
140
480
THS

This is to certify that the
thesis entitled

Bacterial vaginosis,
vaginal fluid defensins, and preterm delivery

presented by

Jia Xu

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Epidemiology

Claudia Holzman

Major Professor's Signature

August 21, 2007

Date

BACTERIAL VAGINOSIS, VAGINAL FLUID DEFENSINS, AND PRETERM DELIVERY

By

Jia Xu

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree for

MASTER OF SCIENCE

Department of Epidemiology

2007

ABSTRACT

BACTERIAL VAGINOSIS,
VAGINAL FLUID DEFENSINS, AND PRETERM DELIVERY

By

Jia Xu

Bacterial vaginosis (BV) has been implicated in preterm delivery (PTD). Defensins, peptides produced by neutrophils, have a wide antimicrobial spectrum and may play a role in vaginal innate immunity. This study explored the relations between BV, vaginal fluid defensins, and subsequent PTD subtypes. Nugent Gram stain scoring for BV and defensin measurements were performed on vaginal fluid specimens obtained at 15 to 27 weeks of gestation from Pregnancy Outcomes and Community Health (POUCH) Study subcohort women [569 non-Hispanic White (Whites), 462 African American (Af-AM) women]. Race/ethnic-specific polytomous logistic regression models, weighted for the subcohort sampling scheme, were used to estimate adjusted odds ratio (AOR) of PTD subtypes with term delivery (>=37 weeks) as the referent. The relations between mid-pregnancy vaginal defensin levels, bacterial vaginosis, and pregnancy outcome were not consistent across racial/ethnical groups. Among Af-AM, vaginal defensin levels above the median were associated with intermediate and positive bacterial vaginosis ($p<0.05$) and with increased risk of PTD initiated by preterm labor (AOR=2.2, 95% CI 1.1-4.6) or premature rupture of membrane (AOR=2.7, 95% CI 0.9-7.6). Once African American women were identified as having vaginal defensin levels above the median, additional information on bacterial vaginosis added nothing to predicting risk of PTL/PROM PTD. By contrast none of the above associations held true for non-Hispanic whites.

ACKNOWLEDGEMENTS

I am indebted to my advisor, Dr. Claudia Holzman for her expert guidance and mentorship, and for her encouragement and support at all levels. Her exemplary character and technical insights have made my graduate study and research fruitful and enjoyable. I feel extremely fortunate to have her as my advisor.

I am deeply grateful to my committee members Dr. Cindy Arvidson and Dr. Hwan Chung for providing valuable advice, revising my thesis draft, understanding, and accommodating my last minute-needs all along the way.

POUCH has always been a friendly and stimulating research and work environment. I want to express my sincere gratitude to the whole POUCH team for being a constant source of friendship, encouragement, and support from the moment I arrived at MSU. I have greatly benefited from working in POUCH.

Last but not the least, I want to thank my family. I would have never reached here without the education and support from my parents.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATION

Abbreviation	**Meaning**
BV	Bacterial vaginosis
PTD	Preterm delivery
POUCH	Pregnancy Outcomes and Community Health Study
Whites	Non-Hispanic White
Af-AM	African American
AOR	Adjusted Odds Ratio
CI	Confidence interval
PTL	Preterm labor
PROM	Premature rupture of membranes
BMI	Body mass index
WBC	White blood cell
HNP	Human neutrophil peptides
HD	Human defensins
HBD	Human β-defensins
MIAC	Microbial invasion of the amniotic cavity
MSAFP	Maternal serum alpha-fetoprotein
HIPAA	Health Insurance Portability and Accountability Act
MoM	Multiples of the median
fFN	Fetal fibronectin
GA	Gestational age
LMP	Last menstrual period
US	Ultrasound

Literature review

Preterm delivery (PTD) refers to the delivery prior to 37 completed weeks of gestation. It is a leading cause of infant mortality and morbidity [1, 2]. In 2004, 84 percent of U.S. premature infants were born between 32 and 36 weeks of gestation. About 10 percent were born between 28 and 31 weeks of gestation, and 6 percent were born at less than 28 weeks of gestation [3]. Most of the deaths and severe illnesses occur in the infants who are born before 32 weeks of gestation [4]. Despite substantial clinical efforts devoted to reducing the incidence of PTD, the incidence rate has not decreased over the last decades [5]. In 2005, the rate in the U.S. rose to 12.7% [6].

PTD is often categorized based on clinical circumstances. Spontaneous preterm labor (PTL) and spontaneous preterm premature rupture of membranes (PROM) account for approximately 80% of all the singleton preterm births, and the other 20% are medically indicated to prevent or minimize adverse maternal and/or fetal outcomes [7]. Although spontaneous PTL and PROM are often conceptualized as two different processes leading to PTD, the risk factors for PTL and PROM are overlapping. Thus PTD resulting from spontaneous PTL and PROM are often considered together as spontaneous PTD.

Research has identified numerous risk factors for spontaneous PTD. Multifetal pregnancy has been repeatedly indicated as a risk factor for spontaneous PTD [8-12]. In singleton pregnancies, one obvious risk factor related with increased risk of spontaneous PTD is previous reproductive history [13-17]. Previous PTD is consistently reported to be related to increased risk of PTD, with the relative risks ranging up to 6.0 [17-22]. The disparity of incidence of PTD among different racial/ethnic groups is also of notice. The

rate of PTD among African American women is found to be twice that of any other racial group of women in the U.S. [23-27]. The other risk factors that have been implicated as PTD risk factors include short cervical length measured by ultrasound [23, 28-30], small pre-pregnancy body mass index (BMI) [21, 25, 31-33], poor weight gain during pregnancy [21, 32-35], and psychosocial stress [24, 36, 37]. A major predictor for spontaneous PTD is increased fetal fibronectin level in cervical or vaginal secretions. Fetal fibronectin is a protein produced by fetal membranes. It functions as an adhesion molecule which binds the placenta and membranes to the decidua. The presence of fetal fibronectin in the cervix or vagina after 20 weeks of gestation may provide evidence for the disruption of the attachment of the membranes to the decidua [38]. Fetal fibronectin in cervical or vaginal secretions has been found strongly and consistently associated with subsequent spontaneous preterm delivery [23, 39-43].

Although extensively studied, mechanisms by which these risk factors influence the incidence of spontaneous PTD remain incompletely understood. The hypothesized pathophysiologic pathways include infection and/or inflammation; premature activation of the maternal or fetal hypothalamic-pituitary-adrenal (HPA) axis in response to maternal or fetal stress; decidual hemorrhage with thrombin-induced protease release; and mechanical stretch due to pathologic uterine and cervical distention [44]. These pathophysiologic pathways may occur independently or collaboratively in the process leading to PTD. The unsuccessful medical interventions to reduce incidence of PTD may be due to the existing diverse pathophysiologic pathways leading to PTD.

Out of all currently suspected pathways to PTD, upper and lower genital tract infection and/or inflammation have been increasingly associated with PTD [45]. Knox et

al's study suggested that infection is related to increased incidence of PTD [46]. Since then, numerous studies have confirmed the finding that infection and/or inflammation has a strong association with preterm delivery. However, the relation between infection and PTD is not consistent throughout pregnancy. It is found that infection is present in most early preterm delivery (at less than 30 weeks of pregnancy), but is relatively rare in late preterm delivery (at 34 to 36 weeks of pregnancy) [4].

The most direct and accurate method to identify the upper genital tract infection and/or inflammation is the analysis of amniotic fluid. Tests include the white blood cell (WBC) and neutrophil counts, glucose concentration, Gram stain and microbial culture. The most commonly found bacteria in relation to spontaneous PTD include *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis*, and *Bacteroides* sp. Most bacteria found in PTD associated upper genital tract infection are thought to be of cervical or vaginal origin [4]. Investigators speculate that vaginal microorganism could ascend to upper genital tract, resulting in gestational tissues infection and PTD [47]. Multiple studies reported links between lower genital tract infections/conditions and PTD risk [48-55]. One frequently studied lower genital tract condition is bacterial vaginosis (BV).

BV, characterized as a decreased concentration of lactobacilli and an overgrowth of bacteria such as *Gardnerella vaginalis, Mycoplasma, Mobiluncus,and Bacteroides* spp, is a prevalent syndrome. About half of all pregnant and non-pregnant women with BV report abnormal vaginal discharge and other symptoms [56]. Most of our current understanding of the prevalence of BV is from selected clinical settings. The prevalence of BV varies by study population. BV has been detected in 17-19% of women attending

family planning clinics, 4-10% in student health clinics, and 24-64% in sexually transmitted disease clinics [57]. In pregnant women, the prevalence ranges from 14 to 33% [49, 58-62]. A recently published study estimated that almost a third (29%) of U.S. women between age 14 and 49 are positive for BV [63]. Asymptomatic women are less likely to be included in above mentioned studies. So, the reports may underestimate the prevalence of BV. Clinically, diagnostic criteria established by Amsel et al has been widely adopted [64]. According to Amsel criteria, BV is diagnosed when three of the following four criteria are met: 1. thin, homogenous, and adherent vaginal fluid; 2. vaginal fluid pH>4.5; 3. a positive sniff/whiff test (release of fishy odor on addition of 10% potassium hydroxide solution); 4. presence of "clue" cells, which are vaginal epithelial cells with obscured borders. In laboratory settings, the diagnostic criteria of BV is usually based on Nugent scoring of vaginal Gram stains [65]. The Nugent standardized scoring system, is a semi-quantitative assessment of the following morphotypes: *Lactobacilli*, *Gardnerella vaginalis* or *Bacteroides spp.*, and curved gram-variable rods. A high number of *Lactobacilli* lowers the score, whereas a high number of other bacteria raises the score. The total score ranges from 0 to 10. Based on the score, BV is typically categorized into negative (0-3), intermediate (4-6), and positive (7-10). Compared to Amsel criteria, the Nugent scoring system to diagnose BV has sensitivity and specificity ranging from 83-97%, and 83-98%, respectively [66-68]. The two diagnostic criteria have close correspondence to each other. Nugent's scoring system needs an experienced diagnostician, so currently it is not feasible to be widely used in clinical practice.

Compared to knowledge on identification and microbiologic change of BV, our information about risk factors of BV is limited. Currently identified factors that could

increase risk of BV include smoking, black ethnic group, vaginal douching, and lower socio-economic status. Smoking has been repeatedly associated with increased risk of BV in a pregnant population [69, 70]. However, it has been suggested smoking may be a marker for poor health care seeking behavior [9]. Thus, to understand the link between smoking and BV, other health behavior should also be included for consideration. BV is also not distributed evenly among race-ethnic groups. In U.S., the prevalence of BV is highest in African American women [63, 71, 72]. However, vaginal douching has been reported to be independently associated with higher risk of BV (HR=2.1, 95% CI: 1.0-4.3), and it is more commonly practiced in African American women [73]. The observed relation between race/ethnicity and BV could be confounded by vaginal douching. A case-control study conducted in the UK found that after adjusting for vaginal douching, history of BV, and some other covariates, being black was not a risk factor for BV (adjusted OR=1.1, 95% CI: 0.5-2.5) [71]. A recent prevalence of BV study, using 2001-2004 National Health and Nutrition Examination Survey Data, suggested that among U.S. aged 14-49 women, the odds of BV is higher in the group living below the federal poverty level (adjusted OR=1.32, 95% CI: 1.05-1.77) [63].

