increases found in abundance of the four primary *Lactobacillus* spp. Healthy vaginal microbiota in pregnancy was also correlated with decreased amounts of anaerobic microbial species throughout progression of normal and health pregnancy (Romero, Nikita et al., 2014).

Normal vaginal microflora primarily consists of four distinct *Lactobacillus* species, L. *crispatus*, L. *jensenii,* L. *gasseri,* and L. *iners*. L. *crispatus* and L. *gasseri* have a stronger defense mechanism against invading bacteria than L. *iners* or L. *jensenii*. Vaginal flora that are found to be protective against preterm labor and preterm birth include L. *crispatus*, L. *gasseri* and mixed dominant *Lactobacillus* spp. These three are considered protective and for the remainder of the paper these organisms will be defined as protective collectively (Kindinger et al., 2017; Stafford et al., 2017; Tabatabaei et al., 2018; Verstraelen et al., 2009; Walther-Antonio et al, 2014; Zheng et al., 2019).

L. crispatus

Kindinger et al. (2017) evaluated the vaginal microbiome of 161 pregnant women in a cross-sectional cohort study. L. *crispatus* (CSTI) dominance was distinctly indicative of term birth (p=0.009). L. *crispatus* excretes elevated concentrations of D-lactic acid. These elevated levels of lactic acid are protective and necessary for healthy vaginal flora in pregnancy. They decrease the pH level and create an acidic environment that reduces the incidence of vaginal infection (Kindinger et al., 2017).

In a prospective pilot study, Stafford et al. (2017) observed 80 asymptomatic women at 20-22 wks. gestation, 41 asymptomatic women at 26-28 wks. gestation and 37 symptomatic women at 24-36 wks. gestation. There was a statistically significant proportion of *L.crispatus* present in the vaginal microbiome of women who delivered at term p=< 0.05 (p<0.05 is considered statistically significant) (Kindinger et al., 2017; Verstraelen et al., 2009). Similar findings were observed by Tabatabaei et al. (2018) in a nested case-control study in 3D cohort of 2366 pregnant women.

A prospective cohort study of 100 pregnant women completed by Verstraelen et al. (2009) observed that among the women who delivered at term, 77 had *Lactobacillus* spp. dominated microbiota with 18 of those women specifically having dominant L. *crispatus*. Normal vaginal microflora containing L. *crispatus* have a five-fold reduction of risk of changing to atypical vaginal microflora compared to non-L. *crispatus* vaginal microflora (p = 0.04). In another prospective cohort study, Walther-Antonio et al. (2014) also identified L. *crispatus* was the dominant species associated with term birth.

L. gasseri

Stafford et al. (2017) observed benefits of L. *crispatus* and L. *gasseri* dominated microbiota. A strong association linked L. *crispatus* and L. *gasseri* (CSTII) with a healthy microbiome. Furthermore, the combination of L. *crispatus/gasseri* domination within the vaginal microbiome compared to the presence of other lactobacilli were found to improve the environment to be very beneficial to the vaginal microbiota (Stafford et al., 2017). Stafford et al. (2017) observed an absence of L. *gasseri* in preterm patient samples at 26-28 weeks gestation ($p=0.03$) compared to term patient samples ($p<0.0001$). The presence of L. *gasseri* is thought to be correlated with the incidence of pregnancies that progress to term. It is one of the health promoting *Lactobacillus* spp. L. *gasseri* that is more limited with its role in the vaginal microbiome associated with preterm birth with some lack of clarity. L. *gasseri* is more frequently seen in white populations and in vaginal microbiomes with more diversity (Stafford et al., 2017).

Lactobacillus dominance

Brown, Nikita et al. (2019) observed 1538 women in a prospective cohort study that found a *Lactobacillus* spp. dominated vaginal microbiota > 24 weeks gestational age (GA) is correlated with a decreased incidence of PPROM risk 0.39 (0.26-0.60), RR-0.43 (0.29-0.63). Relative risk is the ratio of risk present in one group compared to another group (Brown, Nikita et al., 2019). Furthermore, in a prospective cohort study of 111 women aged 18-40 with low-risk singleton pregnancies, Petricevic et al. (2014), found that dependent upon the *Lactobacillus* spp. present in pregnant women there are statistically significant differences in outcomes of term and preterm delivery ($p=0.004$). Women with normal vaginal microflora in early pregnancy have a 75% decreased incidence of delivering prior to 35 weeks (GA). Mixed dominant *Lactobacillus* spp. show statistical difference between term and preterm. The vaginal microbiota is healthier when

colonized with two or more *Lactobacillus* spp. Higher diversity of *Lactobacillus* spp. contributes to health and protection in pregnancy ($p<0.0009$). Approximately, 56% of the women in this study that delivered at term had vaginal microbiota with more than one *Lactobacillus* spp. (Petricevic et al., 2014).

In a cross-sectional cohort study, Zheng et al. (2009) evaluated 83 healthy pregnant women in all trimesters of pregnancy. Lactobacillus represented the majority of the vaginal microbiome in healthy pregnancy. L. *jensenii* (CST V), L. *iners* (CST III), and L. *crispatus* were the primary recurrent species. The amount of L. *iners* and L. *crispatus* was notably different among the three trimesters. L. *iners* decreased during the second and third trimester when compared to the first trimester ($p < 0.001$), while L. *crispatus* notably increased during the second trimester ($p=0.030$). L. *crispatus* is distinctly correlated with term delivery. Similar results were reported by Romero, Biede et al. (2014) in a nested case-control study.

An association was observed by Stout et al. (2017) that an increase in vaginal community richness, diversity, and stability are highly associated with term birth. Other studies have confirmed these same findings (Freitas et al., 2018 & Romero, Nikita et al., 2014).

Non-protective microbiome

Ten studies evaluated the microbiome of women who delivered preterm (Brown et al., 2019; Brown et al., 2018; Honda et al., 2014; Hyman et al., 2014; Jayaprakash et al., 2016; Kindinger et al., 2017; Petricevic et al., 2014; Stafford et al., 2017; Tabatabaei et al., 2018; Zheng et al., 2019). An unhealthy or imbalanced vaginal microbiome means that unhealthy microorganisms increase and reproduce rapidly in the vagina.

Lactobacillus depletion

Lactobacillus spp. depletion has been identified as an increased risk for preterm birth. When *Lactobacillus* spp. depletion is noted there is increased incidence of vaginal dysbiosis demonstrating an association with negative pregnancy outcomes such as preterm birth and late miscarriage (Kindinger et al., 2017). A vaginal microbiome that has a decreased abundance of *Lactobacillus* spp. (<75%) is correlated with a mean relative probability of preterm labor and preterm birth. A vaginal microbiome community that is dominated by other species than *Lactobacillus* spp. is associated with PPROM in all trimesters of pregnancy (Brown et al., 2019).

Brown et al. (2018) identified in a prospective cohort study of 337 pregnant women that a vaginal microbiome depleted in *Lactobacillus* spp., independent of specific species is correlated with probability of risk of PPROM in approximately 25% of cases, despite maternal attributes or other preterm birth risks. Vaginal dysbiosis associated with the depletion of *Lactobacillus* spp. was found to be present before rupture of fetal membranes in nearly one third of cases and continued following the rupture of membranes (31%, p=0.005). Thus, Lactobacillus depletion is an associated risk factor for subsequent PPROM correlated with negative short-term maternal/neonatal outcomes (Brown et al., 2018). In a cohort study of 1538 women evaluated by Brown et al. (2019) similar results were found.

Other dominant vaginal flora

Other dominant vaginal flora that have been implicated include L. *iners*, L. *jensenii*, *Mycoplasma* sp., *Ureaplasma* sp., *Provotella* genus, G. *vaginalis*, and A. *vaginae*. Honda et al. (2014) evaluated 1735 pregnant women in a randomized control study finding in the incidence of preterm birth the vaginal flora often shifts from one of normal flora to that of an intermediate flora state. Vaginal microbial communities cluster in a CST IV-B (mixed anaerobes) state are associated

with a lack of *Lactobacillus* spp. and increased amounts of G. *vaginalis*, *BVAB1*, A. *vaginae,* and *Megasphaera* sp. Freitas et al. (2019), in a retrospective cohort study of 216 pregnant women, found *Prevotella* sp. to be a biomarker for preterm birth. Increased diversity of G. *vaginalis*, A. *vaginae,* and V. *bacterium* were also reported to be correlated with increased risk of early sPTB in a nested case-control study of 2366 observed by Tabatabaei et al. (2018) ($p<0.0001$). Zheng et al. (2019) found similar results with *Gardnerella, Atopobium, Megasphaera, Eggerthella, Leptotrichia/Sneathia*, and *Prevotella*, accounting for the vaginal pathogenic community. In the incidence of Lactobacillus depletion these bacteria increased significantly. L.*iners* ($p<0.001$), A. *vaginae* ($p=0.005$), G. *vaginalis* ($p=0.003$), and L. *jensenii* ($p=0.010$) (Zheng et al., 2019).

In a longitudinal study by Hyman et al. (2014), 1572 pregnancies were evaluated for composition of the vaginal microbiota present in preterm birth. In the preterm women, several taxa including *BVAB1* ($p=0.0031$), *Prevotella* ($p=0.0037$), P. *amnii* ($p=0.0031$) and Sneathia *amnii* ($p=0.0015$) were found in abundance and associated with incidence of preterm birth.

Jayaprakash et al., (2016) found the mean gestational age of PPROM was 28.8 weeks gestation. *Mycoplasma* sp. and *Ureaplasma* sp. were found in 81% of the participants with PPROM. *Mycoplasma* sp. significantly increased the incidence of preterm birth ($p<.001$) (Jayaprakash et al., 2016). The presence of L. *iners* at antenatal testing at 16 weeks GA was highly correlated with a short cervix <25mm ($p<0.05$) and preterm birth <34 weeks GA ($p<0.01$; 69% PPV) in a cross-sectional cohort study of 161 women completed by Kindinger et al. (2017) in assessment of the connection linking the vaginal microbiota, cervical length, and preterm birth risk.

L. iners/L. jensenii

Petricevic et al. (2014) in a prospective cohort study of 111 pregnant women found L. *iners* to be correlated with vaginal dysbiosis during pregnancy, subsequent preterm delivery, and low birth weight. L. *iners* is associated with dysbiosis and is considered a marker indicative of microbial imbalance leading to bacterial vaginosis (Kindinger et al., 2017). If L. *iners* is noted to be the dominate species at sixteen weeks gestation, there is an elevated risk of preterm birth ($p<0.01$) (Kindinger et al., 2017). L. *iners* is considered the smallest group of the *Lactobacillus* spp. group and is a common bacterial species within the vagina. This species is found to demand special nutrient requirements (Petricevic et al., 2014).

In a prospective pilot study of 158 pregnant women, Stafford et al. (2017) examined differences that exist in the vaginal microbiome and metabolite profiles of term deliveries versus preterm deliveries. Vaginal pH associated with health varies from 3-4.5. Vaginal pH is a determinant of a healthy vaginal environment with elevated pH levels indicating an imbalanced vaginal environment (Stafford et al., 2017). pH levels ranged between 3.6 and 6.1 with relationship between community state types observed. pH levels are found to be higher in women who deliver preterm with increased association with the presence of L. *iners* or L. *jensenii* (CSTV) (Stafford et al., 2017). A 25%, two-fold increase was found in the presence of L. *jensenii* in women who delivered preterm. L. *jensenii* is considered a more unstable vaginal microbiome and is thought to be detrimental to a healthy vaginal microbiome during pregnancy. It is also found to be associated with L. *iners* as an organism that transitions between community state types becoming detrimental (Stafford et al., 2017).

Zheng et al. (2019) completed a cross-sectional cohort study of 83 women to assess vaginal microbiome in pregnancy. Specific species in the vaginal microbiome were found to decrease the

acidity of the vagina causing elevation of the vaginal pH leading to overgrowth of pathogenic bacteria ($p<0.05$) (Zheng et al., 2019).

Lactobacillus spp. depletion, L. *iners*, L. *jensenii*, *Mycoplasma* sp., *Ureaplama* sp., *Provotella* genus, G. *vaginalis,* A. *vaginae*, *Veillonellaceae* sp. *Molliculite* sp., *BVAB1*, V. *bacterium*, M. *curtsii/mulieris*, S. *sanquinegens*, *Atropobium* sp., and *Megasphaera* sp. are all considered nonprotective microbiome organisms. For the remainder of this paper these organisms will be referred to as nonprotective microbiome collectively.

Variations

Body Mass Index

Body Mass Index (BMI) is an individual's weight measured in kilograms divided by the square of height in meters. An elevated BMI can be an index of elevated body fat. BMI is used for screening specific weight categories that may be associated with increased incidence of health problems. It is not indicative of the body fat or an individual's health status. Two studies evaluated the association of BMI and preterm birth (Wen et al., 2014 & Oh et al., 2015). BMI is adversely associated with the existence of *Mycoplasma* sp. (mean BMI difference p=.018). and BVAB2 (mean BMI difference p=.004). This is more significant in the African American population (Wen et al., 2014).

Genetic and environmental factors are associated with obesity as well as amounts and types of food eaten, cultural practices, and socioeconomic characteristics. Within the pregnant population, women who have less gestational weight gain tend to have greater amounts of the presence of protective microbiome organisms. Dietary changes and increased activity are important factors in appropriate weight maintenance in pregnancy (Oh et al., 2015).

Wen et al., (2014) found that BMI was highly correlated with the vaginal microbiome in African American women. *Mycoplasma* sp. was found to significantly increase sPTB (OR=5.70 [2.40, 14.4], P<.001) opposed to *BVAB3* that was found to drastically decrease the incidence of sPTB (OR=0.13 [0.036, 0.38], p<.001). Oh et al. (2015) observed that negative microbiome organisms increased, and positive microbiome organisms decreased associated with increased body mass index (BMI). L. *iners* dominance in the vaginal microbiome was highly associated with obesity (odds ratio [OR], 7.55 [95% confidence interval [CI], 1.18 to 48.2]), in comparison to L. *crispatus* dominance. Thus, obesity may be a contributing factor in bacterial community structure. Women with obesity have other physiological characteristics associated with dysbiosis. These characteristics include elevated estrogen levels, systemic inflammation, and decreased immune function (Oh et al., 2015).

Pre-pregnancy BMI and leptin levels can impact the outcome of pregnancy leading to preterm birth. This is related to a non-protective vaginal microbiome in early pregnancy and an increased presence of any bacteria. In underweight women the ratio of protective microbiome dominance (65%) was higher than negative microbiome dominance (35%). In obese women the ratio of protective organisms (17%) was less than non-protective organisms (67%). Hydrogen peroxide (H202), a significant antimicrobial product of the vaginal microbiome is produced at different amounts dependent upon the *Lactobacillus* spp. present. L. *crispatus* produces 95%, L. *jensenii* 94%, and L. *iners* 9%. It is unknown why obese women have higher levels of non-protective organisms in the vaginal microbiome (Oh et al., 2015).