BV has been indicated to be associated with a number of obstetrical complications, such as PTL, PROM, spontaneous abortion, amniotic fluid infection, and postpartum endometritis. Studies repeatedly support the association between BV and increased risk for spontaneous PTL [74, 75] and PROM [76-79]. Eschenbach et al. conducted the first study to implicate BV as a risk factor for PTD. Their results suggested that the presence of BV in the mid-third trimester (mean 32.6 weeks of pregnancy) is associated with increased risk for preterm labor (OR=2.0, 95% CI: 1.1-3.5) and PROM (OR=2.0, 95%

CI: 1.1-3.7). Of note, several prospective cohort studies suggest that BV could be detected up to months before the onset of PTD [49, 80]. Significantly increased risks of PTD, PROM, and PTL have been detected in women with non-treated BV between 8 and 17 weeks of gestation [81]. Similar results were also observed in Hay et al.'s study, which reported increased risk of PTD (RR=5.2, 95% CI: 2.0-13.5) in women with BV before 16 weeks of gestation [69]. These research results inspire investigators to explore the possibility of preventing PTD by treating BV with anti-microbial agents.

Previously reported randomized, controlled trials aimed at treating BV or other sexually transmitted infections with antimicrobial agents to prevent PTD have produced primarily negative results, although these treatments were effective in treating these conditions/infections [69, 72, 82-84]. Several reasons may lead to the mixed results. First, treatment methods adopted in trials were different. McGregor et al. observed 50 percent risk reduction in clinically identified BV women who received oral clindamycin treatment, compared to the untreated controls [60]. In contrast, a randomized, placebo-controlled trial to prevent PTD by treating BV with 2% intravaginal clindamycin cream did not show a reduction in PTD risk [85]. Second, the varied results from trials could also be caused by different study populations. A randomized, placebo-controlled clinical trial reported that treating asymptomatic BV women between 16 and 24 weeks of gestation with metronidazole is not effective in the prevention of PTD [86]. In Hauth et al.'s trial, women at higher risk of PTD (prepregnancy weight of less than 50 kg) were screened for BV. The study population received metronidazole and erythromycin therapy or placebo during second trimester. Results showed that the BV treatment significantly

reduced PTD risk in the population who had BV, but not in the population who did not have BV [83].

The mechanisms linking BV and PTD are not clearly understood. Some studies showed that BV could cause both local genital symptoms and upper reproductive tract pathology, so it is proposed that BV could cause PTD through upper reproductive tract infection and/or inflammation. Also, because amniocentesis is invasive and not routinely conducted in clinical practice, cervicogenital specimens are usually used to document genital tract infection and/or inflammation. Several studies have shown the links between cervicogenital biomarkers (fetal fibronectin, interleukin-6, and interleukin-8) and upper genital tract infection and/or inflammation [30, 87-89]. Consequently, investigators have focused on investigating links between vaginal milieu and PTD.

Investigators have hypothesized that the BV-PTD relation is complex, and includes interplay between characteristics of the bacterial overgrowth and host immune response [90]. Host defense against microorganisms include innate and adaptive immunity. Innate immunity is thought to form the first, non-specific line of host defense [91]. It is also a critical precursor to an adaptive immune response [92]. In contrast to adaptive immunity, innate immunity is characterized as an immediate act, non-specific and with no need of previous experience with the same microorganism.

Antimicrobial peptides are members of the innate immunity system. Defensins are a family of the endogenous antimicrobial peptides. Defensins exhibit a wide antimicrobial spectrum, including activities against Gram negative and Gram positive bacteria, fungi, and enveloped viruses, and also act as regulators of inflammatory response and adaptive immune response [93-97]. In human, defensins have been found expressed in neutrophils,

macrophages, epithelial cells (lung, reproductive tract, urogenital tissues), and Paneth cells of the small intestine [98].

Defensins are cationic, arginine-rich, and highly disulfide-bonded peptides. They have molecular masses of 3.5-6 kDa and contain six to eight cysteine residues that form three to four intramolecular disulfide bridges [91, 99]. In humans, two categories of defensins have been found: α-defensins and β-defensins. Currently, six α-defensins and seven β-defensins have been discovered in humans [91]. Among the six α-defensins, four [human neutrophil peptide (HNP) 1-4] are found in azurophilic granules of neutrophils. HNP 1-3 compose half of the azurophilic protein, while HNP-4 is in lower concentrations [100]. HNP-1 and HNP-3 have also been found in B cells and natural killer cells. The other two α-defensins [human defensin (HD) 5 and 6] are found in granules of Paneth cells of the small intestine and the HD-5 has also been found in the epithelial cells of the female urogenital tract [101-103]. β-defensins (HBD) are found expressed at epithelial surfaces (kidney, female reproductive tract, respiratory tract, pancreas), parotid gland, tongue, leukocytes, and the bone marrow [104-106].

Human defensins exhibit activities against bacterial, fungi, and viruses. The mechanisms of the antimicrobial activity of human defensins are not clearly understood. It has been proposed that defensins permeabilize bacterial cells membranes through the formation of multimeric pores to impose antibacterial effect [107]. Defensins could also exhibit antibacterial effects on several bacterial cell targets. The precise mechanism is not understood; however, it is recognized that this effect is dependent on interaction between defensins and bacterial cell membranes [108]. The antiviral activity involves viral

adsorption and entry process [109, 110]. The antifungal activity appears to be related to either fungal cell lysis or interfence with fungal cell wall synthesis [111].

In studies of pregnant women, defensins have been found to be elevated in amniotic fluid during inflammatory processes like intra-amniotic infection [112]. The first study on defensins in amniotic fluid was conducted by Heine et al [113]. They reported a high amniotic fluid concentration of neutrophil defensins (HNP 1-3) in patients who had a cultural and/or histological genital tissues infection [113]. Espinoza and colleagues conducted a cross-sectional study to examine the relation of defensins (HNP 1-3) in amniotic fluid with microbial invasion of the amniotic cavity (MIAC), intra-amniotic inflammation, PTL, and PROM. Their results showed that MIAC, PTL, and PROM were associated with increased amniotic fluid concentrations of HNP 1-3. In the PTL patients with intact membranes, elevated HNP 1-3 were associated with intra-amniotic inflammation and histological chorioamnionitis. Also, defensins in plasma have been studied in relation to adverse pregnancy outcomes. Grable et al's study found that maternal plasma defensin levels were greater in women with PROM compared to that in controls [114]. The functional and pathologic significance of defensin elevations in maternal plasma may be different from that in other fluids such as amniotic or vaginal or in tissues.

The vagina is a space routinely exposed to a variety of microbes that could elicit both local neutrophil activation and defensins production. Accumulating evidence indicates that most bacteria isolated from upper genital tract in women with PTD are of vagina or cervix origin. Unfortunately, randomized clinical trials aimed at treating lower genital tract infections and/or conditions to prevent PTD have yielded primarily negative results.

Consequently, there are ongoing efforts by investigators to examine the interplay between characteristics of bacterial overgrowth and host immune response to better understand the problem and devise new interventions. As part of this process, defensins in vaginal fluid, components of the host immune response, may be useful for assessing host immune response.

One study which measured HNP 1-3 at 7-22 weeks of gestation found that the concentrations of vaginal fluid defensins were greater in women with asymptomatic *Trichomonas* vaginalis [115]. Balu RB et al. used a cohort study to assess the association between BV status (negative, intermediate, and positive), vaginal fluid defensins (HNP-2) at 24 to 29 weeks of gestation, and risk of PTD [107, 116]. They found an association between intermediate BV status and elevated vaginal fluid defensins, but no association between positive BV status and defensins. In a subsequent analysis, they showed that defensin levels were positively associated with risk of early PTD (<32 weeks of gestation) after adjusting for race/ethnicity, which was not modified by BV status, and no associations between defensin levels and PTD before 37 weeks of gestation. Their study measured HNP-2 at 24 to 29 weeks of gestation, which was close to early PTD (<32 weeks of gestation); and therefore it was difficult to tell if the elevated defensin levels were part of a process involving preparation for labor.

Though investigators have begun to unravel clues to underlying PTD pathways, PTD remains a leading cause of perinatal mortality and long-term neurological morbidities. Particularly concerning PTD rates in African Americans are almost twice that in other racial/ethnical groups. Evidence suggests that racial/ethnical differences in prevalence of genital tract infections and host immune response may explain part of the racial/ethnical

disparity in PTD risks. Based on previous studies, vaginal fluid defensins could be a mediator on the pathway from BV to PTD, or on PTD pathway associated with other vaginal infections that co-exist with BV. Alternatively, defensins may not be a mediator but may serve as a marker of an inflammatory process linked to PTD. Studies focusing on relations between vaginal fluid defensin levels, BV, and PTD may contribute to greater understanding of the racial/ethnical disparity in PTD risks.

Reference

1. Berkowitz, G.S. and E. Papiernik, *Epidemiology of preterm birth.* Epidemiol Rev, 1993. **15**(2): p. 414-43.

2. Hack, M. and A.A. Fanaroff, *Outcomes of extremely immature infants--a perinatal dilemma.* N Engl J Med, 1993. **329**(22): p. 1649-50.

3. March of Dime. [cited; Available from: http://www.marchofdimes.com/prematurity/5196_5799.asp.

4. Goldenberg, R.L., J.C. Hauth, and W.W. Andrews, *Intrauterine infection and preterm delivery.* N Engl J Med, 2000. **342**(20): p. 1500-7.

5. Goldenberg, R.L. and D.J. Rouse, *Prevention of premature birth.* N Engl J Med, 1998. **339**(5): p. 313-20.

6. Hamilton, B.E., A.M. Minino, J.A. Martin, et al., *Annual summary of vital statistics: 2005.* Pediatrics, 2007. **119**(2): p. 345-60.

7. Tucker, J.M., R.L. Goldenberg, R.O. Davis, et al., *Etiologies of preterm birth in an indigent population: is prevention a logical expectation?* Obstet Gynecol, 1991. **77**(3): p. 343-7.

8. Strauss, A., B.W. Paek, O. Genzel-Boroviczeny, et al., *Multifetal gestation--maternal and perinatal outcome of 112 pregnancies.* Fetal Diagn Ther, 2002. **17**(4): p. 209-17.

9. Roberts, W.E., J.C. Morrison, C. Hamer, et al., *The incidence of preterm labor and specific risk factors.* Obstet Gynecol, 1990. **76**(1 Suppl): p. 85S-89S.

10. Mercer, B.M., L.G. Crocker, W.F. Pierce, et al., *Clinical characteristics and outcome of twin gestation complicated by preterm premature rupture of the membranes.* Am J Obstet Gynecol, 1993. **168**(5): p. 1467-73.

11. Wen, S.W., K. Demissie, Q. Yang, et al., *Maternal morbidity and obstetric complications in triplet pregnancies and quadruplet and higher-order multiple pregnancies.* Am J Obstet Gynecol, 2004. **191**(1): p. 254-8.

12. Ludwig, M., M. Kohl, A. Kruger, et al., *Complications in high order multiple pregnancies* Geburtshilfe Frauenheilkd, 2004. **64**(2): p. 168-77.

13. Wen, S.W., R.L. Goldenberg, G.R. Cutter, et al., *Intrauterine growth retardation and preterm delivery: prenatal risk factors in an indigent population.* Am J Obstet Gynecol, 1990. **162**(1): p. 213-8.

14. Goldenberg, R.L., S.K. Mayberry, R.L. Copper, et al., *Pregnancy outcome following a second-trimester loss.* Obstet Gynecol, 1993. **81**(3): p. 444-6.

15. Rawlings, J.S., V.B. Rawlings, and J.A. Read, *Prevalence of low birth weight and preterm delivery in relation to the interval between pregnancies among white and black women.* N Engl J Med, 1995. **332**(2): p. 69-74.

16. Feresu, S.A., S.D. Harlow, and G.B. Woelk, *Risk factors for prematurity at Harare Maternity Hospital, Zimbabwe.* Int J Epidemiol, 2004. **33**(6): p. 1194-201.

17. Carlini, L., E. Somigliana, G. Rossi, et al., *Risk factors for spontaneous preterm birth: a Northern Italian multicenter case-control study.* Gynecol Obstet Invest, 2002. **53**(3): p. 174-80.

18. Mercer, B.M., R.L. Goldenberg, A.H. Moawad, et al., *The preterm prediction study: effect of gestational age and cause of preterm birth on subsequent obstetric outcome. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network.* Am J Obstet Gynecol, 1999. **181**(5 Pt 1): p. 1216-21.

19. Adams, M.M., L.D. Elam-Evans, H.G. Wilson, et al., *Rates of and factors associated with recurrence of preterm delivery.* Jama, 2000. **283**(12): p. 1591-6.

20. de Haas, I., B.L. Harlow, D.W. Cramer, et al., *Spontaneous preterm birth: a case-control study.* Am J Obstet Gynecol, 1991. **165**(5 Pt 1): p. 1290-6.

21. Lang, J.M., E. Lieberman, and A. Cohen, *A comparison of risk factors for preterm labor and term small-for-gestational-age birth.* Epidemiology, 1996. **7**(4): p. 369-76.

22. Adams, M.M., A.P. Sarno, F.E. Harlass, et al., *Risk factors for preterm delivery in a healthy cohort.* Epidemiology, 1995. **6**(5): p. 525-32.

23. Goldenberg, R.L., J.D. Iams, B.M. Mercer, et al., *The preterm prediction study: the value of new vs standard risk factors in predicting early and all spontaneous preterm births. NICHD MFMU Network.* Am J Public Health, 1998. **88**(2): p. 233-8.

24. Copper, R.L., R.L. Goldenberg, A. Das, et al., *The preterm prediction study: maternal stress is associated with spontaneous preterm birth at less than thirty-five weeks' gestation. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network.* Am J Obstet Gynecol, 1996. **175**(5): p. 1286-92.

25. Mercer, B.M., R.L. Goldenberg, A. Das, et al., *The preterm prediction study: a clinical risk assessment system.* Am J Obstet Gynecol, 1996. **174**(6): p. 1885-93; discussion 1893-5.

26. DuPlessis, H.M., R. Bell, and T. Richards, *Adolescent pregnancy: understanding the impact of age and race on outcomes.* J Adolesc Health, 1997. **20**(3): p. 187-97.

27. Schieve, L.A. and A. Handler, *Preterm delivery and perinatal death among black and white infants in a Chicago-area perinatal registry.* Obstet Gynecol, 1996. **88**(3): p. 356-63.

28. Iams, J.D., R.L. Goldenberg, P.J. Meis, et al., *The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network.* N Engl J Med, 1996. **334**(9): p. 567-72.

29. Grimes-Dennis, J. and V. Berghella, *Cervical length and prediction of preterm delivery.* Curr Opin Obstet Gynecol, 2007. **19**(2): p. 191-5.

30. Bittar, R.E., E.B. da Fonseca, M.H. de Carvalho, et al., *Predicting preterm delivery in asymptomatic patients with prior preterm delivery by measurement of cervical length and phosphorylated insulin-like growth factor-binding protein-1.* Ultrasound Obstet Gynecol, 2007. **29**(5): p. 562-7.

31. Hickey, C.A., S.P. Cliver, S.F. McNeal, et al., *Low pregravid body mass index as a risk factor for preterm birth: variation by ethnic group.* Obstet Gynecol, 1997. **89**(2): p. 206-12.

32. Ehrenberg, H.M., L. Dierker, C. Milluzzi, et al., *Low maternal weight, failure to thrive in pregnancy, and adverse pregnancy outcomes.* Am J Obstet Gynecol, 2003. **189**(6): p. 1726-30.

33. Chang, S.C., K.O. O'Brien, M.S. Nathanson, et al., *Characteristics and risk factors for adverse birth outcomes in pregnant black adolescents.* J Pediatr, 2003. **143**(2): p. 250-7.

34. Schieve, L.A., M.E. Cogswell, K.S. Scanlon, et al., *Prepregnancy body mass index and pregnancy weight gain: associations with preterm delivery. The NMIHS Collaborative Study Group.* Obstet Gynecol, 2000. **96**(2): p. 194-200.

35. Hickey, C.A., S.P. Cliver, S.F. McNeal, et al., *Prenatal weight gain patterns and spontaneous preterm birth among nonobese black and white women.* Obstet Gynecol, 1995. **85**(6): p. 909-14.

36. Alder, J., N. Fink, J. Bitzer, et al., *Depression and anxiety during pregnancy: a risk factor for obstetric, fetal and neonatal outcome? A critical review of the literature.* J Matern Fetal Neonatal Med, 2007. **20**(3): p. 189-209.

37. Dayan, J., C. Creveuil, M.N. Marks, et al., *Prenatal depression, prenatal anxiety, and spontaneous preterm birth: a prospective cohort study among women with early and regular care.* Psychosom Med, 2006. **68**(6): p. 938-46.

38. Feinberg, R.F., H.J. Kliman, and C.J. Lockwood, *Is oncofetal fibronectin a trophoblast glue for human implantation?* Am J Pathol, 1991. **138**(3): p. 537-43.

39. Vetr, M., M. Kudela, and J. Prasilova, *Fetal fibronectin in patients at increased risk for premature birth.* Acta Univ Palacki Olomuc Fac Med, 1996. **140**: p. 55-7.

40. Lockwood, C.J., A.E. Senyei, M.R. Dische, et al., *Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery.* N Engl J Med, 1991. **325**(10): p. 669-74.

41. Goldenberg, R.L., B.M. Mercer, P.J. Meis, et al., *The preterm prediction study: fetal fibronectin testing and spontaneous preterm birth. NICHD Maternal Fetal Medicine Units Network.* Obstet Gynecol, 1996. **87**(5 Pt 1): p. 643-8.

42. Iams, J.D., D. Casal, J.A. McGregor, et al., *Fetal fibronectin improves the accuracy of diagnosis of preterm labor.* Am J Obstet Gynecol, 1995. **173**(1): p. 141-5.

43. Nageotte, M.P., D. Casal, and A.E. Senyei, *Fetal fibronectin in patients at increased risk for premature birth.* Am J Obstet Gynecol, 1994. **170**(1 Pt 1): p. 20-5.

44. Lockwood, C.J. and E. Kuczynski, *Markers of risk for preterm delivery.* J Perinat Med, 1999. **27**(1): p. 5-20.

45. Romero, R., J. Espinoza, L.F. Goncalves, et al., *Inflammation in preterm and term labour and delivery.* Semin Fetal Neonatal Med, 2006. **11**(5): p. 317-26.

46. Knox, I.C., Jr. and J.K. Hoerner, *The role of infection in premature rupture of the membranes.* Am J Obstet Gynecol, 1950. **59**(1): p. 190-4, illust.

47. Haggerty, C.L., S.L. Hillier, D.C. Bass, et al., *Bacterial vaginosis and anaerobic bacteria are associated with endometritis.* Clin Infect Dis, 2004. **39**(7): p. 990-5.

48. Kimberlin, D.F. and W.W. Andrews, *Bacterial vaginosis: association with adverse pregnancy outcome.* Semin Perinatol, 1998. **22**(4): p. 242-50.

49. Svare, J.A., H. Schmidt, B.B. Hansen, et al., *Bacterial vaginosis in a cohort of Danish pregnant women: prevalence and relationship with preterm delivery, low birthweight and perinatal infections.* Bjog, 2006. **113**(12): p. 1419-25.

50. Hillier, S.L., R.P. Nugent, D.A. Eschenbach, et al., *Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group.* N Engl J Med, 1995. **333**(26): p. 1737-42.

51. Cotch, M.F., J.G. Pastorek, 2nd, R.P. Nugent, et al., *Trichomonas vaginalis associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group.* Sex Transm Dis, 1997. **24**(6): p. 353-60.

52. Meis, P.J., R.L. Goldenberg, B. Mercer, et al., *The preterm prediction study: significance of vaginal infections. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network.* Am J Obstet Gynecol, 1995. **173**(4): p. 1231-5.

53. Regan, J.A., M.A. Klebanoff, R.P. Nugent, et al., *Colonization with group B streptococci in pregnancy and adverse outcome. VIP Study Group.* Am J Obstet Gynecol, 1996. **174**(4): p. 1354-60.

54. Leitich, H., B. Bodner-Adler, M. Brunbauer, et al., *Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis.* Am J Obstet Gynecol, 2003. **189**(1): p. 139-47.

55. Krohn, M.A., S.L. Hillier, M.L. Lee, et al., *Vaginal Bacteroides species are associated with an increased rate of preterm delivery among women in preterm labor.* J Infect Dis, 1991. **164**(1): p. 88-93.

56. Eschenbach, D.A., *Vaginal infection.* Clin Obstet Gynecol, 1983. **26**(1): p. 186-202.

57. Hillier, S.L. and K.K. Holmes, *Bacterial vaginosis.*, in *Sexually Transmitted Diseases, ed.* 1999, McGraw-Hill: New York. p. 563-87.

58. Cristiano, L., N. Coffetti, G. Dalvai, et al., *Bacterial vaginosis: prevalence in outpatients, association with some micro-organisms and laboratory indices.* Genitourin Med, 1989. **65**(6): p. 382-7.

59. Eschenbach, D.A., *Bacterial vaginosis: emphasis on upper genital tract complications.* Obstet Gynecol Clin North Am, 1989. **16**(3): p. 593-610.

60. McGregor, J.A., J.I. French, R. Parker, et al., *Prevention of premature birth by screening and treatment for common genital tract infections: results of a prospective controlled evaluation.* Am J Obstet Gynecol, 1995. **173**(1): p. 157-67.

61. Tolosa, J.E., S. Chaithongwongwatthana, S. Daly, et al., *The International Infections in Pregnancy (IIP) study: variations in the prevalence of bacterial vaginosis and distribution of morphotypes in vaginal smears among pregnant women.* Am J Obstet Gynecol, 2006. **195**(5): p. 1198-204.

62. Thanavuth, A., A. Chalermchockcharoenkit, D. Boriboonhirunsarn, et al., *Prevalence of bacterial vaginosis in Thai pregnant women with preterm labor in Siriraj hospital.* J Med Assoc Thai, 2007. **90**(3): p. 437-41.

63. Allsworth, J.E. and J.F. Peipert, *Prevalence of bacterial vaginosis: 2001-2004 National Health and Nutrition Examination Survey data.* Obstet Gynecol, 2007. **109**(1): p. 114-20.

64. Amsel, R., P.A. Totten, C.A. Spiegel, et al., *Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations.* Am J Med, 1983. **74**(1): p. 14-22.

65. Nugent, R.P., M.A. Krohn, and S.L. Hillier, *Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation.* J Clin Microbiol, 1991. **29**(2): p. 297-301.

66. Navarrete, P., M. Dominguez, E. Castro, et al., *[Evaluation of Nugent and Amsel criteria for the diagnosis of bacterial vaginosis].* Rev Med Chil, 2000. **128**(7): p. 767-71.

67. Coppolillo, E.F., B.E. Perazzi, A.M. Famiglietti, et al., *Diagnosis of Bacterial Vaginosis During Pregnancy.* J Low Genit Tract Dis, 2003. **7**(2): p. 117-121.

68. Schwebke, J.R., S.L. Hillier, J.D. Sobel, et al., *Validity of the vaginal gram stain for the diagnosis of bacterial vaginosis.* Obstet Gynecol, 1996. **88**(4 Pt 1): p. 573-6.