Race/Ethnicity/Genetics

Four studies evaluated differences in racial and ethnic groups in relation to the vaginal microbiome and preterm birth (Elovitz et al., 2019; Hyman et al., 2014; Stout et al., 2017; Wylie et al., 2014). Two studies observed genetic variations associated with vaginal microbiome (Hyman et al., 2014; Walther-Antonio et al., 2014).

Differences of ethnicity and geographical location are significant factors. Race and ethnicity show substantial variation. African American women experience a preterm birth rate of 18% compared with 12% of white women, 10% of Hispanic women, and 11% of Asian women (Hyman et al., 2014). Elovitz et al. (2019) evaluated the CSTs according to race or ethnicity. African American were predominantly CST I or CST IV A/IV B. Hispanic women were predominantly CST IV, and white women were predominantly CST I, II, III. Elovitz et al. (2019) found that the frequency of CSTs was significantly different between African American and non-African American women. At the primary visit, African American women were found to be 20% CST I and 45% CST IV A/IV B compared to non-African American women at 50% and 15%. These differences remained at visit 2 and visit 3. The presence of CST III was higher for African American women at visit 3 but the presence of CST V was consistently decreased among African American women compared to non-African American women throughout the study (Elovitz et al., 2019).

Among all participants at all visits, several nonprotective microbiome organisms were found that were statistically significant with an association to an increased incidence of sPTB. The rate of sPTB was over 55% when the non-protective bacterial taxa were present (Elovitz et al., 2019). Stout et al. (2017) completed a study where 69% of the participants of the study were African American with a preterm birth rate of 31%. Those who delivered at term were found to

have a vaginal microbiome that had stable community richness and Shannon diversity. Community richness is a microbiology term that means how many different species can be detected in the microbial ecosystem (Morris et al., 2014). Shannon diversity means is the species evenness (equal in abundance) or do some species dominate others? How evenly are the microbes distributed in a sample. (Morris et al, 2014). Those that delivered preterm were found to have significantly lower vaginal richness and diversity throughout pregnancy ($p<.01$). Beginning early during the first and second trimesters, instability of the vaginal microbiome was highly associated with sPTB. In the African American women who delivered at term, diversity, vaginal community richness, and evenness were stable ($p=.11$, $p=.09$, and $p=.08$). Evenness is a comparison of the similarity of the population size of each of the species present within the vaginal microbiome (Morris et al., 2014). In comparison among those who delivered preterm, richness, diversity, and evenness were significantly decreased ($p<0.001$, $p=.003$, and $p<.001$) (Stout et al., 2019).

African American women have two times increased risk for preterm birth compared to Caucasian women. The underlying components for this disparity are not well understood but cannot be explained merely by sociodemographic influences. Additionally, underlying components may include complicated interactions among maternal, paternal, and fetal genetics, epigenetics, and the microbiome. Within these sociodemographic influences may be probable basis for the differences found in racial groups. Mixed results have been found in studies of Asian and Hispanic women (Hyman et al., 2014). Caucasians are often noted to have higher amounts of protective microbiome organisms. Asian ethnicities tend to have increased amounts of nonprotective microbiome organisms. Hispanic and African American women are found to have vaginal microbiomes that have a diverse mixture of bacteria with little to no *Lactobacillus* spp. (Hyman et al., 2014).

Significant association was also found among the Hispanic women in connection with the vaginal microbiome and birth outcomes. Several non-protective microbiome organisms were present and associated with preterm birth (OR=4.45 [1.69, 11.97], p<.01) and (OR=0.19 [0.0076, 1.01], p=.068) (Wen et al., 2014). Comparing these two ethnicities, following management of maternal behavior and biological attributes, there continued to be differences that remained within the vaginal microbiome that markedly contributed to the incidence of preterm birth. Wylie et al. (2018) found similar results. Increased viral richness in African American women was correlated with sPTB (p=.0015 and p=.0019). Increased integration of bacterial diversity was also correlated with preterm delivery (p=.01) and a reduction in diversity was correlated with proceeding through the trimesters of pregnancy (p<.0001), to term delivery (Wylie et al., 2014).

Hyman et al. (2014) observed that among Asian women, 22% were lacking *Lactobacillus* spp. in the vaginal microbiome compared to other ethnicities at 0-8%. A significant difference in African American women during pregnancy was vaginal microbiomes that contained <50% of Lactobacillus compared to other ethnicities with vaginal microbiomes that contained at one species of Lactobacillus >50% with average of 63-91%. The percentage of the presence of at least one Lactobacillus in the vaginal microbiome in Caucasian women (93%), African American women (69%), Asian women (70%), and Hispanic women (74%).

Disparities continue even after considering commonly known preterm birth risk factors including smoking, education level, and socioeconomic status. African American women have a higher rate of reoccurrence of sPTB and preterm rupture of membranes (PROM). Sociodemographic influences account for some risk associated with sPTB, but do not account for many of the differences found within differing ethnic groups. Women who live in disadvantaged conditions are found to have higher risk of sPTB, both of which are higher among African

American women. Disparities associated with sPTB are found in different racial and ethnic groups even when they have the same access to healthcare (Elovitz et al., 2019).

Viruses

Viruses are small collections of genetic code which can be either DNA or RNA with a surrounding protein coat. Viruses are unable to reproduce; therefore, they infect cells and use components of these cells to make duplicates of themselves. In this process they kill the host cell and cause damage to the host organism. Bacteria in the vaginal microbiome have been studied more than viruses. Only one study evaluated viruses within the vaginal microbiota (Wylie et al., 2018). Wylie et al. (2018) refers to the Human Microbiome Project which studied vaginal DNA viruses and bacterial structures together. Viruses are common in the samples of healthy, asymptomatic women. DNA viruses were found in women with high-diversity bacterial groups. Wylie et al. (2018) suggests that this data shows the importance of the presence of viruses in the vaginal microbiome which has been thought to be unappreciated components.

Wylie et al. (2018) hypothesized that the presence of DNA viruses may be a significant portion of the microbiota correlated with preterm birth. Interactions linking bacteria and viruses is significant in assessment of the vaginal microbiome. The study examined a possible correlation between the vaginal virome and preterm birth. This was completed with the vaginal virome alone and with the bacterial microbiome. Bacteria and viruses may trigger a maternal inflammation cascade which can lead to PTL and PTB. These microbial features may be prospective biomarkers of a familiar underlying physiology in women at higher risk of PTL and PTB (Wylie et. al, 2018).

Viral pathogens are studied elements of the vaginal microbiome. No relationship was noted in bacterial groups with <70% *Lactobacillus* spp. and the existence of viruses during pregnancy. No specific bacterial groups were correlated with the existence of any specific virus. Wylie et al,

(2018) found no specific viruses correlated with PTB but did find that in the presence of higher viral richness had an important correlation with PTB. The incidence of both high bacterial diversity and high viral diversity increased probability of PTB. This was particularly evident among AA women (Elovitz, 2019).

Examination of bacterial and viral communities is an important part of elucidating correlations of the vaginal microbiota and PTB (Wylie et. al, 2018). The specimen patterns of viral diversity in pregnancy are comparable to specimen patterns of bacterial diversity. Physiological changes in the vaginal microbiota in pregnancy can impact bacterial and viral groups. According to Wylie et al. (2018) there is greater viral diversity in the first trimester of pregnancy between term and preterm births, increased viral diversity is correlated with sPTB (Wylie et al., 2018).

Stress

Stress is indicative of how an individual may feel overwhelmed or unable to cope. One study assessed the correlation of stress and vaginal dysbiosis in the microbiota (Wylie et al., 2018). When the body is under mental and physical stress, increased pro-inflammatory cytokines and cortisol are produced. Acute inflammation contributes to increases of the incidence of sPTB. Chronic inflammation can also occur resulting in the same outcome (Burris et al., 2020).

Low-income African American women experience increased chronic social, financial, and racial stress which is linked to the significant racial disparity rate in the incidence of infection and PTB. Stress can influence the occurrence of depression and psychological response stressors which are also associated with negative pregnancy outcomes including PTB (Burris et al., 2020).

Stress stimuli are inevitable. The physiologic response of stress can be detrimental to the vaginal microbiome when stress stimuli are prolonged. Continuous stress stimulates the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) axes which

increase hormones that can cause a genitourinary infection. A cortisol-induced hindrance of vaginal glycogen accumulation leads to the incidence of vaginal infection (Burris et al., 2020).

Increases in estrogen and epithelial maturation are necessary for maintenance of a healthy vaginal microbiome (eubiosis). Cortisol can disturb this process and plays a role in pathogenesis of vaginal dysbiosis leading to infection and inflammation. This process is found to be significant in the presence of a healthy vaginal microbiome dominated by *Lactobacillus* spp. which should not be interrupted. Increased corticotropin-releasing hormone (CRH) is produced by the fetal membranes, decidua, and placenta (Burris et al., 2020).

Interventions

Interventions are actions completed to improve a situation, such as a medical disorder. They are actions that can be changed to improve or alter the microbiome. For this review interventions included antibiotics, nutrition/probiotic foods and supplements, progesterone. Five articles described potential interventions that may improve vaginal microbiome composition (Bellard et al., 2018; Brown et al, 2019); Elovitz et al., 2019; Honda et al., 2014; Myhre et al., 2011).

Antibiotics

Antibiotics are medications used to fight bacterial infections in the human body. Antibiotics work by killing bacteria or by stopping them from growing or multiplying. Two studies explored the effects of antibiotics on the vaginal microbiome (Brown et al., 2019; Wylie et al., 2018).

The primary cause of disease responsible for preterm birth is infection. Many cases of intrauterine infection that cause preterm birth occur from ascending infection. Attempts have been made to determine specific women with probability for preterm birth through assessment of the

vaginal microbial state. The use of antibiotics has been evaluated. Bellad et al. (2018) observed that in women with bacterial vaginosis if clindamycin was administered prior to 22 weeks gestation the rate of sPTB before 37 weeks gestation significantly decreased. Statistical significance was only found for oral clindamycin but not for vaginal clindamycin. The cure rate of bacterial vaginosis in pregnancy with clindamycin is 90%. The use of metronidazole was correlated with negative pregnancy outcomes and increased incidence of preterm birth. Clindamycin is only effective for few microbial species and is not effective against other numerous microbial species that cause preterm birth (Bellad et al., 2018).

Brown et al. (2019) observed that the use of erythromycin in treating vaginal dysbiosis caused exacerbation of the dysbiosis 47% ($p=0.00009$) especially in women who were primarily colonized with *Lactobacillus* spp. Following erythromycin treatment, dysbiosis of the vaginal microbiota stayed constant and bacterial richness and diversity remained unchanged. The use of erythromycin promotes depletion of *Lactobacillus* spp. and increases the bacterial diversity of the vaginal microbiota (Brown et al., 2019).

Nutrition, probiotic foods, and supplements

Nutrition is the intake of food and nutrients that are necessary for optimal health and growth. One study evaluated the relationship between nutrition, probiotic foods and supplements in relation to the vaginal microbiome (Myhre et al., 2011). The consumption of probiotic rich foods such as yogurt, kefir, sauerkraut, pickles, sourdough bread, traditional buttermilk, gouda/mozzarella/cheddar and cottage cheese in pregnancy is correlated with decreased incidence of preterm birth. Probiotics are noted to change the composition of the vaginal microbiota to allow for inhibition of pathogens as well as modulate inflammation that is commonly associated with preterm birth (Myhre et al., 2011).

Probiotic supplements, especially *Lactobacillus* spp., play a significantly beneficial role in maintaining healthy urinary and reproductive tracts. Emerging evidence is showing safety and efficacy in the use of probiotics in treatment and prevention of numerous infections or inflammatory conditions in pregnancy. They help to decrease the incidence of vaginal infections and help to increase colonization of *Lactobacillus* spp. within the vagina (Myhre et al., 2011).

The effect that *Lactobacillus* spp. have on the immune system and vaginal colonization is both species and strain dependent. L. *rhamnosus* GR1 and L. *reuteri* RC14 were found to have an excellent capability of colonization and are considered the preferred Lactobacilli for the use in the treatment of infections within the urogenital tract (Myhre et al., 2011). Myhre et al. (2011) completed a nationwide cohort study of 18,888 pregnancies in Norway. In the group that did not take probiotics, 950 incidences of sPTB occurred (70%) compared to the women who had taken probiotics (21%). The conclusion of this study was that the use of probiotics is associated with a decreased incidence of sPTB. Probiotics work to lower overall inflammation and help to provide a healthy microbial vaginal environment. Reduction of sPTB is thought to be able to be achieved through targeting dietary health concerns and evaluation of intake of probiotics (Myhre et al., 2011).

Consideration of interventions in regard to nutrition in early pregnancy are thought to be significant. Using probiotics that contain L. *rhamnosus* GR1 and L. *reuteri* RC14 has the potential to decrease vaginal infections as well as the incidence of sPTB by approximately 50% (Myhre et al., 2011).

Progesterone

Progesterone is a steroid hormone that the corpus luteum releases leading to stimulation of the uterus in preparation for pregnancy. One study evaluated the use of progesterone in altering the vaginal microbiome to prevent preterm birth (Kindinger et al., 2017). Currently used clinical methods to recognize and decrease the probability of sPTB include cervical length screening and treatment with 17-alpha hydroxyprogesterone caproate. Vaginal progesterone does provide some protection in women with a short cervix but is a strategy that will not have a significant impact on sPTB rates (Elovitz et al., 2019).

Kindinger et. al. (2017) investigated the role of progesterone within the vaginal microbiome in women with a short cervix. Progesterone supplementation had no effect on the structure of the vaginal microbiota during pregnancy. Richness and alpha diversity measurements were not altered. Progesterone also did not impact the amount of L. *iners* or L. *crispatus* throughout pregnancy (Kindinger et. al., 2017).

Critique of strengths and weaknesses

Strengths

The first strength of the review of the literature is that many of the studies are able to similarly identify new insights about the vaginal microbial profile in pregnancy and the association with the incidence of preterm labor and preterm birth. The majority of the studies identified consistent results which makes these findings reliable. Several other strengths of the review included studies with large cohorts and studies with intervention and control groups.

Implications were also provided in many of the studies for future stratifications of preterm labor and preterm birth risks and suggested targeted interventions. The majority of the studies were of high and good quality. While the number of participants in some of the studies were small, the

nature of the studies allowed for saturation of the results and the identification of common themes. All studies were limited within the last eleven years with the majority of the studies occurring in the past five years, providing the most current available information. Besides identifying common themes of the correlation of the vaginal microbiome and its role in preterm labor and preterm birth, several studies provided key strategies and recommendations for practice to improve prenatal outcomes associated with vaginal microbiome. The microbiome is almost invisible, and frequently overlooked. The fact that it can contribute to pregnancy outcomes is a relatively new idea that deserves attention.