69. Ugwumadu, A., I. Manyonda, F. Reid, et al., *Effect of early oral clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: a randomised controlled trial.* Lancet, 2003. **361**(9362): p. 983-8.

70. Alnaif, B. and H.P. Drutz, *The Association of Smoking with Vaginal Flora, Urinary Tract Infection, Pelvic Floor Prolapse, and Post-Void Residual Volumes.* J Low Genit Tract Dis, 2001. **5**(1): p. 7-11.

71. Rajamanoharan, S., N. Low, S.B. Jones, et al., *Bacterial vaginosis, ethnicity, and the use of genital cleaning agents: a case control study.* Sex Transm Dis, 1999. **26**(7): p. 404-9.

72. Goldenberg, R.L., M.A. Klebanoff, R. Nugent, et al., *Bacterial colonization of the vagina during pregnancy in four ethnic groups. Vaginal Infections and Prematurity Study Group.* Am J Obstet Gynecol, 1996. **174**(5): p. 1618-21.

73. Aral, S.O., W.D. Mosher, and W. Cates, Jr., *Vaginal douching among women of reproductive age in the United States: 1988.* Am J Public Health, 1992. **82**(2): p. 210-4.

74. Silver, H.M., R.S. Sperling, P.J. St Clair, et al., *Evidence relating bacterial vaginosis to intraamniotic infection.* Am J Obstet Gynecol, 1989. **161**(3): p. 808-12.

75. Subtil, D., V. Denoit, F. Le Goueff, et al., *The role of bacterial vaginosis in preterm labor and preterm birth: a case-control study.* Eur J Obstet Gynecol Reprod Biol, 2002. **101**(1): p. 41-6.

76. Lamont, R.F., *Recent evidence associated with the condition of preterm prelabour rupture of the membranes.* Curr Opin Obstet Gynecol, 2003. **15**(2): p. 91-9.

77. McGregor, J.A., J.I. French, and K. Seo, *Premature rupture of membranes and bacterial vaginosis.* Am J Obstet Gynecol, 1993. **169**(2 Pt 2): p. 463-6.

78. Reynolds, H.D., *Bacterial vaginosis and its implication in preterm labor and premature rupture of membranes. A review of the literature.* J Nurse Midwifery, 1991. **36**(5): p. 289-96.

79. Gravett, M.G., H.P. Nelson, T. DeRouen, et al., *Independent associations of bacterial vaginosis and Chlamydia trachomatis infection with adverse pregnancy outcome.* Jama, 1986. **256**(14): p. 1899-903.

80. Daskalakis, G., A. Papapanagiotou, S. Mesogitis, et al., *Bacterial vaginosis and group B streptococcal colonization and preterm delivery in a low-risk population.* Fetal Diagn Ther, 2006. **21**(2): p. 172-6.

81. Kekki, M., T. Kurki, J. Pelkonen, et al., *Vaginal clindamycin in preventing preterm birth and peripartal infections in asymptomatic women with bacterial vaginosis: a randomized, controlled trial.* Obstet Gynecol, 2001. **97**(5 Pt 1): p. 643-8.

82. Morales, W.J., S. Schorr, and J. Albritton, *Effect of metronidazole in patients with preterm birth in preceding pregnancy and bacterial vaginosis: a placebo-controlled, double-blind study.* Am J Obstet Gynecol, 1994. **171**(2): p. 345-7; discussion 348-9.

83. Hauth, J.C., R.L. Goldenberg, W.W. Andrews, et al., *Reduced incidence of preterm delivery with metronidazole and erythromycin in women with bacterial vaginosis.* N Engl J Med, 1995. **333**(26): p. 1732-6.

84. McDonald, H.M., J.A. O'Loughlin, R. Vigneswaran, et al., *Impact of metronidazole therapy on preterm birth in women with bacterial vaginosis flora (Gardnerella vaginalis): a randomised, placebo controlled trial.* Br J Obstet Gynaecol, 1997. **104**(12): p. 1391-7.

85. McGregor, J.A., J.I. French, W. Jones, et al., *Bacterial vaginosis is associated with prematurity and vaginal fluid mucinase and sialidase: results of a controlled trial of topical clindamycin cream.* Am J Obstet Gynecol, 1994. **170**(4): p. 1048-59; discussion 1059-60.

86. Carey, J.C., M.A. Klebanoff, J.C. Hauth, et al., *Metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units.* N Engl J Med, 2000. **342**(8): p. 534-40.

87. Hitti, J., S.L. Hillier, K.J. Agnew, et al., *Vaginal indicators of amniotic fluid infection in preterm labor.* Obstet Gynecol, 2001. **97**(2): p. 211-9.

88. Peipert, J.F., R.B. Ness, D.E. Soper, et al., *Association of lower genital tract inflammation with objective evidence of endometritis.* Infect Dis Obstet Gynecol, 2000. **8**(2): p. 83-7.

89. Goldenberg, R.L., E. Thom, A.H. Moawad, et al., *The preterm prediction study: fetal fibronectin, bacterial vaginosis, and peripartum infection. NICHD Maternal Fetal Medicine Units Network.* Obstet Gynecol, 1996. **87**(5 Pt 1): p. 656-60.

90. Romero, R., T. Chaiworapongsa, H. Kuivaniemi, et al., *Bacterial vaginosis, the inflammatory response and the risk of preterm birth: A role for genetic epidemiology in the prevention of preterm birth.* American Journal of Obstetrics and Gynecology, 2004. **190**(6): p. 1509-1519.

91. De Smet, K. and R. Contreras, *Human antimicrobial peptides: defensins, cathelicidins and histatins.* Biotechnol Lett, 2005. **27**(18): p. 1337-47.

92. Medzhitov, R. and C.A. Janeway, Jr., *Innate immune induction of the adaptive immune response.* Cold Spring Harb Symp Quant Biol, 1999. **64**: p. 429-35.

93. Selsted, M.E., D. Szklarek, and R.I. Lehrer, *Purification and antibacterial activity of antimicrobial peptides of rabbit granulocytes.* Infect Immun, 1984. **45**(1): p. 150-4.

94. Ganz, T., M.E. Selsted, D. Szklarek, et al., *Defensins. Natural peptide antibiotics of human neutrophils.* J Clin Invest, 1985. **76**(4): p. 1427-35.

95. Selsted, M.E., D. Szklarek, T. Ganz, et al., *Activity of rabbit leukocyte peptides against Candida albicans.* Infect Immun, 1985. **49**(1): p. 202-6.

96. Aley, S.B., M. Zimmerman, M. Hetsko, et al., *Killing of Giardia lamblia by cryptdins and cationic neutrophil peptides.* Infect Immun, 1994. **62**(12): p. 5397-403.

97. Lehrer, R.I., K. Daher, T. Ganz, et al., *Direct inactivation of viruses by MCP-1 and MCP-2, natural peptide antibiotics from rabbit leukocytes.* J Virol, 1985. **54**(2): p. 467-72.

98. Jenssen, H., P. Hamill, and R.E. Hancock, *Peptide antimicrobial agents.* Clin Microbiol Rev, 2006. **19**(3): p. 491-511.

99. Chen, H., Z. Xu, L. Peng, et al., *Recent advances in the research and development of human defensins.* Peptides, 2006. **27**(4): p. 931-40.

100. Ganz, T., M.E. Selsted, and R.I. Lehrer, *Defensins.* Eur J Haematol, 1990. **44**(1): p. 1-8.

101. Jones, D.E. and C.L. Bevins, *Paneth cells of the human small intestine express an antimicrobial peptide gene.* J Biol Chem, 1992. **267**(32): p. 23216-25.

102. Jones, D.E. and C.L. Bevins, *Defensin-6 mRNA in human Paneth cells: implications for antimicrobial peptides in host defense of the human bowel.* FEBS Lett, 1993. **315**(2): p. 187-92.

103. Quayle, A.J., E.M. Porter, A.A. Nussbaum, et al., *Gene expression, immunolocalization, and secretion of human defensin-5 in human female reproductive tract.* Am J Pathol, 1998. **152**(5): p. 1247-58.

104. Valore, E.V., C.H. Park, A.J. Quayle, et al., *Human beta-defensin-1: an antimicrobial peptide of urogenital tissues.* J Clin Invest, 1998. **101**(8): p. 1633-42.

105. Mathews, M., H.P. Jia, J.M. Guthmiller, et al., *Production of beta-defensin antimicrobial peptides by the oral mucosa and salivary glands.* Infect Immun, 1999. **67**(6): p. 2740-5.

106. Krisanaprakornkit, S., A. Weinberg, C.N. Perez, et al., *Expression of the peptide antibiotic human beta-defensin 1 in cultured gingival epithelial cells and gingival tissue.* Infect Immun, 1998. **66**(9): p. 4222-8.

107. Balu, R.B., D.A. Savitz, C.V. Ananth, et al., *Bacterial vaginosis, vaginal fluid neutrophil defensins, and preterm birth.* Obstet Gynecol, 2003. **101**(5 Pt 1): p. 862-8.

108. Hancock, R.E. and A. Rozek, *Role of membranes in the activities of antimicrobial cationic peptides.* FEMS Microbiol Lett, 2002. **206**(2): p. 143-9.

109. Aboudy, Y., E. Mendelson, I. Shalit, et al., *Activity of two synthetic amphiphilic peptides and magainin-2 against herpes simplex virus types 1 and 2.* Int J Pept Protein Res, 1994. **43**(6): p. 573-82.

110. Belaid, A., M. Aouni, R. Khelifa, et al., *In vitro antiviral activity of dermaseptins against herpes simplex virus type 1.* J Med Virol, 2002. **66**(2): p. 229-34.

111. De Lucca, A.J. and T.J. Walsh, *Antifungal peptides: novel therapeutic compounds against emerging pathogens.* Antimicrob Agents Chemother, 1999. **43**(1): p. 1-11.

112. Ganz, T., *Defensins in the urinary tract and other tissues.* J Infect Dis, 2001. **183 Suppl 1**: p. S41-2.

113. Heine, R.P., H. Wiesenfeld, L. Mortimer, et al., *Amniotic fluid defensins: potential markers of subclinical intrauterine infection.* Clin Infect Dis, 1998. **27**(3): p. 513-8.

114. Grable, I.A. and R.P. Heine, *Neutrophil granule products: can they identify subclinical chorioamnionitis in patients with preterm premature rupture of membranes?* Am J Obstet Gynecol, 2003. **189**(3): p. 808-12.

115. Simhan, H.N., B.L. Anderson, M.A. Krohn, et al., *Host immune consequences of asymptomatic Trichomonas vaginalis infection in pregnancy.* Am J Obstet Gynecol, 2007. **196**(1): p. 59 e1-5.

116. Balu, R.B., D.A. Savitz, C.V. Ananth, et al., *Bacterial vaginosis and vaginal fluid defensins during pregnancy.* Am J Obstet Gynecol, 2002. **187**(5): p. 1267-71.

Bacterial vaginosis, vaginal fluid defensins, and preterm delivery

INTRODUCTION

Preterm delivery (PTD), delivery prior to 37 completed weeks of gestation, is a leading cause of perinatal mortality and long-term neurological morbidity [1, 2]. In 2005, the PTD rate in the U.S. rose to 12.7%, which was higher than the previous decade [3]. PTD is more common in poor women, and African American women have twice the PTD risk of that found in most other racial/ethnic groups in the U.S. [3, 4].

PTD is often categorized based on clinical circumstances. Spontaneous PTD, initiated by either preterm labor (PTL) or spontaneous preterm premature rupture of membranes (PROM), accounts for approximately 80% of all singleton PTDs. The other 20% are medically indicated to prevent or minimize adverse maternal and/or fetal outcomes [5]. Although extensively studied, the pathways to PTD remain incompletely understood. Out of all suspected PTD pathways, genital tract infection/inflammation has been increasingly associated with PTD, especially early PTD that begins with PTL or PROM [6-11]. Most bacteria isolated from the upper genital tract in women with spontaneous PTD are thought to be of vaginal or cervical origin [7] and multiple studies have reported links between the vaginal microbial milieu and risk of PTD [12-19].

One such frequently studied link involves a condition known as bacterial vaginosis (BV), which is characterized by a decreased concentration of lactobacilli and an overgrowth of a diverse community of bacteria (e.g. *Gardnerella vaginalis, Mycoplasma, Mobiluncus, Bacteroides* spp, and *Atopobium vaginae*) [20-23]. BV has been associated with upper genital tract infection, immunologic markers of infection in upper genital

tract, and PTD [12-14, 18, 29-32]. The prevalence of BV in pregnant women ranges from 14% to 33% [13, 24-28]. BV prevalence is higher in African-Americans compared with non-Hispanic whites, irrespective of other socioeconomic characteristics [33-35], leading some to conclude that this condition may be related to race/ethnicity disparities in PTD risk. Unfortunately, randomized clinical trials aimed at treating BV and/or other sexually transmitted infections with antimicrobial agents to prevent PTD have primarily yielded negative results [36-50]. Consequently investigators have tried to step back and re-examine the relation between vaginal milieu and PTD risk, looking deeper into the complex interplay between characteristics of bacterial overgrowth and host immune response.

As part of studying innate immune response, there is growing interest in a group of peptides known as defensins. Defensins are a family of endogenous 3 to 5 kDa, three-disulfide cationic peptides [51] that exhibit a wide antimicrobial spectrum, including activity against gram-negative and gram-positive bacteria, fungi, and enveloped viruses [52-56]. Defensins also act as regulators of inflammation and of the adaptive immune response. Human neutrophil peptides (HNP) belong to the family of defensins. HNP 1-3 are found in azurophilic granules of neutrophils and compose half of the azurophilic protein. HNP-1 and HNP-3 have also been found in B cells and natural killer cells. Elevations in HNP 1-3 levels in aminiotic fluid were found in pregnant women with PTL and subclinical intra-uterine infection [57]. More recently, increased levels of HNP 2 in vaginal fluid measured at 24-29 weeks of pregnancy were linked to increased risk of delivery at less than 32 weeks [58, 59]. These studies raise the possibilities that defensins could be important mediators or markers of an inflammatory pathway leading to PTD.

Building on these previous studies, this study evaluated defensins (HNP 1-3) in vaginal fluid earlier in pregnancy (15-26 weeks). The goal was to assess relations between vaginal defensin levels, BV, and risk of PTD in a cohort of low and high risk non-Hispanic white and African American women. In light of mounting evidence that race/ethnicity differences in prevalence of BV and reproductive tract infections and in host immune response may account for a significant proportion of the racial/ethnical disparity in PTD risk [60-63]. A secondary goal included examining effect modification of the BV, defensins, PTD associations by race/ethnicity.

MATERIAL AND METHOD

Study population

This study included participants from the Pregnancy Outcomes and Community Health (POUCH) Study, a community-based prospective cohort study [64]. The POUCH study enrolled women in the 15-27th week of pregnancy from 52 participating clinics in five Michigan communities from 1998 to 2004. All five communities encompassed urban, suburban, and rural areas. Eligibility criteria included maternal age >14 years, screening for maternal serum alpha-fetoprotein (MSAFP) between 15-22 weeks' gestation, English-speaking, a singleton pregnancy with no known congenital or chromosomal abnormalities, and no history of pre-pregnancy diabetes. Of the 3,038 women enrolled, 19 were lost to follow-up, leaving 3,019 (99.4 %) in the cohort. Study protocols were approved by institutional review boards at Michigan State University and at eight hospitals in five Michigan communities. Before enrollment, all participants provided written consent to participate in the POUCH study.

Due to Health Insurance Portability and Accountability Act (HIPAA) regulations, it was not feasible to find out the exact response rate or compare characteristics of participants with those of women who declined to participate in POUCH study. However, we compared POUCH study data with data recorded on birth certificates of women who delivered in the five POUCH study communities in 2000. Race/ethnicity-specific analyses (non-Hispanic White, African American), weighted by the proportion of women enrolled from each POUCH study community, suggested that the POUCH sample was very similar to community mothers on most factors measured, including age, parity, education levels, the proportions of women with Medicaid insurance, preterm delivery, previous stillbirth, previous preterm infant, and previous low birth weight infant. The only exception was that the percentage of African American over 30 years of age in POUCH study was 14%, lower than that in community birth certificate (21%) [64].

A sub-cohort was selected for more intense assessment to maximize resources and statistical power for studying subgroups of interest. Cohort women were stratified by race/ethnicity (i.e. African-American and white/other), and by MSAFP levels (i.e. normal < 2 multiples of the median (MoM), unexplained high >= 2 MoM) and sampled into the sub-cohort. The sampling goal was to include all women who delivered preterm, all women with high MSAFP, and a random sample of women who delivered at term with normal MSAFP, with an over-sampling of African-Americans in this latter category. All sub-cohort analyses incorporated weights to reflect this sampling scheme. In the POUCH study sub-cohort (N=1,371), 692 (50.5%) women reported their race/ethnicity as non-Hispanic whites, 579 (42.2%) as African Americans, and 100 (7.3%) as other racial/ethnic groups. Because of the broad racial/ethnic mix in the "other" group and the

small number of women in this group, we excluded this group from the following analyses. We also excluded 240 non-Hispanic White and African American women who declined vaginal fluid sampling or whose samples were not sufficient to be analyzed, leaving a sub-cohort sample of 1,031 women in the current analyses.

Data collection

At 15-27 week's gestation, participating women met with a study nurse in their respective community and provided information about demographics, current pregnancy, reproductive history, health behaviors, and social and psychosocial factors. In addition, vaginal fluid samples were collected via a fetal fibronectin (fFN) specimen collection kit (Adeza International, Sunnyvale, CA, USA). After placement of a vaginal speculum, a sterile Dacron swab was used to collect vaginal fluid from the posterior fornix. The swab was then placed in the kit's 1 ml extraction buffer and refrigerated (4°C) for at least 24 hours. At the end of the refrigeration period, buffer was expressed from the swab by pressing the swab against the inside of the collection tube. The specimen was then filtered using a 10.25mm x 4" serum filter (Fisherbrand® Serum Filter System, Fisher Scientific, Pittsburgh, PA, USA), divided into 0.5 ml aliquots, and stored at -80°C.

Vaginal fluid specimens were later thawed and 50 µl aliquots were prepared and refrozen for shipment to the laboratory at the University of Alabama-Birmingham for defensin measurements. HNP 1-3 were assayed using an ELISA kit (HyCult Biotechnology, The Netherlands). The measurable concentration ranges were 41-10,000 pg/ml, however, values below 50 pg/ml were considered less reliable. Thirteen women had vaginal fluid defensin levels below the level of detection, which were imputed as half

the detection level in the analyses. In the text that follows, the total level of HNP 1-3 is referred to as vaginal fluid defensins.

At the time of vaginal fluid sampling, study nurses also obtained vaginal smears. To assess BV, vaginal smears were Gram-stained and evaluated by a microbiologist using the Nugent scoring system [20]. The scores ranged from 0 to 10, and BV status was categorized as negative (0-3), intermediate (4-6), and positive (7-10).

PTD definition and subtypes

PTD was defined as births before completion of 37 weeks' gestation. Gestational age (GA) was determined by the first day of the last menstrual period (LMP) or early ultrasound (US) data. US data was used only when a GA based on LMP differed from the US estimated GA by at least two weeks. PTD was divided into three groups based on clinical circumstances: 1) Preterm spontaneous labor (PTL) included women with regular contractions that led to cervical changes ($\geq$2 cm dilatation); 2) premature rupture of membranes (PROM) included women with rupture of membranes before or simultaneously with onset of contractions; and 3) Medically indicated included women induced or C-sectioned before either PTL or PROM.

Analytical strategy

The overall strategy was to first assess relations between vaginal defensin levels and BV, as well as other maternal characteristics, and then examine associations between vaginal defensins and risk of PTD. In addition, variations in these relations across race/ethnic groups were of particular interest. All analyses used sub-cohort sampling weights. The initial description of vaginal defensins levels at mid-pregnancy included comparisons of levels between African Americans and non-Hispanic whites. Because

distributions were skewed, defensin levels were transformed to the natural log scale and analyzed using SAS surveymeans and surveyreg procedures.

A dichotomous defensin variable was created using the median value, i.e. high >=median, low <median, from the distribution of defensin levels in women who delivered at term and had normal MSAFP. Race/ethnic specific categorical analyses (SAS surveyfreq) were then performed to evaluate BV Nugent score and other maternal characteristics (i.e., maternal age, education, smoking, Medicaid Insured status, weeks of gestation at enrollment, and parity) in relation to high and low defensin levels. Rao-Scott Chi-square test was applied to test for statistically significant associations. Maternal characteristics that were associated with defensin levels at $p<0.10$ were considered as potential confounders to be included in models relating defensin levels to PTD risk.

Weighted polytomous logistic regression models were constructed to test associations between covariates (high/low defensin levels, BV, and race/ethnicity) and a four-level outcome variable, i.e., PTD divided into three subtypes (PTL, PROM, MI) and term as the referent. In these analyses the BV variable was the race/ethnicity-specific mean Nugent score for each BV group (negative, intermediate, and positive) and treated as a continuous measure. Results showed a statistically significant three-way interaction between race/ethnicity, defensin levels, and BV status, leading to the development of race/ethnicity-specific polytomous logistic regression models. These models were used to calculate unadjusted and adjusted odds of PTD subtypes for high versus low defensin levels. Beta parameters from the two race/ethnicity-specific, polytomous logistic regression models that included BV and defensins as covariates were then used to

calculate race/ethnicity-specific probabilities of each PTD subtype by BV status and defensin level (high/low). Results are displayed in graph format.

RESULTS

In Table 1, maternal characteristics of this sub-cohort sample are presented with and without sampling weights to describe the sub-cohort sample and to compare across race/ethnicity groups using weighted data. In the sub-cohort, 45 percent were African American. Analyses of weighted data showed that the PTD risk was significantly greater in African American women (14%) than in non-Hispanic white women (10%) but the mean log vaginal defensin level was significantly higher in non-Hispanic whites compared to that in African Americans.

With the exception of 13 women, all study paticipants' defensin levels were detectable, ranging from 2.0 ng/ml to 90,340 ng/ml, with median of 2,441 ng/ml, and mean of 7,130 ng/ml. The median log defensin among women who delivered term and had normal MSAFP was 7.79 ng/ml. Using this median, all women were categorized into two groups, "low defensin" (<median) and "high defensin" (>=median).

In non-Hispanic whites, only maternal age was significantly associated with defensin levels (Table 2). Approximately 59% of women aged 20-29 and only 46% of women of over 39 years of age were in the high defensin group. Surprisingly, as BV status changed from negative, to intermediate, and to positive, the percentage of women in the high defensin group decreased (56%, 44%, and 42%, respectively), but this inverse relation was not statistically significant.

In African American women, week of pregnancy at enrollment and BV status were significantly associated with defensin levels. Women who enrolled later in pregnancy

were more likely to be in the high defensin group. The BV positive group had the largest percentage of high defensin women (57%), followed by the BV intermediate group (53%), and the BV negative group (43%).