Weaknesses

The qualitative nature of some of the studies is a weakness of this research article review. In these studies it is more difficult to analyze and measure causal relationships within variables related to quantity, intensity, amount, and frequency. Another weakness is the small sample sizes in some of the studies as well as correlations not being fully interpreted or needing further trials for validation. Many studies did not discuss how screening for all the microbiomes could be incorporated into regular prenatal care. These weaknesses cause difficulty in generalizing the results to larger populations. PTL/PTB is a very complex, multifactorial problem. By focusing only on the microbiome, many other factors that contribute to PTL/PTB can be missed.

Summary

A thorough search of the literature was completed, and 23 articles were selected for review. This chapter outlined the synthesis of major findings. Several microbiome environments were found to be associated with PTL/PTB. These primarily were correlated with microbiomes influenced by L. *iners* and L. *jensenii* as well as other pathogens and *Lactobacillus* spp. depleted environments (Brown et al., 2018; Freitas et al., 2018; Jayaprakash et al., 2016; Petricevic et al.,

2014; Santos de Freitas, 2019; Tabatabaei et al., 2018; Verstraelen et al., 2009). Microbiome environments that were found to be protective against PTL/PTB and were found to be predominantly influenced by L. *crispatus* and L. *gasseri* (Kindinger et al., 2017; Romero, Nikita et al., 2014; Stafford et al., 2017; Stout et al., 2017; Walther-Antonio et al., 2014).

Evidence is growing that the vaginal microbiome may have a significant role in maintaining pregnancy. Differences were noted among various racial and ethnic groups with higher incidence of negative microbial composition and PTB in the African American and Hispanic population (Elovitz et al., 2019; Wen et al., 2013; Wylie et al., 2018). Risk factors in all ethnic groups included high BMI, poor diet, negative health behaviors, and stress. Potential interventions were discussed among these as well as the potential for the use of oral probiotics to decrease the incidence of PTL/PTB (Myhre et. al, 2011).

Presence of multiple *Lactobacilli spp*. is a significant determinant of stability of the vaginal microbiome during pregnancy. These are important observations in light of current disease burden linked with depleted Lactobacilli. Approximately 50% of women have vaginal microflora containing L. *gasseri*/L. *iners* which are the least effective colonizers and defenders. There appears to be a significant number of women with lactobacilli-driven defenses that are less optimal than presumed (Verstraelen et al, 2009).

Chapter four will address nurse-midwifery practice implications, examination of recommendations and directions for future research studies, as well as integration of The Life Perspective Rhythm Model developed by theorist Dr. Joyce Fitzpatrick in relation to microbial composition and its association with PTL/PTB.

Chapter IV: Discussion, Implications and Conclusions

The purpose of this literature review was to examine the CSTs that are associated with the incidence of PTL and PTB and to identify any interventions or treatments that could successfully alter the vaginal microbiome and decrease the incidence of PTL and PTB. A hostile vaginal microbiome is identified as an aspect that can be modifiable in correlation with PPROM. Application of the Johns Hopkins Research of Evidence Level and Guide (Dearholt & Dang, 2012) assisted in the selection and appraisal of 23 scholarly peer-reviewed articles shown in the Matrix. These articles were evaluated on their research methodology, results, strengths, limitations. Implications for nurse-midwifery practice and recommendations for future research will be addressed.

Literature Synthesis & Implications for Midwifery Practice

The research questions for this review of the literature explore: "How do the vaginal microbiome community states relate to preterm labor or birth?" and "If the microbiome is implicated, are there interventions that can alter the microbiome to reduce preterm birth?" Through the critical appraisal of the literature, a significant amount of information related to abnormal vaginal microbial CSTs and increased incidence of PTL and PTB was identified. The variety of methods that are most successful in the determination of abnormal CSTs and interventions to prevent PTL and PTB reveal that there is not just one single method that has been found the most effective and consistently used. Utilizing the information from the 23 research articles made it possible to determine the bacterial taxa associated with PTL and PTB.

To determine which women are at an increased risk of PTL and PTB, assessment of vaginal bacterial CSTs is essential. In women who deliver at term, the vaginal bacterial community states show stability of richness and diversity throughout pregnancy. In women who

deliver prematurely, the vaginal bacterial CSTs have significantly decreased community richness and diversity that can already be identified in early pregnancy. The change in richness and diversity associated with PTB occurs near the end of the first or the beginning of the second trimester with a greater diversity found in the first trimester. When diversity was diminished at five weeks it was predictive of PTL that progressed to PTB later in pregnancy (Stout et. al, 2017).

Consideration must be given to differing region-specific factors as well as the differing racial/ethnic groups. Hormonal, nutritional, and immunological changes during pregnancy may alter the vaginal microbiota. They may also help to maintain maternal and fetal health and well-being during pregnancy. Understanding these factors and the role they may have in the incidence of PTL and PTB is significant for the nurse-midwife (Elovitz et al., 2019).

Knowledge of the vaginal microbiome that is associated with PTL and PTB is necessary to determine strategies that will prevent this reproductive outcome (Jayaprakash et. al, 2016). Fixed point of references of vaginal microbiome patterns of stability in pregnancy were determined. This provides a basis for evaluation of the connections between the vaginal microbiome and adverse pregnancy outcomes. Awareness of the vaginal microbiome during pregnancy can provide valuable prognostic, diagnostic, and therapeutic information (Romero, Biede et. al, 2014). These observations are pertinent to developing a comprehensive view of the changes within the vaginal ecosystem that occur during healthy pregnancy. These changes can be meaningful in assessment of health and for identifying a predisposition to any adverse outcomes (Romero, Biede et. al, 2014).

Important links have been found between microbial CSTs and metabolite profiles associated with PTL and PTB. This has clinical significance that helps improve comprehension

of the processes of inflammation correlated with PTL and PTB (Stafford et. al, 2017). Studies also identify the significance of taking into consideration race/ethnicity when assessing vaginal bacteria and risk for PTL and PTB. (Wen et. al, 2013). In pregnancy and non-pregnancy, African American women have a higher proportion of Lactobacillus depleted vaginal microbiomes. Their commonly noted higher incidence of PTL and PTB can be associated with Lactobacillus depletion. When Lactobacillus sp. is present it is commonly L. iners which is a marker for PTB (Elovitz et. al, 2019).

Wylie et al. (2018) hypothesized that the presence of DNA viruses may be a significant constituent related to the vaginal microbiome and probability of preterm birth. Interactions linking bacteria and viruses is significant in assessment of the vaginal microbiome. Bacterial and viral groups may trigger a maternal inflammation cascade which can lead to PTL and PTB. Microbial features can be potential biomarkers of a familiar underlying physiology that is correlated with probability of PTL and PTB (Wylie et. al, 2018). Viral pathogens are familiar elements of the vaginal microbiome. Wylie et al, (2018) found no specific virus responsible for PTB but did find that higher viral richness had a significant association with PTB. Increased bacterial diversity combined with increased viral diversity have the highest probability of PTB. Examination of bacterial and viral groups is a significant aspect of interpreting correlations of the vaginal microbiome and PTB (Wylie et. al, 2018).

Due to the increasing evidence that discusses how the human microbiome influences health, it is significant for the nurse-midwife to have a basic comprehension of the microbiome and how to apply it when providing patient care. There are vast differences among individuals in the mixed types of bacteria that make up various microbiome communities. There is much more to learn. Research needs to occur in relationship to the vaginal microbiome. Future evidence may

help to provide insight into how to develop more effective interventions for treating vaginal dysbiosis and for promoting positive maternal/fetal outcomes.

The literature review identified various bacteria associated with PTB, but no trials attempted to alter the vaginal microbiome. Safety and efficacy are found for the use of probiotic rich foods in pregnancy for treatment and prevention of numerous infections or inflammatory conditions (Myhre et al., 2011). Probiotics that contain L. *rhamnosus* GR1 and L. *reuteri* RD14 have the potential to decrease vaginal infections and the incidence of PTB by approximately 50% (Myhre et al., 2011). Factors found to impact the vaginal microbiome negatively are smoking, substance abuse and hygiene practices, such as douching (Brown et al., 2018b). Physiological effects of stress lead to the incidence of genitourinary infection, vaginal dysbiosis, and the loss of Lactobacillus dominance.

Prevention strategies are aimed at alleviating stress through lifestyle and nutrition modifications (Burris et al., 2020). The use of metronidazole was correlated with negative pregnancy outcomes and increased incidence of preterm birth. Clindamycin is only effective for few microbial species and is not effective against other numerous microbial species that cause preterm birth (Bellard et al., 2018). The use of erythromycin promotes depletion of *Lactobacillus* spp. and increases the bacterial diversity of the vaginal microbiota (Brown et al., 2019). The use of progesterone also did not impact the amount of L. *iners* or L. *crispatus* throughout pregnancy and does not decrease the PTB incidence related to vaginal dysbiosis (Kindinger et. al., 2017).

As a nurse-midwife it is important to contribute to this research and to incorporate the most up to date evidence-based recommendations into patient care. Several modifiable factors contribute to vaginal dysbiosis, including hygienic practices and sexual behavior that can alter the composition of the microbiome in the vagina. The nurse-midwife has the responsibility to

provide education aimed at decreasing potentially harmful behaviors that exist in high-risk populations. Douching occurs in some cultures. This practice is associated with vaginal dysbiosis (McElroy et al., 2017). The stability of the vaginal microbiota in pregnancy is significant due to its association with the increased incidence of PTL and PTB (McElroy et. al, 2017).

The body of evidence continues to grow, showing that the microbiome profoundly influences human health. Nurse-midwives need to be aware of any practices such as antibiotic administration or frequency of vaginal examinations when providing antenatal care or in preterm labor that may alter the composition of the vaginal microbiota. To provide comprehensive and evidence-based care it is prudent for the nurse-midwife to follow practices that incorporate what is known about the vaginal microbiome (Cahill et al., 2012).

Recommendations for Future Research

Much of the current research reached similar conclusions. The research that does exist from the past ten years has consistent results and is of good quality. *L. iners* is distinctly correlated with vaginal dysbiosis. Further research studies are needed to recognize this microbiome and quantify the relative number of L. *iners* in the presence of differing conditions during pregnancy. This will further the understanding of the potential harmful outcomes associated with this microbiome (Zheng et. al, 2019). Overall specific causal relationships have not been able to be determined. Recommendations for further study utilizing sequencing methodology are needed. Additionally, assessment of microbial metabolite production and host response might additionally explain the elements associated with the incidence of sPTB. Answers to that question could improve the ability to recognize women at risk earlier in pregnancy (Freitas et al., 2018). Further research is needed to determine practical methods to build assessing CST's into routine patient care. Some of this research has been completed as a

part of studies in major healthcare centers but has limitation as information has not been provided for general use.

Across all studies, L. *iners* was correlated with preterm birth and L. *crispatus* is correlated with term birth. Mechanisms of how the increased presence of L. *iners* contributes to the incidence of PTB are not completely understood. Further studies that investigate the presence of differing Lactobacilli within the vaginal microflora to provide a longitudinal picture of the vaginal microbiota throughout pregnancy are indicated (Petricevic et. al, 2014). Another important aspect to study is the immunology of the host (Tabatabaei et. al, 2018).

Further evidence is needed to provide insight into how to develop effective strategies and interventions that improve the vaginal microbiome for promotion of positive maternal/fetal outcomes. Further research is also indicated in studies of all racial/ethnic groups and bacterial communities as it is important to understand the differing diverse populations. The etiology of infections associated with PTB, can be a focus as many of these studies have shown powerful structural dissimilarities among differing racial/ethnic groups. Racial/ethnic groups would benefit from research identifying bacterial communities among diverse populations. Also to be taken into account is the potential analytical value of community ecology methods to improve understanding of the associations related to differing racial/ethnic groups (Wen et al., 2013).

Vaginal viral diversity has a strong association with PTB. The changes within the vaginal virome follow similar patterns that allude to an underlying physiology of pregnancy itself that can affect bacterial/viral communities. The first trimester presence of vaginal dysbiosis has shown to have the most significant association with PTB suggesting it is of critical importance to have better understanding of bacterial/viral communities during this time period (Wylie et al., 2018).

Current standards of care for decreasing the incidence of PTL and PTB are not decreasing the public health burden of sPTB. These studies as well as further studies will help to develop prevention strategies. Further research will help to develop innovative therapeutic opportunities for prevention of sPTB that may include microbiome-based therapeutics and immune modulators (Elovitz et al., 2019).

With rapidly evolving technologies, cervicovaginal fluid sampling can be an easy, quick, and cost-effective point of care testing that can assess the *Lactobacillus* spp. abundance as well as the presence of any pathobionts. All women could be tested in the first trimester and when preterm labor symptoms occur. Factors such as genetics, antepartum hemorrhage and anatomical abnormalities are unmodifiable risk factors compared to an unfavorable vaginal microbiome that can be a modifiable factor for the incidence of PPROM. The ability to identify this subset of patients and initiate interventions such as manipulation of the bacterial communities through prebiotic or probiotics requires further investigation as these may be promising strategies for decreasing or preventing the incidence of PPROM and PTB (Brown et al., 2018).

Many associations found to this point between microbiomes and PTB have been qualitative. Future goals are to identify unique bacterial communities that can be targeted in working toward prevention of PTB. Important considerations need to be made of the ecological dynamics of differing bacterial species and the interactions these play in the host environment. Current therapies used in treating infection or inflammation associated with PTB focus on the causal microbiomes. This requires timely and accurate diagnostic measures or the targeting of cytokines and inflammation pathways that cause preterm labor (Brown et al., 2019).

Due to emerging findings of the contribution of the vaginal microbiota to PTL and PTB, it is necessary to determine therapeutics that will alter this microbiota in differing niches. Long

term, further understanding of the vaginal microbiome and the role it plays in PTL and PTB can help to define what is a healthy microbiome during pregnancy. That knowledge can lead to development of strategies to restore normal microbiota during pregnancy (Walther-Antonio et al., 2014).

Application and Integration of Theoretical Framework

When considering the use of The Life Perspective Rhythm Model in association with vaginal microbiomes, PTL, and PTB, the model provides a unique way to analyze what is necessary to ensure successful outcomes in pregnancy for each woman by understanding negative outcomes, benefits, barriers, and cues to action. Understanding negative outcomes allows the woman and the midwife to look at her current situation and surrounding environment. Understanding benefits allows the woman to value how the assessment and recommendations of the nurse-midwife can help her to have more optimal pregnancy outcomes. The nurse midwife should recognize the barriers that a woman faces that the nurse midwife, and the patient can discuss and find ways to overcome them. Understanding the barriers allows the woman and midwife to assess lifestyle and dietary behaviors, and to identify any modifiable factors that may be present that can decrease the incidence of PTL or PTB. Finally, understanding cues to action are seen clearly by the woman through the external stimulus of the midwife providing counseling.