In the weighted polytomous logistic regression model with the four-level outcome variable (i.e. 3 PTD subtypes and term referent), there was a statistically significant three-way interaction between three covariates (i.e., BV status, defensin levels, and race/ethnicity), which led to the development of race/ethnicity-specific models (Table 3 and Table 4). Defensin levels were significantly associated with PTD subtypes in African Americans (p-value=0.04), but not in non-Hispanic whites. In African Americans with high defensin levels, the unadjusted odds ratios (OR) for PTL was 2.1 (95% confidence interval (CI): 1.0-4.3), and for PROM was 2.5 (95% CI: 0.9-7.1). Adjustment for potential confounders, maternal age for non-Hispanic white and weeks of pregnancy at enrollment for African Americans, had little effect on the odds ratios. In a final set of analyses, the relation between defensins and PTD was considered within the context of BV. Because the sub-cohort was sampled from a prospective cohort study, weighted analyses permitted calculations of the probabilities (risks) of each PTD subtype given defensin and BV status at mid-pregnancy (Figure 1). Results showed that no matter what the BV score was, the risk of medically indicated PTD was not significantly associated with defensin levels in non-Hispanic whites and in African Americans (Figure 1a). The risk of PROM in African Americans appeared higher among women with high defensins (though not statistically significant), and the magnitude of this relation was not influenced by BV status (Figure 1b). In non-Hispanic whites, risk of PROM was not related to high defensin levels. On the contrary, there was the suggestion that low

defensin levels and high BV score may increase the risk of PROM, but the combination of these two exposures in non-Hispanic whites was rare and this study lacked statistical power to adequately assess this subgroup. The risk of PTL was significantly associated with high defensin levels in African Americans, and this association persisted regardless of BV status (Figure 1c). There was no link between PTL and defensin levels in non-Hispanic whites.

DISCUSSION

We found that the relations between mid-pregnancy vaginal defensin levels, bacterial vaginosis, and pregnancy outcome were not consistent across racial/ethnical groups. Among African Americans, vaginal defensin levels above the median were associated with intermediate and positive bacterial vaginosis and with increased risk of PTD initiated by PTL or PROM. Once African American women were identified as having vaginal defensin levels above the median, additional information on bacterial vaginosis added nothing to predicting risk of PTL/PROM PTD. By contrast none of the above associations held true for non-Hispanic whites.

To our knowledge, one other cohort study reported findings on vaginal defensin levels, BV, and pregnancy outcome in a series of two papers authored by Balu et al [58, 59]. Their results showed that vaginal defensin levels were positively associated with risk of early PTD (<32 weeks of gestation), but only after excluding women with vaginal bleeding or adjusting for bleeding and race/ethnicity in the final model. There was no association between defensin levels and all PTD before 37 weeks. Elevated defensins were linked to intermediate BV but not to positive BV and, as in our study, BV status did not modify the relation between defensins and PTD risk. In contrast to our findings, Balu

et al did not detect effect modification by race/ethnicity for the above association. Several methodological differences between our study and the Balu et al study are worth noting. First, we assessed HNP1-3 in vaginal fluid whereas they measured only HNP 2. Perhaps for this reason, or due to study differences in assaying approaches, we found only about 1% of the 1,031 women had non-detectable defensin levels compared with 69.5% of 749 women with non-detectable levels in the other study. This may also indicate the vaginal fluid HNP 1-3 are not equally expressed. The interval from vaginal fluid sampling (24-29 weeks) to early delivery (i.e. < 32 weeks) was short in the other study, raising the possibility that increases in vaginal defensin levels accompany preparation for labor. In our study, defensin levels were measured earlier in pregnancy (15-27 weeks with about 90% before 25 weeks), offering some assurance that higher vaginal defensin levels are not just a marker of impending labor. In their analyses, Balu et al found that dividing PTD by weeks (<32, <34, <37) was more informative relative to defensins than dividing PTD by subtypes (i.e., PTL, PROM, medically indicated PTD), which was the approach we used. It is likely that the greatest insights would come from dividing PTD by weeks and subtypes simultaneously, but few pregnancy cohort studies have an adequate sample size to analyze their data this way.

Other studies have examined defensins in plasma and amniotic fluid in relation to adverse pregnancy outcomes. Grable et al reported that maternal plasma defensin levels were higher in women with histologic chorioamnionitis after preterm PROM [65], and Hein et al [57] and Espinoza et al [66] found higher amniotic fluid defensins in women with histologic and/or culture-based evidence of infection in gestational tissues. By comparison, the functional or pathological significance of high defensin levels in vaginal

fluid during pregnancy seems less clear. The vagina is a space routinely exposed to a variety of microbes that may elicit local neutrophil activation and defensin production, which could be protective or contribute to the risk of PTD. In the case of undetected, subclinical membrane ruptures, defensins from the amniotic fluid may enter the vaginal fluid.

The uncertainty surrounding the meaning of high or low vaginal defensin levels in pregnancy raises challenges in interpreting our study findings. Defensins could be true mediators in a causal pathway to PTD, or mainly markers of an underlying inflammatory process. One of our most intriguing findings was the effect modification by racial/ethnic group. Defensin levels above the median were positively linked to BV in African-Americans but not in non-Hispanic whites. This might be explained by confounding due to the prevalence of other vaginal infections that often co-occur with BV and may have been more prevalent in African American POUCH study participants [35, 67]. One such infectious organism, *Trichomonas vaginalis*, has been linked with PTD and low birth weight [15]. In at least one study, asymptomatic trichomoniasis was associated with higher vaginal fluid defensin levels measured at 7 to 22 weeks of pregnancy [68]. Another explanation could be race/ethnic group differences in level of host innate immune response to BV. Alternatively, because BV is broadly defined, higher defensin levels might signify the presence of specific BV-related bacteria which may be more prevalent in African-American BV cases than in non-Hispanic white BV cases. We also observed a positive association between vaginal defensin levels above the median and PTL/PROM PTD in African Americans but not in non-Hispanic whites. It is possible that higher defensins were a marker of a more vigorous underlying inflammatory process in

the gestational tissues of African American participants compared to that in non-Hispanic whites. Or, as observed in the study be Balu et al, higher vaginal defensin levels may be most strongly linked to the earliest PTL/PROM PTDs, and these earlier spontaneous PTDs occur more frequently in African Americans compared with non-Hispanic whites.

Expressions of defensins could be constitutive, inducible by infectious and/or inflammatory stimuli, or both [69, 70]. HNP 1-3 in male urethral lavages have been indicated to be inducible by *N. gonorrhoeae* and *Chlamydia* [70].It is not clear if defensins in vaginal fluid can be inducible by pathogens. It is possible that vaginal fluid defensins are induced by some specific BV-related bacteria. Defensins have been shown to be inducible by proinflammatory cytokines [71]. In Simhan et al's study, vaginal fluid defensins were reported to be positively associated with IL-8. Incorporating information on both defensins and cytokines networks will aid in revealing the relations between vaginal defensins and the related inflammatory process.

A major strength of our study was the composition of the cohort, which consisted of high and low-risk women, sampled from many settings and representing a wide range of socioeconomic backgrounds. In addition, sampling of vaginal fluid for defensins was performed approximately at mid-pregnancy, providing evidence for the early appearance of an underlying inflammatory process and a maternal immune response linked to PTD in African Americans. The use of non-invasive methods (i.e. vaginal fluid sampling) for detecting a maternal inflammatory response offers greater clinical applications. We also separated medically indicated PTD from spontaneous PTD (PTL and PROM), the latter considered to occur more frequently in association with infection and/or inflammation [7, 72, 73]. The specificity of our study results, i.e. defensins related to PTL/PROM PTD but

not medically indicated PTD, offers biologic coherence to our findings and supports the link between defensins and the infection/inflammation pathway.

An important limitation is that BV status and defensin levels were measured only once and at the same time, thereby precluding the establishment of a temporal relation between BV and elevated defensin levels. We did not incorporate information on antimicrobial use in pregnancy, or systematically measure other relevant vaginal infections such as trichomoniasis, and these factors may have had some bearing on vaginal defensin levels. The sample size of the subcohort did not provide enough statistical power to detect a 2.5 odds ratio for PROM among African American women with defensins above the median, and did not allow us to further partition PTD subtypes into early (i.e. < 32 weeks) versus later. Our defensin level cutoff for 'exposure', i.e., above the median, was based on defensins distribution among both non-Hispanic white and African American women who delivered at term and had normal MSAFP. Had we used the race-specific median, only 5% of non-Hispanic white and 4% of African American women would have been assigned to a different category above or below the median. The median cutoff is appropriate for an association study but less informative for purposes of clinical screening. However this cutoff may prove useful in future clinical studies that use a combination of biomarkers to predict PTD or that select at-risk subsets for targeted clinical trials.

Our study reinforces previous findings suggesting that an inflammatory process in the lower genital tract is associated with subsequent PTD, and this process may be marked by higher vaginal defensin levels. The observed effect modification by race/ethnic groups in the relations between BV status, defensin levels, and PTD is a new finding and requires

additional studies to discover underlying explanations for these results. The findings invite further investigations comparing the vaginal milieu across different race/ethnic groups in relation to defensin levels and associated bacteria. This is particularly relevant because African Americans have about twice the rate of PTD as other racial/ethnical groups and there is strong evidence that an infection/inflammation pathway contributes to this disparity [60, 61]; however, the details of how and why have been difficult to uncover. Detecting variations in vaginal microbial ecology and host immune responses across racial/ethnic groups may provide important clues.

Table 1. Maternal characteristics of 1031 non-Hispanic White and African-American POUCH study women with vaginal fluid analyses

Maternal Characteristics	Non-Hispanic White (n=569)	African-American (n=462)
	No. (%) (%)#	No. (%) (%)#
Age (years)		
<20	51 (9) (9)	126 (27) (27)§
20-29	319 (56) (57)	272 (59) (60)
≥30	199 (35) (34)	64 (14) (13)
Education		
<12 yrs (age<20)	30 (5) (5)	90 (20) (20)§
<12 yrs (age≥20)	33 (6) (6)	88 (19) (19)
12yrs	166 (29) (27)	139 (30) (31)
> 12 yrs	340 (60) (62)	145 (31) (30)
Smoking		
No smoke during pregnancy	404 (71) (73)	325 (71) (69)§
Stopped before enrollment	62 (11) (10)	38 (8) (9)
Smoked <1/2 pack/day at enrollment	56 (10) (9)	84 (18) (19)
Smoked at least 1/2 pack/day at enrollment	47 (8) (8)	15 (3) (3)
Medicaid Insured*		
No	359 (63) (65)	74 (16) (16)§
Yes	209 (37) (35)	388 (84) (84)
Week of Pregnancy At Enrollment		
<20 wks	85 (15) (14)	91 (20) (20)§
20-24 wks	414 (73) (75)	319 (69) (69)
25-27 wks	70 (12) (11)	52 (11) (11)
Parity†		
0 live birth	231 (41) (39)	192 (42) (42)
>=1 live birth	337 (59) (61)	270 (58) (58)
Pregnancy Outcome		
Term	412 (72) (90)	375 (81) (86)§
Medically Indicated PTD	49 (9) (3)	28 (6) (5)
PTL	60 (10) (4)	39 (9) (6)
PROM	48 (9) (3)	20 (4) (3)
Bacterial Vaginosis		
Negative	482 (85) (85)	318 (69) (68)§
Intermediate	46 (8) (9)	54 (12) (12)
Positive	41 (7) (6)	90 (19) (20)
	Mean (SD) Mean#	**Mean (SD) Mean#**
Defensin (log transformed)	7.6 (0.1) 7.7	7.4 (0.1) 7.3§

* Data missing for 1 woman
† Data missing for 1 woman
§ p<0.05
# Weighted percent/mean

Table 2. Race/ethnicity-specific relations between maternal characteristics and defensin levels