The core ideas of the Life Perspective Rhythm Model include the significance of person, health, wellness, and the metaparadigm of nursing. It is important to understand the woman as a whole person including her environment, that is, the culture and the social structures that exist within her environment. In focusing on health, assessment is made of all those around the patient. The concept is that the woman needs the provider to ensure optimal health. The patient is

also willing to make needed changes and follow recommendations to ensure improved health and optimal outcomes. Wellness is the optimal state of health the nurse-midwife hopes to provide through education and health promotion. This would include any lifestyle factors or behaviors that the woman may have that may be modifiable to improve the composition of her vaginal microbiome to ensure the positive pregnancy outcome of decreased potential for PTL or PTB. The patient must be willing to provide information and follow the nurse midwife's recommendations. The nurse-midwife plays a significant role in providing education that will help to decrease the incidence of negative maternal or neonatal outcomes related to vaginal dysbiosis during pregnancy.

The application of the nursing metaparadigm allows the nurse-midwife to fully understand the patient and their life, environment, and social constructs. The nurse-midwife thoroughly assesses current health status and environment to identify the needs of the woman. The nurse-midwife will then incorporate theory, practice, and procedures that are individualized to each woman and each situation to ensure the most optimal outcomes for each person in her care. In doing this, the goal is to decrease the incidence of PTL or PTB for each woman and ensure a positive and optimal pregnancy outcome.

Conclusion

The purpose of this review was to investigate vaginal microbiome CSTs that are found to be associated with the incidence of PTL and PTB and any modifiable risk factors. Using the Johns Hopkins Research Evidence Level and Guide (Dearholt & Dang, 2012), 20 scholarly peer-reviewed articles were thoroughly appraised and examined. The articles were examined for their implications into nurse-midwifery practice. The information presented shows the significant need and opportunity for further research on this topic in order to be relevant to the women of this generation and working toward decreasing the current health burden of PTL and PTB. Integration and application of The Life Perspective Rhythm Model showed the critical elements needed to guide the discussion pertaining to the vaginal microbiome and association with PTL and PTB. This review will inform CNMs and other healthcare professionals on the need to further educate themselves in regard to vaginal microbiome and encourage them to participate in research to develop strategies to decrease the current high percentage of PTL and PTB that occur.

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Appendix A. Microbiology terms and definitions

Anaerobic Microbes

Lactobacillus crispatus (L. crispatus), Lactobacillus gasseri (L. gasseri), Lactobacillus iners (L. iners), Lactobacillus jensenii (L. jensenii), Lactobacillus acidophilus (L. acidophilus), Leptotrichia buccalis (L. buccalis) , Sneathia sanquinegens (S. sanquinegens), Eggerthella lenta (E. lenta), Megasphaera species (Megasphaera spp.), Veillonellaceae bacterium (V. bacterium), Molliculite species (Molliculite spp.), Mageebacillus indolicus (M. indolicus), Actinomyces neuii (A. neuii), Peptoniphilus harei (P. harei), Dialister pneumosintes (D.pneumosintes), Varibaculum cambriense (V. cambriense) and bacterial vaginosis-associated *bacterium 1(BVAB1).*

Grow in anaerobic and aerobic conditions

Mycoplasma pneumoniae (M. pneumoniae), Ureaplasma urealyticum (U. urealyticum), Gardnerella vaginalis (G. vaginalis), Provetella bivia (P. bivia), Atopobium vaginae (A. vaginae), Mobiluncus curtsii/mulieris (M. curtsii/mulieris), Aerococcus christensenii (A. christensenii), Bacteroides ureolyticus (B. ureolytics).

Definitions

Aerobic-Bacteria that require free oxygen.

Anaerobic- Bacteria that require the absence of free oxygen.

Alpha diversity- Variation of microbes in a single sample (Chu et al., 2018).

Beta diversity- Variation of microbial communities between samples. What are the differences in microbial composition from one environment to another (Chu et al., 2018).

Community state types (CSTs)- The most common isolated species found in a healthy vaginal microbiome are *L. crispatus, L. gasseri, L. jensenii*, and *L. iners*. Community state types is a system to grade the vaginal microbiota patterns in relationship to which dominant *Lactobacillus spp.* is present. This system was developed through the use of gram staining, sequencing of 16S rRNA genes and cultures. In CSTIa *L. crispatus* is the dominant species followed by *L. jensenii*. In CSTIb the dominant species are *L. iners* and *L. gasseri*. CSTII is considered an intermediate state between CSTI and CSTIII. Within CSTII are the presence of *L. iners*, *L. gasseri, L. crispatus, A. vaginae, G. vaginalis, A. neuii* and *P. harei.* CSTIII are the bacterial vaginosis associated species including *G. vaginalis, A. christensenii, A. vaginae, B. ureloyticus, D. pneumosintes, M. curtisii, P. bivia* and *V. cambriense* as well as *Lactobacillus spp*., primarily *L.*

iners. CSTIV is a group of strictly anaerobic bacteria, including *P. bivia, D. pneumosintes, A. vaginae, G. vaginosis, Megasphaera, P. harei*, bacterial vaginosis associated bacteria I, II, III (BVABI, BVABII, BVABIII), as well as *L. crispatus* and *L. iners* (VandeWijgert, 2017).
CSTI- *Lactobacillus crispatus*/CSTII- *Lactobacillus gasseri*/CSTIII- *Lactobacillus iners*/CSTIV-mixed anaerobes/CSTV- *Lactobacillus jensenii.*

Diversity- Is their species evenness (equal abundance) or do some species dominate others? Diversity states how evenly the microbes are distributed in a sample. (Morris et al, 2014).

Evenness- A comparison of the similarity of the population size of each of the species present within the vaginal microbiome (Morris et al., 2014).
Vaginal cleanliness- Vaginal cleanliness is used to determine inflammation status as it can lead to further inflammation. Degree II-III indicate normal microecological vaginal status. Degree III-IV indicate a status of abnormality. (Ting et al., 2019).

Free oxygen- Is oxygen that is not combined with other elements such as carbon or nitrogen.

P value- P-values tell us whether an observation is a result of a change that was made or is a result of random occurrences. It is a test for significance. In order to accept a test result the p-value should be low. Value below 0.05 is significant. Value greater than 0.05 is not significant (Nahm, 2017).

Shannon diversity index- Combines richness and diversity. It measures both the number of species and the inequality between species abundances (Morris et al., 2014).
Shannon evenness index- Independent of species richness, measures how evenly the microbes are distributed in a sample without considering the number of species (Morris et al., 2014).

Vaginal dysbiosis- Shift from the most favorable vaginal microflora by bacterial diversity which is associated with adverse health outcomes. (VandeWijgert, 2017).
Richness- How many different species can be detected in the microbial ecosystem (Morris et al., 2014).

The terms microbiome and microbiota may be used interchangeably.

Appendix B. Literature Review Matrix

<table>
<tr><td colspan="4">Source: Bellad, M., Hoffman, M., Mallapur, A., Charantimath, U., Katageri, G., Ganachdri, M., Kavi, A., Ramdurg, U., Bannale, S., Revankar, A., Sloan, N., Kodkany, B., Goudar, S., & Derman, R. (2018). Clindamycin to reduce preterm birth in a low resource setting: A randomized placebo-controlled clinical trial. An International Journal of Obstetrics and Gynaecology, 125(12), 1601-1609. https://doi.org/10.1111/1471-0528.15290</td></tr>
<tr><td>Purpose/
Sample:</td><td>Design
(Method/
Instruments):</td><td>Results:</td><td>Strengths/
Limitations:</td></tr>
<tr><td>Purpose: To determine whether oral clindamycin reduces the risk of preterm birth (PTB) in women with abnormal vaginal microflora as evidenced by a vaginal pH ≥5.0.

Sample/Setting: Randomized double-blind placebo-controlled trial. Rural southern India. Pregnant women with a singleton fetus between 13+0/7 weeks and 20+6/7 weeks. Pregnant women were recruited during prenatal visits in Karnataka, India, from October 2013 to July 2015.

Level of Evidence: I

Quality of Evidence: B</td><td>Women were required to have a singleton fetus between 13+0/7 weeks and 20+6/7 weeks and an elevated vaginal pH (≥5.0) by colorimetric assessment. Participants were randomized to either oral clindamycin 300 mg twice daily for 5 days or an identical-appearing placebo.</td><td>Results: Of the 6476 screened women, 1727 women were randomized (block randomized in groups of six; clindamycin n = 866, placebo n = 861). The demographic, reproductive, and anthropomorphometric characteristics of the study groups were similar. Compliance was high, with over 94% of capsules being taken. The rate of PTB before 37 weeks was comparable between the two groups [clindamycin 115/826 (13.9%) versus placebo 111/806 (13.8%), between-group difference 0.2% (95% CI −3.2 to 3.5%, P = 0.93)], as was PTB at less than 34 weeks [clindamycin 40/826 (4.8%) versus placebo group 37/806 (4.6%), between-group difference 0.3% (95% CI −1.8 to 2.3%, P = 0.81)]. No differences were detected in the incidence of birthweight of<2500 g, <1500 g, miscarriage, stillbirth or neonatal death.

Conclusion: In this setting, oral clindamycin did not decrease PTB among women with vaginal pH ≥5.0.</td><td>Strengths: Randomized control trial. Large cohort size. Use of placebo group.

Limitations: Specific setting. Specific population.</td></tr>
<tr><td colspan="4">Author Recommendations: Oral clindamycin between 13+0/7 and 20+6/7 weeks does not prevent preterm birth in women with a vaginal pH ≥5.0.</td></tr>
<tr><td colspan="4">Summary for current clinical practice question: Further studies are needed. Other methods need to be researched to find effective strategies for prevention of preterm birth.</td></tr>
</table>

Source: Brown, R., Al-Memar, M., Marchesi, J., Lee, Y., Smith, A., Chan, D., Lewis, H., Kindinger, L., Terzidou, V., Bourne, T., Bennett, P., & MacIntyre, D. (2019). Establishment of vaginal microbiota composition in early pregnancy and its association with subsequent preterm prelabor rupture of the fetal membranes. *Translational Research, 207*, 30-43. https://doi.org/10.1016/j.trsl.2018.12.005			
Purpose/ Sample:	**Design (Method Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: A high diversity, Lactobacillus spp. deplete vaginal microbiome is a risk factor for PPROM. It is unknown when in pregnancy this is established. **Sample/Setting:** Preterm surveillance clinics at Queen Charlotte's, St. Mary's, and Chelsea and Westminster hospitals, London (n=535). Early pregnancy unit Queen Charlotte's Hospital, London (n=1003). **Level of evidence:** II **Quality of evidence:** A	Prospective cohort study. Cervicovaginal fluid sample from the vaginal fornix. Repeat samples were taken where possible within the gestational time window of 12-17, 18-23, 24-29, 30-36 weeks of gestation.	A vaginal microbiome with reduced Lactobacillus spp. abundance (<75%) is associated with increased relative risk; 2.56 (1.66–3.88) and 2.34 (1.59–3.42) at 24–29+6 and 30–35+6 weeks. A vaginal microbiome dominated by Lactobacillus spp. > 24 weeks is associated with a decrease in PPROM risk 0.39 (0.26–0.60), RR-0.43 (0.29–0.63) at 24–29+6 and 30–35+6 weeks. A vaginal microbiome dominated by any species other than Lactobacillus is associated with subsequent preterm premature rupture of membranes (PPROM) at all gestational time windows (RR 1.63 (1.27–2.80), 1.28 (1.10–1.47), 1.39 (1.17–1.66), 2.11 (1.52–2.95), 1.8 (1.28–2.52). **Conclusion:** The study revealed that a vaginal microbiome depleted of Lactobacillus spp. is a risk factor for PPROM in roughly 25% of cases, independent of maternal characteristics and preterm birth risk.	**Strengths:** Findings of this study provide implications for future stratification of PTB risk and targeted, preventative interventions, the success of which are highly reliant upon accurate identification of the underlying etiology. **Limitations:** Examination of vaginal microbiota composition across patient groups preceding PPROM was performed using relative abundance comparisons determined by 16S rRNA gene sequencing.
Author Recommendations: Cervicovaginal fluid can be easily sampled. Quick and cost-effective point of care testing to assess Lactobacillus spp. abundance and the presence of pathobionts may be available in the near future.			
Summary for current clinical practice question: Unlike genetic factors, such as antepartum hemorrhage and anatomical abnormalities, an unfavorable vaginal microbiome is a modifiable risk factor for PPROM. Identification of this subset of patients followed by manipulation of bacterial communities through a combination of antibiotic, prebiotic and probiotic therapies warrants further investigation and may represent a promising strategy for the reduction and/or prevention of PPROM and preterm birth.			

Source: Brown, R., Marchesi, J., Lee, Y., Smith, A., Lehne, B., Kindinger, L., Terzidou, V., Holmes, E., Nicholson, J., Bennett, P. & MacIntyre, D. (2018). Vaginal dysbiosis increases risk of preterm fetal membrane rupture, neonatal sepsis and is exacerbated by erythromycin. *BMC Medicine, 16*(9), 1-15. doi:10.1186/s12916-017-0999-x			
Purpose/ Sample:	**Design (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: PPROM precedes 30% of preterm births and is a risk factor for early onset neonatal sepsis. As PPROM is strongly associated with ascending vaginal infection, prophylactic antibiotics are widely used. The evolution of vaginal microbiota compositions associated with PPROM and the impact of antibiotics on bacterial compositions are unknown. **Sample/Setting:** Antenatal clinics of Queen Charlotte's and Chelsea Hospital and Chelsea and Westminster Hospital (n=250). A second cohort (n = 87). **Level of Evidence**: I **Quality of Evidence**: C	Prospective cohort study. Assessment was made of vaginal microbiota prior to and following PPROM using MiSeq-based sequencing of 16S rRNA gene amplicons and examined the impact of erythromycin prophylaxis on bacterial load and community structures.	Vaginal dysbiosis characterized by Lactobacillus spp. depletion was present prior to the rupture of fetal membranes in approximately a third of cases (0% vs. 27%, P = 0.026) and persisted following membrane rupture (31%, P = 0.005). Vaginal dysbiosis was exacerbated by erythromycin treatment (47%, P = 0.00009) particularly in women initially colonized by Lactobacillus spp. Lactobacillus depletion and increased relative abundance of Sneathia spp. were associated with subsequent funisitis and early onset neonatal sepsis. **Conclusion:** The data showed that vaginal microbiota composition is a risk factor for subsequent PPROM and is associated with adverse short-term maternal and neonatal outcomes.	**Strengths:** A unique assessment of vaginal microbiota prior to rupture of fetal membranes and is the largest study of the vaginal microbiota in the context of PPROM to date. **Limitations:** Study size is limited. Given the observational nature of the study, it was not possible to longitudinally sample a cohort of women following PPROM who did not receive erythromycin as part of treatment guidelines. Difficulty was noted in separating the potential temporal impact of membrane rupture on shaping vaginal community structure from the pharmacological effect of erythromycin.
Author Recommendations: This study highlights vaginal microbiota as a potentially modifiable antenatal risk factor for PPROM and suggests that routine use of erythromycin for PPROM be re-examined.			
Summary for current clinical practice question: It is hypothesized that prophylactic erythromycin would lead to a reduction of vaginal bacterial load, diversity, and richness. Treatment was associated with a shift towards vaginal dysbiosis, particularly in women initially colonized predominately by Lactobacillus species. The sub-analysis showed that in women with Lactobacillus spp. dominance, erythromycin exposure was associated with a shift towards a dysbiotic community structure in most cases. Erythromycin treatment was associated with a reduction in both richness and diversity in women with a Lactobacillus spp. depleted vaginal microbiota.			

Source: Burris, H., Riis, V., Schmidt, I, Gerson, K., Brown, A., & Elovitz, M. (2019). Maternal stress, low cervicovaginal B-defensin, and spontaneous preterm birth. *American Journal of Obstetrics & Gynecology, 2*(2). https://doi.org/10.1016/j.ajogmf.2020.100092			
Purpose/ Sample:	**Design (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To determine whether psychosocial stress is associated with a mediator of the immune system in the cervicovaginal space, β-defensin–2, and to examine the combined impact of high stress and low cervicovaginal β-defensin–2 levels on the odds of sPTB. Nested case control study. **Sample/Setting:** The Motherhood and Microbiome (M&M) cohort. Penn Medicine in Philadelphia. 519 women with perceived stress assessments, CV-βD measured, and either a sPTB or a term (≥38 completed weeks of gestation) delivery. **Level of Evidence:** I **Quality of Evidence:** B	Psychosocial stress was assessed using Cohen's Perceived Stress Scale (PSS-14). Analyzed cervicovaginal fluid collected on Dacron swabs between 16 0/7 weeks and 20 0/7 for human β-defensin–2. Bivariate analyses of characteristics among sPTB cases and term controls. Among the term and sPTB births, analyzed CV-βD levels for 430 and 123 women, that were frequency matched by race/ethnicity.	**Results:** Concurrent stress data for 409 women who delivered at term and 110 with sPTB who were included in the final analytic dataset. The majority of women in the study were non-Hispanic black (72.8%), were insured by Medicaid (51.1%), and had PSS-14 scores <30 (80.2%). Counter to the hypothesis, high stress was associated with reduced odds of low CV-βD levels (adjusted odds ratio [aOR], 0.63; 95% confidence interval [CI], 0.38−0.99) (Table 2). The effect estimates between high stress and reduced odds of low CV-βD were similar among women with sPTB (aOR, 0.66; 95% CI, 0.28−1.58) and term birth (aOR, 0.56; 95% CI, 0.56−0.90), interaction P = .41. **Conclusion:** The maternal psychosocial stress was associated with reduced odds of low CV-βD, but the combination of high stress and low CV-βD conferred significantly higher odds of sPTB. CV-βD may serve as a biological resilience factor to protect women from adverse exposures, including stress in pregnancy, and improve chances of a full-term delivery.	**Strengths:** Prospective enrollment of women in pregnancy, careful phenotyping of preterm births as sPTB, and racial/ethnic diversity. Combined psychosocial stress assessments with a biologic biomarker of immune function related to reproductive health. **Limitations:** Potential for residual confounding and reliance on perceived stress as opposed to a biomarker of stress. Positive findings could be due to chance in the setting of multiple testing. Secondary analysis of an already-completed cohort study was performed.
Author Recommendations: Stress can contribute to depression and anxiety, and that pregnant women should be screened for these conditions during the course of prenatal care to improve outcomes.34 It is possible that biomarkers such as CV-βD may eventually serve as additional screening and risk stratification tools for sPTB.			
Summary for current clinical practice question: Although stress and low CV-βD may be independent risk factors additively resulting in higher sPTB risk, it is also possible that stress causes increases in CV-βD in some women who are thus protected from sPTB but not in other women who remain at higher risk. Larger cohorts, combining several risk factors including psychosocial and molecular biomarkers may improve prediction and eventually target interventions to reduce sPTB.			

Source: Elovitz, M., Gajer, P., Riis, V., Brown, A., Humphrys, M., Holm, J. & Ravel, J. (2019). Cervicovaginal microbiota and local immune response modulate the risk of spontaneous preterm delivery. *Nature Communications, 10*(1305),1-8. https://doi.org/10.1038/s41467-019-09285-9			
Purpose/ Sample:	**Designs (Methods Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To accurately identify women at risk for spontaneous preterm birth early in pregnancy and determine therapeutic strategies to reduce this significant health burden. **Sample/Setting**: 2000 women with singleton pregnancies. The population studied was mostly African American with a mean maternal age of 28 years old. Philadelphia, PA. **Level of Evidence:** III **Quality of Evidence**: A	A prospective cohort study. Biospecimens were obtained. Cervicovaginal specimens were self-collected by the participants or collected by a research coordinator at three different prenatal visits: 16-20 weeks, 20-24 weeks, and 24-28 weeks. Subjects were followed to delivery. All delivery outcomes were recorded. Controls were frequency matched by self-reported race to the cases.	Six major cervicovaginal community state types (CSTs) were noted. Four were predominated by either Lactobacillus crispatus (CST I), Lactobacillus gasseri (CST II), Lactobacillus iners (CST III) or Lactobacillus jensenii (CST V), and two (CST IV-A and CST IV-B) comprised a wide array of strict and facultative bacterial anaerobes. The frequency of CSTs was significantly different in African American (AA) and non-African American women. At visit 1, 20% and 45% of AA women were in CST I or CST IVA/IVB, as compared to 50% and ~15% of non-AA women. These differences persisted at visit 2 and visit 3. The frequency of CST III was higher in AA women at visit 3. CST V was consistently lower in AA than in non-AA women throughout the study. **Conclusion:** The study shows that immune factors, such as β-defensin-2, can modulate the risk associated with the lack of Lactobacillus spp., but are also critical even when Lactobacillus spp. are in high relative abundance	**Strengths**: It identified specific signatures combining both immune and microbial factors associated with spontaneous preterm birth. **Limitations:** The clinical significance of any differences or microbial immune correlations cannot be fully interpreted until these biomarkers are validated in another clinical trial outside of this case-control study.
Authors recommendations: The current standards of care and targeting women with a prior spontaneous preterm birth will not significantly decrease the public health burden from spontaneous preterm birth. This study will help lead to prevention strategies. Most importantly, this work will lead to innovative therapeutic opportunities to prevent spontaneous preterm birth including combination of microbiome-based therapeutics and immune modulators earlier in pregnancy.			
Summary for current clinical practice question: The findings address the long-held belief that not having Lactobacillus spp dominated cervicovaginal microbiota is strongly associated with adverse pregnancy outcomes. In pregnancy and non-pregnancy, a larger proportion of African American women compared to non-African American women do not have Lactobacillus spp in high relative abundance in the cervicovaginal microbiota.			

Source: Freitas, A., Bocking, A., Hill, J., & Money, D. (2018). Increased richness and diversity of the vaginal microbiota and spontaneous preterm birth. *BMC, 6*(117), 1-15. https://doi.org/10.1186/s40168-018-0502-8			
Purpose/Sample:	**Designs (Methods Instruments):**	**Results:**	**Strengths/Limitations:**
Purpose: To characterize the vaginal microbiota of pregnant women who had spontaneous preterm birth and compare to those of pregnant women who delivered at term. **Sample/Setting:** Ontario, Canada. Clinics. Term deliveries (n=170), preterm (n=46) **Level of Evidence**: III **Quality of Evidence**: A	Retrospective cohort study. Analyzed vaginal microbiota of women who experienced spontaneous preterm birth. Compared resulting microbial profiles to those of pregnant women who delivered at term. Self-administered vaginal swabs were taken at 16 weeks gestation. Specimens from all cohorts were processed similarly in terms of sample collection, storage, DNA extraction, library preparation and sequencing. Total bacterial DNA (qPCR) and detection of Mollicutes (PCR). Quantitative PCR (qPCR). CPN60 Universal Target (UT)PCR and pyrosequencing. Analysis of operational taxonomic units (OTU). Statistical analysis.	Assessment of alpha diversity revealed that microbiomes of women who delivered preterm were richer (Chao1 richness 46.3 ± 24.1) and more diverse (Shannon diversity index 1.8 ± 1.1) when compared to those of women in the term group (36.2 ± 14.8; 1.2 ± 0.8) (t test, $p < 0.01$). Higher bacterial loads were detected in samples from the preterm group (7.7 ± 0.9) compared to term group (8.0 ± 0.7) (t test, $p = 0.049$). Most microbial profiles from the preterm group (80.5%) were assigned to Lactobacillus-dominated CST: CST I (37% of profiles), CST III (17.4%), CST V (15.2%) and CST II (10.9%). The remaining profiles (19.5%) were assigned to CST IVA, IVC or IVD. **Conclusion:** The results confirm previous reports of an association between Mollicutes and spontaneous preterm birth and further suggest that a more diverse microbiome may be important in the pathogenesis of some cases.	**Strengths:** The overall findings were similar to two other studies, which provided the ability to compare different study designs that addressed the same research question. **Limitations:** The study design included comparison of samples collected in previously published studies and the observational clinical trial (OBS) due to the availability of foundational data on women who delivered at term and the infeasibility of collecting large numbers of samples at 11-16 weeks gestation from women who would go on to deliver pre-term.
Authors Recommendations: Future study should include evaluation of the microbial metabolite production and host response to further elucidate factors leading to spontaneous preterm birth and identify women at risk early in pregnancy.			
Summary for current clinical practice question: This study provides valuable evidence of subtle alterations in the microbiome associated with preterm birth that requires further study utilization sequencing methodology.			

Source: Honda, H., Yokoyama, T., Akimoto, Y., Tanimoto, H., Teramoto, M. & Teramoto, H. (2014). The frequent shift to intermediate flora in preterm delivery cases after abnormal vaginal flora screening. *Scientific Reports, 4*:4799. doi:10.1038/srep04799			
Purpose/ Sample:	**Design (Methods/Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To evaluate whether specific screening reduces the preterm delivery rate for general-population pregnant women. **Sample/Setting:** A total of 1,735 pregnant women, 574 as the Intervention group and 1,161 as the Control group, were analyzed in the present study. The medical records of the pregnant women who delivered at Hiroshima City Asa Hospital. **Level of Evidence**: I **Quality of Evidence**: A	Randomized control study. The pregnant women were divided into two groups: The Intervention group, i.e., the pregnant women who participated in service A, and the Control group, i.e., the pregnant women who participated in service B. For each woman in the Intervention group, a vaginal smear was taken in the second trimester and Gram-stained for the assessment of abnormal flora, as diagnosed by the Nugent scoring system. Nugent scores of 0–3 were graded as normal flora, 4–6 as intermediate flora, and 7–10 as bacterial vaginosis.	In the Intervention group, the frequency of normal flora was 67.4%, that of intermediate flora was 19.0%, and that of bacterial vaginosis was 13.6%. The admission rates for threatened preterm delivery in the Intervention group and Control group were 8.36% and 11.0%, and the mean gestational ages at the admission for threatened preterm delivery of the Intervention and Control groups was 28.1 +/- 5.01 weeks and 30.1 +/- 4.15 weeks. The preterm delivery rates in the Intervention group and Control group were 3.48% and 4.31%. The mean gestational ages at the preterm delivery in the intervention and Control groups were 34.6 +/- 4.15 weeks and 36.2 +/- 0.72 weeks. **Conclusion:** The screening test and the treatment of abnormal vaginal flora in the present study did not contribute to the reduction in the proportion of intermediate flora, or to the admission rate with a threatened preterm delivery, or to the preterm delivery rate.	**Strengths:** There are very few trials in which the pregnant subjects, regardless of their normal or abnormal vaginal flora, were divided into an intervention group and a control group. **Limitations:** The screening test and the treatment of abnormal vaginal did not contribute to the reduction in the proportion of intermediate flora, or to the admission rate with a threatened preterm delivery, or to the preterm delivery rate. Without screening control group no way to know in control group how many had abnormal flora.
Author Recommendations: Increasing evidence indicates that intermediate flora is more closely associated with preterm delivery compared to bacterial vaginosis. The shift of vaginal flora from normal to intermediate flora among the cases of preterm delivery, suggests that the choice of antimicrobial agents and the timing of the screening and the treatment of abnormal vaginal flora in pregnant women should be reconsidered.			
Summary for current clinical practice question: These findings strongly suggest that preterm delivery is associated with intermediate flora rather than bacterial vaginosis.			

Source: Hyman, R., Fukushima, M., Jiang, H., Fung, E., Rand, L., Johnson, B., Vo, K., Caughey, A., Hilton, J., Davis, R., & Guidice, L. (2014). Diversity of the vaginal microbiome correlates with preterm birth. *Reproductive Sciences, 21*(1), 32-40. doi: 10.1177/19337/9113488838			
Purpose/ Sample:	**Design (Methods/Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: Composition of the vaginal microbiome has a significant population-specific impact on PTB risk. Several studies have focused on populations predominantly of European descent. Replicated in a cohort of predominantly African descent. Longtitudinal cohort study. **Sample/Setting:** Community resource samples collected during longitudinally 1,572 pregnancies of women from diverse ancestries, and data generated from samples collected from 597 pregnancies in a collaborative effort under the umbrella of the National Institutes of Health's integrative Human Microbiome Project. Analysis of the longitudinal, comprehensive, multi-omic profiling of vaginal samples from 45 women who experienced spontaneous PTB and 90 case-matched controls, in a cohort of women of predominantly African ancestry. **Level of Evidence:** III **Quality of Evidence:** A	Analyzed 45 single gestation pregnancies that met the criteria for spontaneous PTB (23–36 weeks 6 days of gestational age) and 90 single gestation pregnancies that extended through term (≥39 weeks). The TB controls in the MOMS-PI PTB study were case matched to the PTB group for age, race and annual household income. The earliest samples were collected at 18 weeks of gestation. The respective mean and median gestational age at delivery was 34, 0/7 and 35, 6/7 for the PTB group and 40, 0/7 and 39, 6/7 for the TB group.	**Results:** Women who went on to deliver at term were more likely to exhibit L. crispatus predominance in the vaginal microbiome ($P = 0.014$). L. crispatus was greatly reduced in PTB samples, and several other taxa, including BVAB1, Prevotella cluster 2 and Sneathia amnii, were more abundant in PTB samples ($q < 0.05$). Many taxa identified as associated with PTB: S. amnii ($P = 0.0015$), Prevotella cluster 2 ($P = 0.0031$), BVAB1 ($P = 0.0037$) and P. amnii ($P = 0.0031$). **Conclusion:** Women of African ancestry have a greatly increased risk of PTB compared with women of European ancestry. BVAB1, which is positively associated with PTBs, is more common in women of African ancestry.	**Strengths:** Observed high concordance in the directionality of differences in abundance levels of preterm and term groups. Able to confirm that BVAB1, Megasphaera phylotype 1 and Sneathia species were elevated in a preterm cohort. **Limitations:** Examined only the spontaneous preterm cases. Sample sizes were small, in the range 5–18 spontaneous PTB cases. Not statistically significant, likely due to sample size, cohort characteristics, and differences in experimental design.
Author Recommendations: The findings contribute to an understanding of how microbial markers for PTB vary across populations.			
Summary for current clinical practice question: Further studies are needed to determine whether the signatures of PTB reported in the present study replicate in other cohorts of women of African ancestry. To establish whether population-specific microbial markers can be ultimately integrated into a generalizable spectrum of vaginal microbiome states linked to the risk for PTB.			