Maternal Characteristics	Non-Hispanic White (n=569)		African-American (n=462)	
	Low defensin (n=272)	High defensin (n=297)	Low defensin (n=242)	High defensin (n=220)
	No. (% #)	No. (%)	No. (%)	No. (%)
Age (years)				
<20	28 (49) §	23 (51)	64 (51)	62 (49)
20-29	134 (41)	185 (59)	137 (51)	135 (49)
≥30	110 (54)	89 (46)	41 (64)	23 (36)
Education				
<12 yrs (age<20)	18 (58)	12 (42)	51 (57)	39 (43)
<12 yrs (age≥20)	17 (52)	16 (48)	43 (52)	45 (48)
12yrs	84 (48)	82 (52)	68 (49)	71 (51)
> 12 yrs	153 (44)	187 (56)	80 (55)	65 (45)
Smoking				
No smoke during pregnancy	183 (44)	221 (56)	165 (51)	160 (49)
Stopped before enrollment	28 (42)	34 (58)	22 (59)	16 (41)
Smoked <1/2 pack/day at enrollment	33 (58)	23 (42)	45 (55)	39 (45)
Smoked at least 1/2 pack/day at enrollment	28 (61)	19 (39)	10 (67)	5 (33)
Medicaid Insured *				
No	165 (44)	194 (56)	45 (60)	29 (40)
Yes	106 (50)	103 (50)	197 (52)	191 (48)
Week of Pregnancy At Enrollment				
<20 wks	44 (47)	41 (53)	60 (67) §	31 (33)
20-24 wks	190 (46)	224 (54)	158 (50)	161 (50)
25-27 wks	38 (49)	32 (51)	24 (46)	28 (54)
Parity †				
0 live birth	110 (49)	121 (51)	96 (50)	96 (50)
>=1 live birth	161 (44)	176 (56)	146 (55)	124 (45)
Bacterial Vaginosis				
Negative	226 (44)	256 (56)	180 (57) §	138 (43)
Intermediate	24 (56)	22 (44)	25 (47)	29 (53)
Positive	22 (58)	19 (42)	37 (43)	53 (57)

* Data missing for 1 woman

† Data missing for 1 woman

§ $p<0.05$

Weighted percent

Table 3. Race-specific unadjusted odds ratio of PTD subtypes in women with high (>=median) vs low (<median) defensin levels

	non-Hispanic White (N=569)	**African-American (N=462)**
	Unadjusted OR	Unadjusted OR
Term	Ref	Ref
Medically Indicated	0.8 (0.4, 1.5)	0.7 (0.3, 1.5)
PTL	1.1 (0.6, 1.9)	2.1 (1.0, 4.3)
PROM	0.6 (0.3, 1.1)	2.5 (0.9, 6.6)

Table 4. Race-specific adjusted odds ratio of PTD subtypes in women with high (>=median) vs low (<median) defensin levels

	non-Hispanic White (N=569)		**African-American (N=462)**	
	Adjusted OR §	Adjusted OR*	Adjusted OR §	Adjusted OR#
Term	Ref	Ref	Ref	Ref
Medically Indicated	0.8 (0.4, 1.5)	0.8 (0.4,1.5)	0.7 (0.3, 1.5)	0.7 (0.3, 1.6)
PTL	1.1 (0.6, 1.9)	1.1 (0.6, 1.9)	2.1 (1.0, 4.3)	2.2 (1.1, 4.6)
PROM	0.6 (0.3, 1.2)	0.6 (0.3, 1.2)	2.5 (0.9, 7.1)	2.7 (0.9, 7.6)