Source: Jayaprakash, T., Wagner, E., Schalkwyk, J., Albert, A., Hill, J. & Money, D. (2016). High diversity and variability in the vaginal microbiome in women following preterm premature rupture of membranes (PPROM): A prospective cohort study. *PLoS ONE, 11*(11), e0166794. doi:10.1371/journal.pone.0166794			
Purpose/ Sample:	**Design (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To characterize the vaginal microbiota of women following preterm premature rupture of membranes (PPROM), and determine if microbiome composition predicts latency duration and perinatal outcomes. **Sample/Setting:** Canada. 51 Women with PPROM between 24+0 and 33+6 weeks gestational age (GA). **Level of Evidence**: II **Quality of Evidence**: A	Randomized control trial. Microbiome profiles, based on pyrosequencing of the cpn60 universal target, were generated from vaginal samples at time of presentation with PPROM, weekly thereafter, and at delivery.	Mean GA at PPROM was 28.8 wk (mean latency 2.7 wk). Microbiome profiles were highly diverse but sequences representing Megasphaera type 1 and Prevotella spp. were detected in all vaginal samples. Only 13/70 samples were dominated by Lactobacillus spp. Mycoplasma and/or Ureaplasma were detected by PCR in 81% (29/36) of women, and these women had significantly lower GA at delivery and correspondingly lower birth weight infants than Mycoplasma and/or Ureaplasma negative women. Mean GA at PPROM was 29 weeks; mean GA at delivery was 32 weeks; mean latency period was 18 days. As expected, latency (days) was negatively correlated ($\rho s = -0.390$, $n = 36$, $p = 0.019$) with GA at PPROM because the number of potential latency days decreases with increasing GA at PPROM. Correlation with body mass index (BMI) was noted ($\rho s = -0.511$, $n = 31$, $p = 0.003$). **Conclusion:** Prevotella spp. and Megasphaera type I were ubiquitous. The presence of Mollicutes in the vaginal microbiome was associated with lower GA at delivery. The microbiome was remarkably unstable during the latency period.	**Strengths:** It is an in-depth study that characterizes the microbiome following preterm premature rupture of membranes, providing new insights into the microbial profile of the hard to culture bacterial community in these high risk pregnancies. **Limitations**: Small number of women followed and the lack of pre-rupture samples. The standard use of broad-spectrum antibiotics in the context of PPROM means that the natural changes of the microbiome during latency can no longer be evaluated.
Author Recommendations: Women with PPROM had mixed, highly variable vaginal microbiota but the specific type of microbiome profile at PPROM did not correlate with latency duration. The highly unstable vaginal microbiota of women in this study demonstrates the need for more intense study of the relationship of genital tract microbiota with PPROM.			
Summary for current clinical practice question: Understanding the microbiome associated with PTB and PPROM is critical to creating strategies to prevent this reproductive outcome and to determine when to initiate delivery.			

<table>
<tr><td colspan="4">Source: Kindinger, L., Bennett, P., Lee, Y., Marchesi, J., Smith, A., Cacciatore, S., Holmes, E., Nicholson, J., Teoh, T. & MacIntyre, D. (2017). The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk. BioMed Central, 5(6), 1-14. doi:10.1186/s40168-016-0223-9</td></tr>
<tr><td>Purpose/Sample:</td><td>Design (Methods/Instruments):</td><td>Results:</td><td>Strengths/ Limitations:</td></tr>
<tr><td>Purpose:
To assess the relationship between vaginal microbiota and cervical length (CL) in the second trimester and preterm birth risk.

Sample/Setting: Two tertiary London maternity units. 161 women.

Level of Evidence: II

Quality of Evidence: B</td><td>A cross-sectional cohort study. 16 weeks of gestation, cervico-vaginal fluid was sampled from the posterior fornix under direct visualization. For the duration of the study both units employed a policy of CL screening every 3 weeks until 25 weeks with the indication for the intervention being a CL <25 mm at TVS measured at <23 weeks gestation.</td><td>Lactobacillus iners dominance at 16 weeks of gestation was significantly associated with both a short cervix <25 mm (n = 15, P < 0.05) and preterm birth <34+0 weeks (n = 18; P < 0.01; 69% PPV). Lactobacillus crispatus dominance was highly predictive of term birth (n = 127, 98% PPV).

Conclusion:
L. iners dominance of the vaginal microbiota at 16 weeks of gestation is a risk factor for preterm birth. L. crispatus dominance is protective against preterm birth.</td><td>Strengths: The study showed a relationship between relative abundance of vaginal Lactobacillus species and risk of subsequent preterm birth. The strength was shown by a high spontaneous preterm birth rate (n=34/161). The study allowed for characterization of microbial profiles associated with both early and late preterm birth providing a broader observational base for microbial-host interactions in pregnancy.

Limitations: Limited by the use of denaturing gradient gel electrophoresis (DGGE) for the characterization of only major Lactobacillus species and could not identify other pathobionts in the samples.</td></tr>
<tr><td colspan="4">Author Recommendations: The clinical relevance of the findings is difficult to establish due to the small sample size and the heterogenous nature of the cohort. The protective role of Lactobacillus species in the context of reproductive health shows major species-specific differences in the capacity to prevent pathobiont colonization and viral infections that are driven largely by maternal host-bacterial metabolite interactions at the vaginal mucosa interface.</td></tr>
<tr><td colspan="4">Summary for current clinical practice question: The use of culture-independent characterization of vaginal bacterial communities in a high-risk population, shows that the perceived benefit of lactobacilli dominance in pregnancy is species specific: L. crispatus is advantageous and associated with term delivery whereas L. iners is associated with increased risk of preterm delivery. L. iners is associated with increased risk of preterm delivery, more specifically risk of early delivery <34 weeks rather than late (34-37 weeks) preterm birth. High relative abundance of L. crispatus is highly specific for term birth.</td></tr>
</table>

Source: Myhre, R., Brantsaeter, A., Myking, S., Gjessing, K., Sengpiel, V., Meltzer, H., Haugen, M., & Jacobsen, B. (2011). Intake of probiotic food and risk of spontaneous preterm delivery. *American Journal of Clinical Nutrition, 93*, 151-157. https://doi.org/10.3945/ajcn.110.004085			
Purpose/ Sample:	**Design (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: Preterm delivery represents a substantial problem in perinatal medicine worldwide. Current knowledge on potential influences of probiotics in food on pregnancy complications caused by microbes is limited. Hypothesized that intake of food with probiotics might reduce pregnancy complications caused by pathogenic microorganisms and, through this, reduce the risk of spontaneous preterm delivery. Prospective cohort study. **Sample/Setting**: This study was performed in the Norwegian Mother and Child Cohort **Level of Evidence:** III **Quality of Evidence:** B	Basis of answers to a food-frequency questionnaire. Studied intake of milk-based products containing probiotic lactobacilli and spontaneous preterm delivery by using a prospective cohort study design (n = 950 cases and 17,938 controls) for the pregnancy outcome of spontaneous preterm delivery < 37 gestational weeks. Analyses were adjusted for the covariates of parity, maternal educational level, and physical activity.	**Results:** Pregnancies that resulted in spontaneous preterm delivery were associated with any intake of milk-based probiotic products in an adjusted model [odds ratio (OR): 0.857; 95% CI: 0.741, 0.992]. By categorizing intake into none, low, and high intakes of the milk based probiotic products, a significant association was observed for high intake (OR: 0.820; 95% CI: 0.681, 0.986) **Conclusion:** Women who reported habitual intake of probiotic dairy products had a reduced risk of spontaneous preterm delivery.	**Strengths:** The prospective design with collection of dietary data and the FFQ completed during 17–22 wk. of gestation, before pregnancy delivery to avoid confounding by retrospectively answered questionnaires. The strict and extensive sample inclusion and exclusion criteria make this a very homogenous set of cases and controls. The sample size was large and represents women from all over Norway with diverse dietary habits and a wide range of intake frequencies of probiotic products. **Limitations**: Because the implicated pregnancy conditions in PTD are presumably atypical and subclinical variants of BV, and the biological dynamics of probiotic food intake and effect of maintaining these dynamics under control are unknown, the amount and concentration of probiotic intake needed for an effect is an important aspect. results thus fit a general hypothesis of subsets of sPTD being caused partly by an increased infection or inflammation state representing an increased level of systemic inflammation.
Author Recommendations: Observed a protective effect of intake of probiotic milk products with sPTD.			
Summary for current clinical practice question: The findings are of importance to perinatal care and has the potential to improve current pregnancy health care. Intake of milk products that contain probiotics might influence and reduce pregnancy complications, possibly through an effect of probiotics on vaginal tract infections and a reduction in overall inflammatory state in keeping with a systemic inflammation hypothesis.			

Source: Oh, H., Seo, S., Kong, J., Lee, J., & Kim, M. (2015). Association between obesity and cervical microflora dominated by Lactobacillus iners in Korean women. *Journal of Clinical Microbiology, 53*, doi:10.1128/JCM.01387-15.			
Purpose/ Sample:	Design/ (Methods/ Instruments):	Results:	Strengths/ Limitations:
Purpose: Assessed the association between obesity and the cervical Lactobacillus composition, which has not been examined previously. **Sample/Setting:** Women 18 to 65 years of age who participated in the Korean HPV cohort study, from 2006 to the present, were included in this study. Korea University Guro Hospital. . 76 women randomly enrolled. **Level of Evidence:** III **Quality of Evidence:** B	Longtitudinal cohort study. Pyrosequencing was performed using cervical swabs collected from 76 normal participants with negative results for cervical intraepithelial neoplasia (CIN) and 57 participants with CIN, based on histological examinations. Cluster analysis of nine Lactobacillus spp. was performed, and five cluster types were identified. The association between obesity and the Lactobacillus community was assessed by logistic regression analysis after adjustment for confounding factors.	**Results:** The proportion of Lactobacillus iners increased and that of Lactobacillus crispatus decreased according to body mass index (BMI) categories, i.e., underweight (BMI of <18.5 kg m2), normal weight (BMI of 18.5 to 22.9 kg m2), overweight (BMI of 23.0 to 24.9 kg m2), and obese (BMI of >25 kg m2). The L. iners-dominant type had a significant association with obesity (odds ratio [OR], 7.55 [95% confidence interval [CI], 1.18 to 48.2]), compared to the L. crispatus-dominant type **Conclusion**: Obesity was associated with cervical microflora dominated by L. iners in reproductive-age women without dysplasia.	**Strengths:** This study was the first to demonstrate that the cervical Lactobacillus microflora of obese women differed from that of nonobese Women. **Limitations:** Cross-sectional studies are limited by the fact that they are carried out at one point in time and give no indication of the sequence of events, i.e., whether exposure occurred before, after, or during the onset of the disease outcome (38). Therefore, it is impossible to infer causality between obesity and the Lactobacillus type dominated by L. iners in this study. The small sample size of this study limited the estimation of the association and led to a wide 95% confidence interval.
Author Recommendations: We suggest that obesity may promote the predominance of L. iners in the cervicovaginal ecosystem and that this state may increase the risk of obstetric and neonatal complications related to obesity, such as preterm birth, in Korean women of reproductive age			
Summary for current clinical practice question: More evidence is needed to reveal the causal link between obesity and the composition of the microbiota and to explain the role of the cervicovaginal microbiota in the maintenance of a healthy reproductive tract.			

<table>
<tr><td colspan="4">Source: Petricevic, L., Domig, K., Nierscher, F., Sandhofer, M. Fidesser, M., Krondorfer, I., Husslein, P., Kneifel, W. & Kiss, H. (2014). Characterization of the vaginal Lactobacillus microbiota associated with preterm delivery. Scientific Reports, 4, 5136. doi:10.1038/srep05136</td></tr>
<tr><td>Purpose/
Sample:</td><td>Design
(Methods/
Instruments):</td><td>Results:</td><td>Strengths/
Limitations:</td></tr>
<tr><td>Purpose: To assess the vaginal microbiome throughout full-term uncomplicated pregnancy. To describe if different diversity of vaginal lactobacilli in first trimester of pregnancy could have an influence on pregnancy outcome.

Sample/Setting:
Medical University of Vienna. The study population consisted of 111 women aged 18–40 years with low-risk singleton pregnancies between 11-14 weeks of gestation scheduled to give birth at our department.

Level of Evidence: III

Quality of Evidence: B</td><td>Prospective cohort study. One vaginal smear lateral vaginal wall and the posterior fornix was taken from each participant, transferred to a microscopy slide, Gram-stained and evaluated. Nugent scoring system was used. The smears were transferred to transport medium to obtain the stability of present vaginal microflora.</td><td>44% of women delivered at term and 92% of women who delivered preterm had only one Lactobacillus spp. detectable by PCR (DGGE) and sequencing in their vaginal specimens. 56% women who delivered at term, and 8% who delivered preterm had a combination of 2, or more Lactobacillus spp. Statistically significant difference (p, 0.0009). Comparing mean number of Lactobacillus species detected from pregnant women were observed to be statistically significant difference between term and preterm birth group, too (p, 0.004), (1.8 6 0.9 vs. 1.2 6 0.8).

Conclusion: There is an association between the vaginal presence of a single vaginal Lactobacillus species in late first trimester of pregnancy, mostly L. iners, and preterm delivery. Displacement of lactobacilli from the vagina frequently leads to an abnormal vaginal microflora which in early pregnancy, is a risk factor for PTB and low birth weight. Women with a normal vaginal microbiota in the first trimester have been found to have a 75% lower risk of delivery before 35 weeks of pregnancy than women with an abnormal vaginal microflora.</td><td>Strengths: The mean gestational age among women with preterm birth was 35 weeks, it was assumed that a mechanism other than first trimester vaginal infection—one involving L. iners as a single Lactobacillus species played a role in these preterm deliveries. There was a statistically significant difference between women with term and preterm deliveries.

Limitations: Sample size.</td></tr>
<tr><td colspan="4">Author Recommendations: There is a need for further research and discussion necessary on the influence of lactic acid bacterium in pregnancy. L. iners is the smallest Lactobacillus discovered to date and is a frequently detected bacterial species in the vagina that demands special nutrient requirements.</td></tr>
<tr><td colspan="4">Summary for current clinical practice question: This study suggests that dominating L. iners alone detected in vaginal smears of healthy women in early pregnancy might be associated with preterm delivery.</td></tr>
</table>

Source: Romero, R., Hassan, S., Gajer, P., Tarca, A., Fadrosh, D., Nikita, L., Galuppi, M.., Lamont, R., Chaemsaithong, P., Miranda, J., Chaiworapongsa, T. & Ravel, J. (2014). The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome, 2*(4), 1-19. http://www.microbiomejournal.com/content/2/1/10			
Purpose/ Sample:	**Design (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To characterize the vaginal microbiota throughout normal human pregnancy using sequence-based techniques. **Sample/Setting:** Non-pregnant women (n = 32) and pregnant women who delivered at term (38 to 42 weeks) without complications (n = 22). Obstetric clinic. Detroit, Michigan. **Level of Evidence**: I **Quality of Evidence**: A	A retrospective case–control longitudinal study. Sample of vaginal fluid was collected under direct visualization from the posterior vaginal fornix by an obstetrician or a midwife using a Dacron swab. Samples were collected every 4 weeks until 24 weeks of gestation, and every 2 weeks until the last prenatal visit. Non-pregnant patients were self-collected sampled twice weekly for 16 weeks using validated methods previously described. All samples were Gram-stained and analyzed using the Nugent score.	The mean within-subject log Jensen-Shannon distance of pregnant women was significantly lower than that for non-pregnant women (difference in means -0.473 log units; that is, 1.6-fold lower Jensen-Shannon distance, $P < 0.001$). Evaluation was made of the ability of a community to shift to CST IV (A or B) by computing the Jensen-Shannon distance between each community state and the mean community state of all samples assigned to CST IV-A and CST IV-B. Jensen-Shannon distances using a GEE model of pregnant women was significantly higher than that for non-pregnant women (difference in means 0.13 log units; that is, 1.14-fold, $P < 0.001$). These results indicate that bacterial communities in pregnancy do shift from one CST dominated by Lactobacillus spp. to another CST dominated by Lactobacillus spp., but rarely to CST IV-A or CST IV-B. **Conclusion:** Differences in the composition and stability of the microbial community between pregnant and non-pregnant women were observed.	**Strengths:** Longitudinal nature of the study. Frequent sampling protocol. Quality of the sequence-based techniques (16S rRNA). Analytical methods used. Inclusion of relevant clinical groups: non-pregnant and normal pregnant women. **Limitations:** The use of primer 27 F could be a limitation of this study; this primer may have underestimated the true relative abundance of 16S rRNA genes of Bifidobacteriaceae in general, and those of the genus G. vaginalis, a bacterium commonly found in the vagina of women who experience bacterial vaginosis.
Author Recommendations: Despite the apparently limited sample size, the identification of significant differences provides evidence that the study of the vaginal microbiota during pregnancy can yield important insights into the relationship between the structure and dynamics of microbial communities and pregnancy outcome. Further studies are required to confirm these findings, extend the observations, and elucidate the role of microorganisms in adverse pregnancy outcome.			
Summary for current clinical practice question: Baseline stability patterns of the vaginal microbiota in pregnancy were established. This could serve as the basis to study the relationship between the vaginal microbiota and adverse pregnancy outcomes. The characterization of the vaginal microbiota in pregnancy has the potential to yield information of prognostic, diagnostic, and therapeutic value.			

Source: Romero, R., Hassan, S., Gajer, P., Tarca, A., Fadrosh, D., Bieda, J., Chaemsaithong, P., Miranda, J., Chaiworapongsa, T., & Ravel, J. (2014). The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at birth. *BioMed Central, 2*(18), 1-15. http://www.microbiomejournal.com/content/2/1/18			
Purpose/ Sample:	**Designs (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To determine whether the vaginal microbiota of pregnant women who subsequently had a spontaneous preterm delivery is different from that of women who had a term delivery. **Sample/Setting**: The study included 18 cases and 72 controls. Obstetric clinic. Detroit, Michigan. **Level of Evidence**: I **Quality of Evidence**: B	Nested case–control study. Cases and controls were selected in a 1:4 ratio. Speculum examination at each visit; a sample of vaginal fluid was collected under direct visualization from the posterior vaginal fornix. Collection every 4 weeks until 24 weeks of gestation, and then every 2 weeks until the last prenatal visit. A comparison of microbial diversity (Shannon Diversity Index; SDI) was used. LME model was used. The SDI values were log-transformed to improve normality of the data.	Two of the CSTs that were most often dominated were L. crispatus (CST I) and L. iners (CST III). Communities that clustered in CST IV-B lacked a substantial number of Lactobacillus spp. and had higher relative abundance of G. vaginalis, BVAB1, A. vaginae and Megasphaera spp. type 1. Frequencies of CST I, CST III and CST IV-B in the entire sample set were 18.6%, 58.5% and 22.9%. There were no differences in the frequency of the different CSTs (CST I, III, IV-B) between women who delivered at term and those who delivered preterm (CST I: 18.4% versus 19.6%; CST III: 59.4% versus 53.6%; CST IV-B: 22.2% versus 26.8%). **Conclusion**: The relative abundance of four Lactobacillus spp. (L. crispatus, L. jensenii, L. gasseri and L. vaginalis) increased as a function of gestational age. The mean relative abundance in the third interval was higher than in the first interval of gestation. The relative abundance of eleven other bacterial taxa were found to decrease with advancing gestational age.	**Strengths**: The longitudinal nature. The quality of the sequence-based techniques (16S rRNA gene) which decreased bias over other methods. The use of analytical and statistical methods specifically designed for the analysis of longitudinal studies. **Limitations:** Sample size. 16S rRNA gene sequence-based techniques were used. A 16S rRNA gene-based survey is a powerful tool to but this approach provides limited information about the function and role of the vaginal microbial community in health and disease.
Authors Recommendations: The composition of the vaginal microbiota during normal pregnancy changed as a function of gestational age, with an increase in the relative abundance of four Lactobacillus spp. and decreased in anaerobe or strict-anaerobe microbial species as pregnancy progressed. Additional studies on the changes in the vaginal microbiome and spontaneous preterm birth are needed.			
Summary for current clinical practice question: The study observations are relevant to understanding the changes in the vaginal ecosystem with normal pregnancy. Moreover, it is possible that these temporal changes may be meaningful in assessing health and predisposition to disease states.			

Source: Stafford, G., Parker, J., Amabebe, E., Kistler, J., Reynolds, S., Stern, V., Paley, M. & Anumba, D. (2017). Spontaneous preterm birth is associated with differential expression of vaginal metabolites by Lactobacilli-dominated microflora. *Frontiers in Physiology, 8*(615), 1-15. doi:103389/fphys.2017.00615			
Purpose/ Sample:	**Design (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To examine the differences in vaginal microbiota and metabolite profiles of women who delivered prematurely compared to their term counterparts. **Sample/Setting:** Antenatal clinics and the labor ward assessment unit of the Jessop Wing Maternity Hospital, Sheffield, UK. Asymptomatic (studied at 20–22, n = 80; and 26–28 weeks, n = 41) and symptomatic women (studied at 24–36 weeks, n = 37). **Level of Evidence**: II **Quality of Evidence**: A	Prospective pilot study. Two high vaginal swab (HVS) samples from the posterior vaginal fornix with dry polystyrene Dacron swabs were collected. 16S rRNA sequencing, the vaginal microbiota from cervicovaginal fluid samples was characterized into five Community State Types (CST) dominated by Lactobacillus spp.: CSTI (Lactobacillus crispatus), CSTII (Lactobacillus gasseri), CSTIII (Lactobacillus iners), CSTV (Lactobacillus jensenii); and mixed anaerobes—CSTIV. This was then related to the vaginal metabolite profile and pH determined by 1H-Nuclear Magnetic Resonance spectroscopy and pH indicator paper.	Observation was made of a greater proportion (>2-fold) of CSTI (L. crispatus) dominated microbiota present in the term than the preterm groups at 20–22 weeks (40.32 vs. 16.66%, Fisher's exact test, P = 0.0002) and a slightly higher proportion in the 26–28 weeks group (20.69 vs. 16.66%, P = 0.03. The proportion of patients' vaginal samples that were categorized as CSTV (L. jensenii dominant) was more than 2-fold lower in the term than preterm groups at 20–22 (9.68 and 22.22%, P = 0.0002) and 26–28 weeks groups (10.34 and 25%, P = 0.03). At 26–28 weeks none of the preterm patient samples were dominated by CSTII (L. gasseri) in contrast to 28% of term patients (P < 0.0001). When the Laud and Dane method was used, these data were supported at 26–28 weeks, with the lack of CSTII in the patients in the preterm group, P = 0.03. There was a trend toward a higher proportion of CSTI in the term women compared to their preterm counterparts (P = 0.06). Using both statistical methods, there appears to be a link between CSTI—L. crispatus and L. gasseri and health. **Conclusion:** The data shows benefits of the presence of an L. crispatus/gasseri-dominated microbiota (CSTI/II) over other lactobacilli, particularly the potentially detrimental L. jensenii (CSTV).	**Strengths:** 16S rRNA gene sequencing is a powerful tool to establish the composition of the microbial community of clinical niches in relation to health and disease and to identify Frontiers in Physiology and differential expression of vaginal metabolites potentially associated organisms. **Limitations**: They did not study any control group of low-risk women, matched for gestation, with no symptoms of PTB.
Author Recommendations: The findings do raise the possibility that further study of the CST Lactobacilli spp. is warranted.			
Summary for current clinical practice question: Important links between microbial community state-types and targeted metabolite profiles in relation to PTB, highlight the potential functional and clinical significance of combining these determinations to improve our understanding of the mechanisms of inflammation associated PTB.			

Source: Stout, M., Zhou, Y., Wylie, K., Tarr, P., Macones, G. & Tuuli, M. (2017). Early pregnancy vaginal microbiome trends and preterm birth. *American Journal of Obstetrics & Gynecology, 356*, e1-e18. http://dx.doi.org/10.1016/j.ajog.2017.05.030			
Purpose/Sample:	**Designs (Methods/Instruments):**	**Results:**	**Strength/ Limitations:**
Purpose: To characterize vaginal microbial community characteristics in a large, predominantly African American, pregnant women and test whether particular vaginal microbial community characteristics are associated with the risk for subsequent preterm birth. **Sample/Setting:** Predominantly (77) African American pregnant women receiving prenatal care at a single tertiary care institution. Washington, U.S. **Level of Evidence**: II **Quality of Evidence**: A	Nested case-control study within a prospective cohort study. Serial mid-vaginal swabs were obtained by speculum exam at their routine prenatal visits. Sequencing of the V1V3 region of the 16S rRNA gene was performed. Alpha diversity community characteristics were compared longitudinally in women who delivered preterm to those who delivered at term.	Women who delivered at term had a vaginal community richness and Shannon diversity that remained stable (P = .14 and P = .07), and Pielou's evenness decreased modestly (P = .04). In women who subsequently delivered preterm, richness (P < .001), Shannon diversity (P < .001), and Pielou's evenness (P < .001) decreased significantly over pregnancy. **Conclusion:** In a predominantly African American population, a significant decrease of vaginal microbial community richness and diversity is associated with preterm birth. The timing of this suppression appears early in pregnancy, between the first and second trimesters.	**Strengths:** The cohort's high proportion of preterm births adds to the literature on the association between changes in the vaginal microbial community and preterm birth. The high proportion of African American women is a unique and informative feature of this studies analysis. **Limitations:** Differences between groups reflect associations and are not necessarily causation. The sample size was too small to allow for reliable statistical comparisons among non-African American women. The study obtained the fewest samples from women in their first trimester.
Authors Recommendations: This cohort study shows that a significant decrease in community richness and diversity and less stability of the vaginal microbiome is associated with preterm birth and is increased in African American women. Future studies should focus on the first- to second-trimester microbial changes, well in advance of the outcome of interest.			
Summary for current clinical practice question: This study of predominantly African American pregnant women shows that a significant decrease in community richness and diversity and less stability of the vaginal microbiome is associated with their increased incidence of preterm birth. Future studies should focus on the first- to second-trimester microbial changes, well in advance of the outcome of interest.			

Source: Tabatabaei, N., Eren, A., Barreiro, L., Yotova, V., Dumaine, A., Allard, C., & Fraser, W. (2018). Vaginal microbiome in early pregnancy and subsequent risk of spontaneous preterm birth: a case-control study. *An International Journal of Obstetrics and Gynaecology, 126*(3), 1-11. https://doi.org/10.1111/1471-0528.15300			
Purpose/Sample:	**Designs (Methods Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To explore differences in the vaginal microbiome between preterm and term deliveries. **Sample/Setting**: Included singleton pregnant women (n=2366) recruited in nine Quebec, Canada hospitals. **Level of Evidence**: II **Quality of Evidence**: B	Nested case-control study in 3D cohort. Sequencing the V4 region of the 16S ribosomal RNA (rRNA). Gene swabs self-collected during early pregnancy. Two vaginal swabs were self-collected in first trimester. Nugent score was used. DNA extraction of the vaginal swabs. Sequencing of barcoded 16S rRNA gene amplicons. Bioinformatic analyses. Clustering of bacterial communities into community state types.	Two of the CSTs were most often dominated by L. crispatus (CST I) and L. iners (CST III). Communities that clustered in CST IV-B lacked a substantial number of Lactobacillus spp. and had higher relative abundance of G. vaginalis, BVAB1, A. vaginae and Megasphaera spp. type 1. Frequencies of CST I, CST III and CST IV-B in the entire sample set were 18.6%, 58.5% and 22.9%, There were no differences in the frequency of the different CSTs (CST I, III, IV-B) between women who delivered at term and those who delivered preterm (CST I: 18.4% versus 19.6%; CST III: 59.4% versus 53.6%; CST IV-B: 22.2% versus 26.8%). **Conclusion**: L. gasseri/L. hohnsonii, L. crispatus/L. acidophilus, L. iners IR solanacearum and B. longum/B. breve are associated with decreased risk of early but not late spontaneous preterm birth. High diversity of BV-associated bacteria (G. vaginalis, A. vaginae and Veillonellaceae bacterium) is associated with an increased risk of early but not late spontaneous.	**Strengths**: Larger sample size than other studies. One of the strengths of the study is the high power for detection of differences compared with previous studies. **Limitations:** The V1-V3 region of the bacterial 16S rRNA versus V4 is commonly used to assess Lactobacillus community composition. The V4 variable region of the 16S rRNA gene provides strong discrimination between most bacterial species. Additional computational methods such as oligotyping may be needed to precisely identify certain species, such as L. crispatus. Selection of the V4 region of 16S rRNA may limit the comparability of these results to studies using other regions.
Authors Recommendations: Further studies exploring the association between the vaginal microbiome across pregnancy and risk of spontaneous preterm birth are recommended while considering the immunology of the host.			
Summary for current clinical practice question: Bifidobacterium are mainly abundant in the intestinal tract but are also detected in the vaginal tract. Meta-analyses have not observed any association between consumption of Bifidobacterium probiotics during pregnancy and gestational age. The observed protective association between Bifidobacterium and early preterm birth requires further research.			

Source: Verstraelen, H., Verhelst, R., Claeys, G., DeBacker, E., Temmerman, M. & Vaneechouette, M. (2009). Longitudinal analysis of the vaginal microflora in pregnancy suggests that L. crispatus promotes the stability of the normal vaginal microflora and that L. gasseri and/or L. iners are more conducive to the occurrence of abnormal vaginal microflora. *BMC Microbiology, 9*(116), 1-10. doi:10.1186/1471-2180-9-116

Purpose/ Sample:	**Design (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To determine to what extent individual differences in vaginal lactobacillus community composition determine the stability of microflora. **Sample/Setting**: 100 women. Outpatient obstetric clinic of the Ghent University Hospital. Belgium. **Level of Evidence**: III **Quality of Evidence**: B	Prospective cohort study. A cotton-tipped wooden vaginal swab was rolled against the lateral vaginal walls, the air-dried vaginal smear was then Gram-stained. A second, sterile cotton-tipped wooden swab was rolled against the lateral vaginal walls and placed in a sterile polypropylene tube for transport. A third swab was obtained in a similar manner and placed into Amies transport medium for anaerobic culture. Gram-stained vaginal smears were categorized as grade I-IV.	Based on Gram stain, 77 women had normal or Lactobacillus-dominated vaginal microflora (VMF) during the first trimester, of which 18 had grade Ia (L. crispatus cell morphotypes) VMF (23.4%), 16 grade Iab (L. crispatus and other Lactobacillus cell morphotypes) VMF (20.8%), and 43 grade Ib (non-L. crispatus cell morphotypes) VMF (55.8%). Thirteen women with normal VMF at baseline, converted in the second or third trimester (16.9%) to abnormal VMF defined as VMF dominated by non-Lactobacillus bacteria. Compared to grade Ia and grade Iab VMF, grade Ib VMF were 10 times (RR = 9.49, 95% CI 1.30 – 69.40) more likely to convert from = 0.009). This was explained by the observation that normal VMF comprising L. gasseri/iners incurred a ten-fold increased risk of conversion to abnormal VMF relative to non-L. gasseri/iners VMF (RR 10.41, 95% CI 1.39–78.12, p = 0.008), whereas normal VMF comprising L. crispatus had a five-fold decreased risk of conversion to abnormal VMF relative to non-L. crispatus VMF (RR 0.20, 95% CI 0.05–0.89, p = 0.04). **Conclusion:** The presence of different Lactobacillus species within the normal vaginal microflora is a major determinant to the stability of this microflora in pregnancy.	**Strengths:** As the study was confined to genotypic characterization of the microflora, it remained to be determined which phenotypic attributes of the different Lactobacillus species explain the observed associations. **Limitations**: Sample size was small. The interval between subsequent sampling occasions was rather large with an average of some 3 months interval time.

Author Recommendations: These observations showed a vast disease burden associated with depleted lactobacilli and bacterial vaginosis. Half of women actually have a microflora characterized by the poorer colonizers and defenders L. gasseri and L. iners. It may be inferred that in a substantial proportion of women lactobacilli-driven antimicrobial defense of the lower female genital tract is actually less optimal than can be assumed by the mere presence of lactobacilli.

Summary for current clinical practice question: L. crispatus is associated with a particularly stable vaginal ecosystem. Microflora comprising L. jensenii elicits intermediate stability, while VMF comprising L. gasseri/L. iners is the least stable.

Source: Walther-Antonio, M., Jeraldo, P., Miller, M., Yeoman, C., Nelson, K., Wilson, B., White, G., Chia, W., & Creedon, D. (2014). Pregnancy's stronghold on the vaginal microbiome. *PLOS ONE, 9*(6), 1-10.			
Purpose/ Sample:	**Design (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations:**
Purpose: To assess the vaginal microbiome throughout full-term uncomplicated pregnancy. **Sample/Setting**: 12 subjects enrolled at the Obstetric Division, Mayo Clinic, Rochester, MN. Caucasian and AA women. **Level of Evidence**: III **Quality of Evidence**: B	Prospective cohort study. Vaginal swabs were obtained from twelve pregnant women at 8-week intervals throughout their uncomplicated pregnancies. Swabs were obtained from the posterior fornix and cervix at 8–12, 17–21, 27–31, and 36–38 weeks of gestation. The microbial community was profiled using hypervariable tag sequencing of the V3–V5 region of the 16S rRNA gene, producing approximately 8 million reads on the Illumina MiSeq. Principal component analysis (PCA) was used. Shannon's diversity was used.	Two species dominated the microbial content (.1% representation) of samples from the entire cohort. The identified species were L. crispatus and L. iners. Among the 12 patients there were 3 profiles that could be distinguished. Eight of the subjects showed a high prevalence (.90%) of L. crispatus throughout pregnancy. Two of the subjects showed a prevalence of L. iners (92–61%); and the remaining 2 subjects showed a transition in dominance after the first trimester of gestation from L. crispatus (70%) to L. iners (52–57%). **Conclusion:** Normal pregnancy is characterized by a microbiome that has low diversity and high stability. Lactobacillus species strongly dominate the vaginal environment during pregnancy across the two studied ethnicities, observed differences between the longitudinal dynamics of the analyzed populations may contribute to divergent risk for pregnancy complications. This helps establish a baseline for investigating the role of the microbiome in complications of pregnancy such as preterm labor and preterm delivery.	**Strengths:** Some technical challenges were present in the study, but important conclusions could be inferred from the results. **Limitations**: Small sample size and homogeneous population. Larger number of Caucasian women were in the study than AA women. Differences in hypervariable 16S rRNA regions amplified (V1-V2 in Romero's dataset and V3-V5 in our study) and sequencing platforms used.
Author Recommendations: Examination of the microbial community dynamics using principal coordinate analysis reveals that Caucasian women cluster by trimester towards a common attractor, suggesting that these subjects share a common microbiome dynamic. On the other hand, African American women cluster by subject and do not show a common attractor.			
Summary for current clinical practice question: Speculation is made that these differences in microbial dynamics may underlie the increased risk of pregnancy complications in particular individuals in the African American population. Due to the multitude of other factors that may also vary across these two populations, it is difficult to isolate the cause. Further research is indicated.			

<table>
<tr><td colspan="4">Source: Wen, A., Srinivasan, U., Goldberg, D., Owen, J., Marrs, C., Misra, D., Wing, D., Ponnaluvi, S., Miles-Jay, A., Bucholz, B., Abbas, K. & Foxman, B. (2013). Selected vaginal bacteria and risk of preterm birth: An ecological perspective. The Journal of Infectious Diseases, 209, 1087-1094. doi://10.1093/infdis/jit632</td></tr>
<tr><td>Purpose/
Sample:</td><td>Design
(Methods/
Instruments):</td><td>Results:</td><td>Strengths/
Limitations:</td></tr>
<tr><td>Purpose: The study examined the community ecology of vaginal microbial samples taken from pregnant women with previous preterm birth experience to investigate whether targeted pathogenic and commensal bacteria are related to risk of preterm birth in the current pregnancy.

Sample/Setting: 374 pregnant women carrying a singleton gestation and who had at least one prior spontaneous preterm birth between 17- and 33-weeks' gestation. Obstetrical clinics, Birmingham, Alabama.

Level of Evidence: I

Quality of Evidence: A</td><td>Nested case control within a prospective study. Cervical length evaluation between 16 weeks 0 days to 21 weeks 6 days gestation. Sterile speculum examination was performed to collect vaginal fluid from the upper one-third of the vaginal sidewalls for pH and Gram stain. Bacterial DNA was extracted from the Gram stain slides. Preamplification with 8F1492R was used, universal bacterial primers based on 16S rDNA was used.</td><td>African American participants had BMI that was significantly correlated with the microbial community. Following subtraction of the effect of all the constraining factors, the correlation (P = .005) between microbial community and birth outcome persisted. An odds ratio (OR) analysis showed that the presence of Mycoplasma significantly increases the chance of preterm birth (OR = 5.70 [2.40, 14.4], P < .001) whereas BVAB3 drastically decreases the risk of preterm birth (OR = 0.13 [0.036, 0.38], P ≤ .001). Among Hispanic participants results of the CCA II showed that Mycoplasma is still strongly correlated with preterm birth. (OR =4.45 [1.69, 11.97], P < .01), but the negative association with BVAB3 was only marginally significant, although the association was in the same direction (OR = 0.19 [0.0076,1.01], P = .068).

Conclusion: Vaginal bacterial community in the second trimester was correlated with birth outcome, with the correlation being dependent upon the race/ethnicity of the mother.</td><td>Strengths: This study, consistent with recent studies on racial/ethnic groups and bacterial communities, reiterates that including diverse populations of pregnant women is critical for understanding the etiology of infection-associated preterm birth, because the microbial community exhibited strong structural differences among racial/ethnic groups.

Limitations: They could only study the association of specific bacteria and preterm birth.</td></tr>
<tr><td colspan="4">Author Recommendations: The discovery of an apparent negative association between BVAB3 and PTB was unexpected and needs further evaluation. Its effect in preterm birth had not been evaluated in published literature previous to this study.</td></tr>
<tr><td colspan="4">Summary for current clinical practice question: Findings from this study affirm the necessity of considering women's race/ethnicity when evaluating the correlation between vaginal bacteria and preterm birth. The study also illustrates the importance of studying the vaginal microbiota from an ecological perspective and demonstrates the power of ecological community analysis to improve understanding of infectious disease.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Wylie, K., Wylie, T., Cahill, A., Macones, G., Tuuli, M., & Stout, M. (2018). The vaginal eukaryotic DNA virome and preterm birth. American Journal of Obstetrics & Gynecology, 189, e1-e12. https://doi.org/10.1016/j.ajog.2018.04.048</td></tr>
<tr><td>Purpose/
Sample:</td><td>Designs
(Methods/
Instruments):</td><td>Results:</td><td>Strengths/
Limitations:</td></tr>
<tr><td>Purpose: To examine associations between vaginal community characteristics and preterm birth.

Sample/Setting: 60 pregnant women receiving prenatal care at a single tertiary care center. Washington, U.S.

Level of Evidence: III

Quality of Evidence: A</td><td>A nested case-control study within a prospective longitudinal cohort. Three swabs of vaginal secretions were taken from the posterior wall of the vaginal fornix at an outpatient service. Two of the swabs were applied onto a slide for determination of vaginal pH and vaginal infection status. The remaining vaginal swab was covered, placed on ice, and used for bacterial genomic DNA extraction. Cleanliness was graded I-IV. The use of ViroCap targeted sequence.</td><td>24 patients delivered preterm. Participants were predominantly African American (65%). Six families of eukaryotic DNA viruses were detected in the vaginal samples. At least 1 virus was detected in 80% of women. No specific virus or group of viruses was associated with preterm delivery. Higher viral richness was significantly associated with preterm delivery in the full group and in the African American subgroup (P = .0005 and P = .0003, respectively). Having both high bacterial diversity and high viral diversity in the first trimester was associated with the highest risk for preterm birth.

Conclusion: Higher vaginal viral diversity is associated with preterm birth. Changes in vaginal virome diversity appear similar to changes in the vaginal bacterial microbiome over pregnancy.</td><td>Strengths: The ability to integrate bacterial community characteristics with viral community characteristics in the same pregnant patients. Majority African American population, allowing for analysis of the full cohort as well as the subgroup of African American patients.

Limitations: Fewest number of swabs from this time point. DNA sequencing detects viral genomes but does not distinguish viral exposure from active replication.</td></tr>
<tr><td colspan="4">Author Recommendations: The study raises the possibility that the physiology driving the changes in the vaginal communities over pregnancy may impact both bacterial and viral communities similarly, a hypothesis that could be tested in future studies. The first trimester appears to have the highest magnitude of difference in viral diversity between term and preterm birth patients. The first trimester time point could be clinically useful to identify women at risk or not at risk for preterm birth and allow enough lead time to individualize surveillance and treatment plans.</td></tr>
<tr><td colspan="4">Summary for current clinical practice question: The findings of this and other studies suggest the interplay between bacteria and eukaryotic DNA viruses is important. The interplay of bacterial and viral communities and maternal inflammation may be a mechanism by which preterm birth is triggered. These microbial community features may not be causal but instead be potential biomarkers of a common underlying physiology in women at risk for preterm birth</td></tr>
</table>

Source: Zheng, N., Guo, R., Yao, Y., Jin, M., Cheng, Y. & Ling, Z. (2019). Lactobacillus iners is associated with vaginal dysbiosis in healthy pregnant women: A preliminary study. *BioMed Research International, 2019*, 1-9. https://doi.org/10.1155/2019/6079734			
Purpose/ Sample:	**Designs (Methods/ Instruments):**	**Results:**	**Strengths/ Limitations**:
Purpose: To investigate the vaginal microbiota in the first, second, and third pregnancy trimester in healthy pregnant women using a cultivation-independent approach that may help to prevent vaginal infections during pregnancy and reduce preterm delivery and PROM. **Sample/Setting**: 83 healthy pregnant women. Department of Obstetrics of Tongde Hospital in Zhejiang Province (Zhejiang, China). **Level of Evidence**: I **Quality of Evidence**: B	Cross-sectional cohort study. Three swabs of vaginal secretions were taken from the posterior wall of the vaginal fornix at an outpatient service. Two of the swabs were applied onto a slide for determination of vaginal pH and vaginal infection status. The remaining vaginal swab was covered, placed on ice, and used for bacterial genomic DNA extraction. Cleanliness was graded I-IV. qRT-PCR was used. N=33 first trimester, N=24 second trimester, N=26 third trimester.	Seven known abundant genera (Lactobacillus, Gardnerella, Atopobium, Megasphaera, Eggerthella, Leptotrichia/Sneathia, and Prevotella) were analyzed. The abundance of Gardnerella, Atopobium, Megasphaera, Eggerthella, Leptotrichia/Sneathia, and Prevotella was significantly different among the three trimesters ($p > 0.05$). The genus Lactobacillus constituted the major proportion of the vaginal microbiota in healthy pregnant women. L. jensenii, L. iners, and L. crispatus were the most frequent species. Among them, the abundance of L. iners and L. crispatus was significantly different among the trimesters. It was found that L. iners decreased significantly in women in the second and third trimester when compared with women in the first trimester ($p < 0.001$), while L. crispatus significantly increased in the second trimester ($p=0.030$). **Conclusion**: L. iners was found to be significantly decreased in the second and third trimester compared with the first trimester, while L. crispatus increased only in the second trimester. It was found that L. iners may be highly associated with vaginal dysbiosis.	**Strengths:** The Lactobacillus species, such as L. iners and L. crispatus, maintained the balance of the vaginal ecosystem. **Limitations**: The limitation of this study is small sample capacity and non-longitudinal design. Number of PTB not listed.
Authors Recommendations: The depletion of lactobacilli, together with the increase of different species of anaerobes, could result in the switch from normal to a dysbiosis vaginal microbiota, which contributes to various adverse outcomes.			
Summary for current clinical practice question: The findings could have important implication when interpreting the varied results of investigations aimed at improving pregnancy outcomes. These data support the observation that the prevalence of vaginal microbiota varies significantly over the course of pregnancy, with a strong trend towards a reduction in infection by the third trimester.			