§ Adjusted for BV status

* Adjusted for maternal age and BV status

Adjusted for weeks of gestation at enrollment and BV status

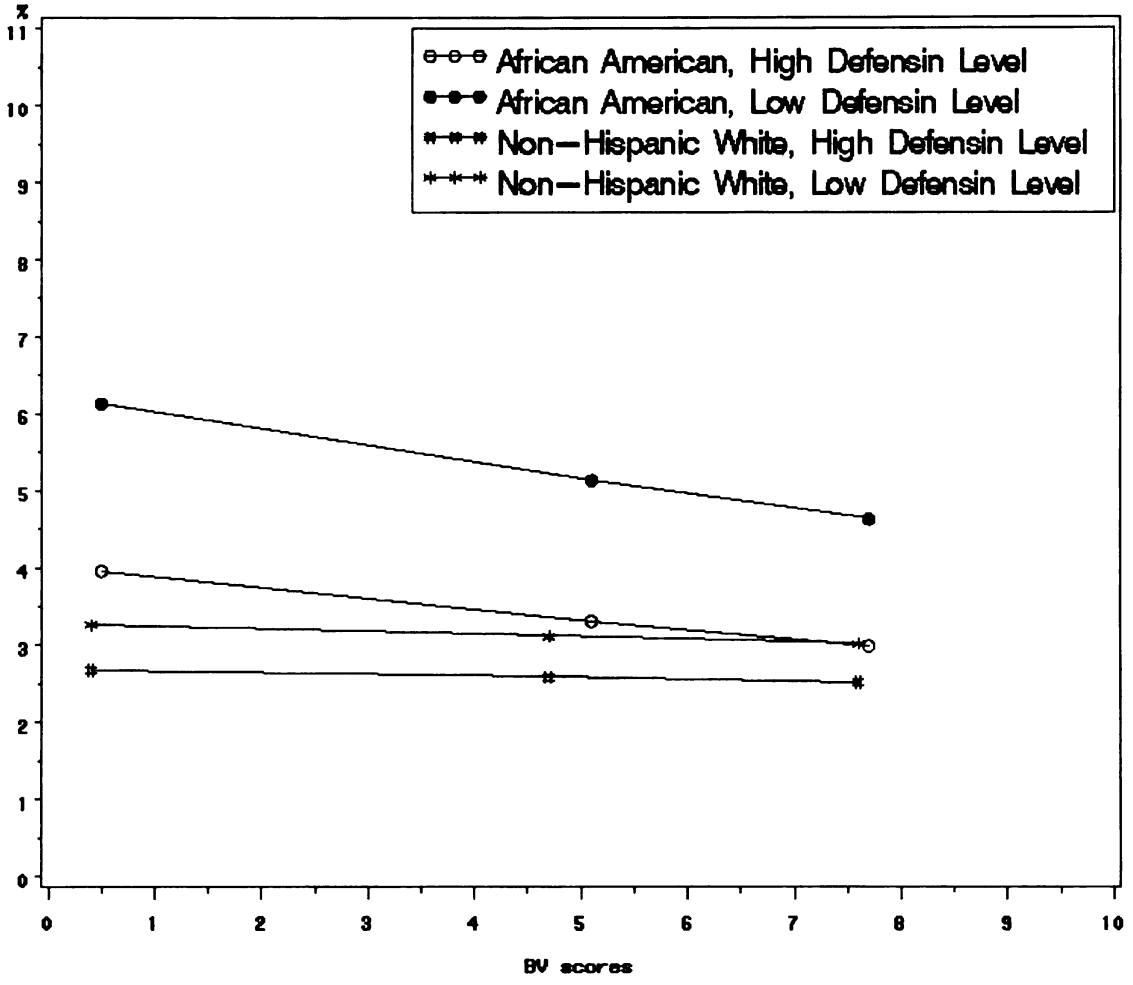

Figure 1a. Medically indicated PTD

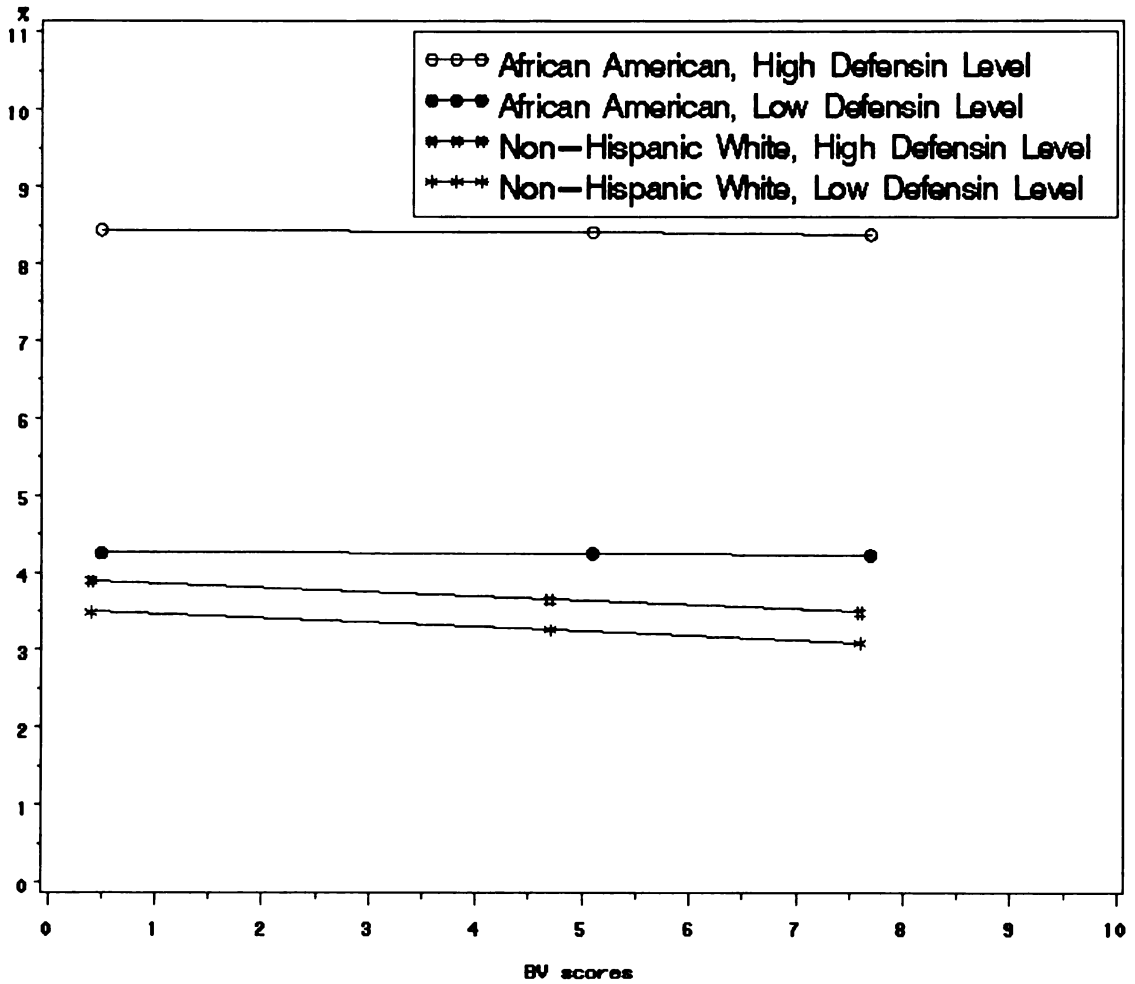

Figure 1b. PTL

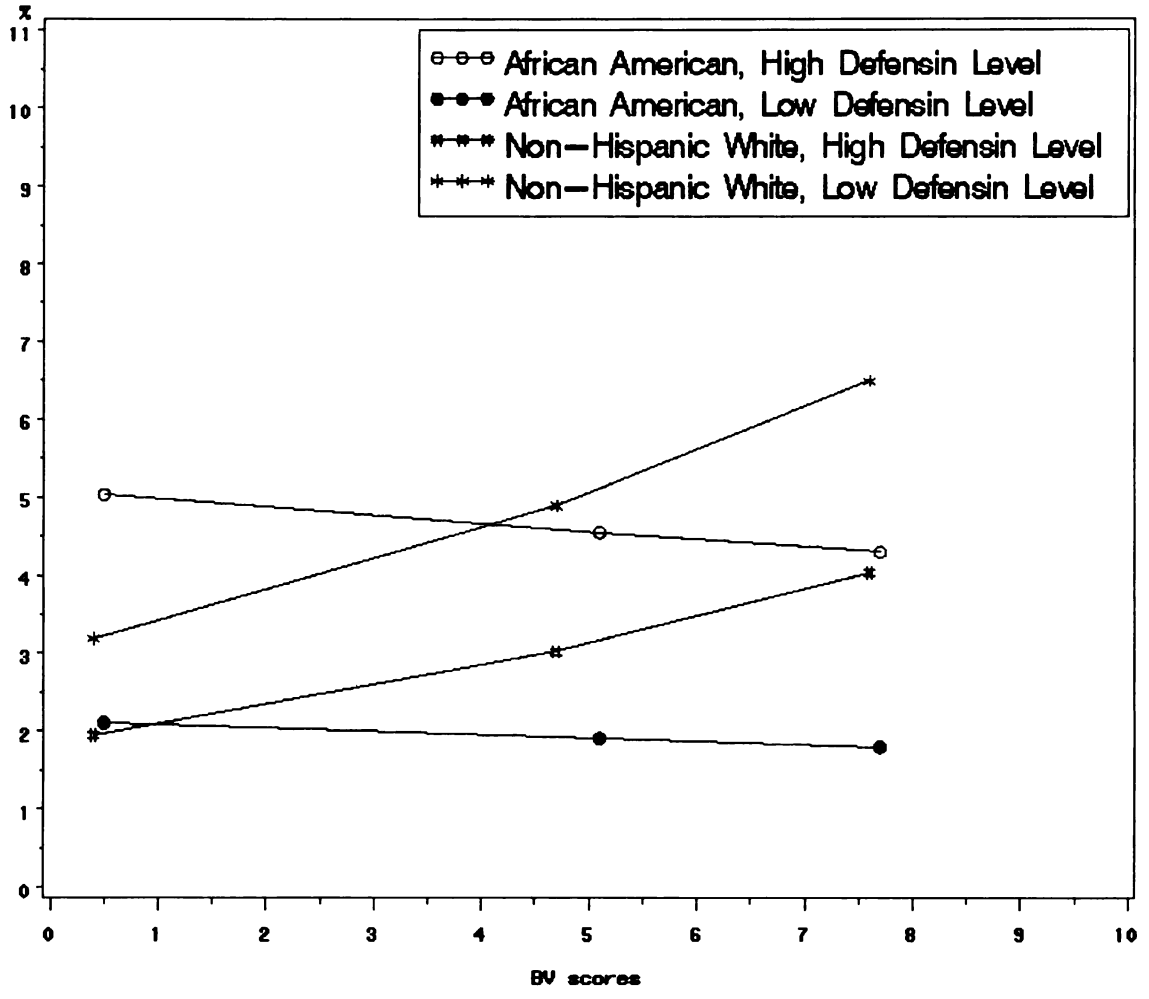

Figure 1c. PROM

Figure 1. Probability of PTD subtypes by BV status among 4 groups; groups defined by defensin levels (high/low) and race/ethnicity (non-Hispanic white/African American)

Reference

1. Berkowitz, G.S. and E. Papiernik, *Epidemiology of preterm birth.* Epidemiol Rev, 1993. **15**(2): p. 414-43.

2. Hack, M. and A.A. Fanaroff, *Outcomes of extremely immature infants--a perinatal dilemma.* N Engl J Med, 1993. **329**(22): p. 1649-50.

3. Hamilton, B.E., A.M. Minino, J.A. Martin, et al., *Annual summary of vital statistics: 2005.* Pediatrics, 2007. **119**(2): p. 345-60.

4. Goldenberg, R.L. and D.J. Rouse, *Prevention of premature birth.* N Engl J Med, 1998. **339**(5): p. 313-20.

5. Tucker, J.M., R.L. Goldenberg, R.O. Davis, et al., *Etiologies of preterm birth in an indigent population: is prevention a logical expectation?* Obstet Gynecol, 1991. **77**(3): p. 343-7.

6. Hillier, S.L., S.S. Witkin, M.A. Krohn, et al., *The relationship of amniotic fluid cytokines and preterm delivery, amniotic fluid infection, histologic chorioamnionitis, and chorioamnion infection.* Obstet Gynecol, 1993. **81**(6): p. 941-8.

7. Goldenberg, R.L., J.C. Hauth, and W.W. Andrews, *Intrauterine infection and preterm delivery.* N Engl J Med, 2000. **342**(20): p. 1500-7.

8. Gibbs, R.S., R. Romero, S.L. Hillier, et al., *A review of premature birth and subclinical infection.* Am J Obstet Gynecol, 1992. **166**(5): p. 1515-28.

9. Sherman, D.J., J. Tovbin, T. Lazarovich, et al., *Chorioamnionitis caused by gram-negative bacteria as an etiologic factor in preterm birth.* Eur J Clin Microbiol Infect Dis, 1997. **16**(6): p. 417-23.

10. Hillier, S.L., J. Martius, M. Krohn, et al., *A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity.* N Engl J Med, 1988. **319**(15): p. 972-8.

11. Romero, R., J. Espinoza, L.F. Goncalves, et al., *Inflammation in preterm and term labour and delivery.* Semin Fetal Neonatal Med, 2006. **11**(5): p. 317-26.

12. Kimberlin, D.F. and W.W. Andrews, *Bacterial vaginosis: association with adverse pregnancy outcome.* Semin Perinatol, 1998. **22**(4): p. 242-50.

13. Svare, J.A., H. Schmidt, B.B. Hansen, et al., *Bacterial vaginosis in a cohort of Danish pregnant women: prevalence and relationship with preterm delivery, low birthweight and perinatal infections.* Bjog, 2006. **113**(12): p. 1419-25.

14. Hillier, S.L., R.P. Nugent, D.A. Eschenbach, et al., *Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group.* N Engl J Med, 1995. **333**(26): p. 1737-42.

15. Cotch, M.F., J.G. Pastorek, 2nd, R.P. Nugent, et al., *Trichomonas vaginalis associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group.* Sex Transm Dis, 1997. **24**(6): p. 353-60.

16. Meis, P.J., R.L. Goldenberg, B. Mercer, et al., *The preterm prediction study: significance of vaginal infections. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network.* Am J Obstet Gynecol, 1995. **173**(4): p. 1231-5.

17. Regan, J.A., M.A. Klebanoff, R.P. Nugent, et al., *Colonization with group B streptococci in pregnancy and adverse outcome. VIP Study Group.* Am J Obstet Gynecol, 1996. **174**(4): p. 1354-60.

18. Leitich, H., B. Bodner-Adler, M. Brunbauer, et al., *Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis.* Am J Obstet Gynecol, 2003. **189**(1): p. 139-47.

19. Krohn, M.A., S.L. Hillier, M.L. Lee, et al., *Vaginal Bacteroides species are associated with an increased rate of preterm delivery among women in preterm labor.* J Infect Dis, 1991. **164**(1): p. 88-93.

20. Nugent, R.P., M.A. Krohn, and S.L. Hillier, *Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation.* J Clin Microbiol, 1991. **29**(2): p. 297-301.

21. Fredricks, D.N., T.L. Fiedler, and J.M. Marrazzo, *Molecular identification of bacteria associated with bacterial vaginosis.* N Engl J Med, 2005. **353**(18): p. 1899-911.

22. Burton, J.P., E. Devillard, P.A. Cadieux, et al., *Detection of Atopobium vaginae in postmenopausal women by cultivation-independent methods warrants further investigation.* J Clin Microbiol, 2004. **42**(4): p. 1829-31.

23. Ferris, M.J., A. Masztal, K.E. Aldridge, et al., *Association of Atopobium vaginae, a recently described metronidazole resistant anaerobe, with bacterial vaginosis.* BMC Infect Dis, 2004. **4**: p. 5.

24. Thanavuth, A., A. Chalermchockcharoenkit, D. Boriboonhirunsarn, et al., *Prevalence of bacterial vaginosis in Thai pregnant women with preterm labor in Siriraj hospital.* J Med Assoc Thai, 2007. **90**(3): p. 437-41.

25. Tolosa, J.E., S. Chaithongwongwatthana, S. Daly, et al., *The International Infections in Pregnancy (IIP) study: variations in the prevalence of bacterial vaginosis and distribution of morphotypes in vaginal smears among pregnant women.* Am J Obstet Gynecol, 2006. **195**(5): p. 1198-204.

26. Cristiano, L., N. Coffetti, G. Dalvai, et al., *Bacterial vaginosis: prevalence in outpatients, association with some micro-organisms and laboratory indices.* Genitourin Med, 1989. **65**(6): p. 382-7.

27. McGregor, J.A., J.I. French, R. Parker, et al., *Prevention of premature birth by screening and treatment for common genital tract infections: results of a prospective controlled evaluation.* Am J Obstet Gynecol, 1995. **173**(1): p. 157-67.

28. Purwar, M., S. Ughade, B. Bhagat, et al., *Bacterial vaginosis in early pregnancy and adverse pregnancy outcome.* J Obstet Gynaecol Res, 2001. **27**(4): p. 175-81.

29. Wiesenfeld, H.C., S.L. Hillier, M.A. Krohn, et al., *Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease.* Obstet Gynecol, 2002. **100**(3): p. 456-63.

30. Bennett, B.B., P. Clark, P. Riley, et al., *The association of asymptomatic bacterial vaginosis with upper genital tract infection.* Prim Care Update Ob Gyns, 1998. **5**(4): p. 150.

31. Peipert, J.F., A.B. Montagno, A.S. Cooper, et al., *Bacterial vaginosis as a risk factor for upper genital tract infection.* Am J Obstet Gynecol, 1997. **177**(5): p. 1184-7.

32. Schwebke, J.R. and H.L. Weiss, *Interrelationships of bacterial vaginosis and cervical inflammation.* Sex Transm Dis, 2002. **29**(1): p. 59-64.

33. Allsworth, J.E. and J.F. Peipert, *Prevalence of bacterial vaginosis: 2001-2004 National Health and Nutrition Examination Survey data.* Obstet Gynecol, 2007. **109**(1): p. 114-20.

34. Rajamanoharan, S., N. Low, S.B. Jones, et al., *Bacterial vaginosis, ethnicity, and the use of genital cleaning agents: a case control study.* Sex Transm Dis, 1999. **26**(7): p. 404-9.

35. Goldenberg, R.L., M.A. Klebanoff, R. Nugent, et al., *Bacterial colonization of the vagina during pregnancy in four ethnic groups. Vaginal Infections and Prematurity Study Group.* Am J Obstet Gynecol, 1996. **174**(5): p. 1618-21.

36. Morales, W.J., S. Schorr, and J. Albritton, *Effect of metronidazole in patients with preterm birth in preceding pregnancy and bacterial vaginosis: a placebo-*

controlled, double-blind study. Am J Obstet Gynecol, 1994. **171**(2): p. 345-7; discussion 348-9.

37. Hauth, J.C., R.L. Goldenberg, W.W. Andrews, et al., *Reduced incidence of preterm delivery with metronidazole and erythromycin in women with bacterial vaginosis.* N Engl J Med, 1995. **333**(26): p. 1732-6.

38. Ugwumadu, A., I. Manyonda, F. Reid, et al., *Effect of early oral clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: a randomised controlled trial.* Lancet, 2003. **361**(9362): p. 983-8.

39. McDonald, H.M., J.A. O'Loughlin, R. Vigneswaran, et al., *Impact of metronidazole therapy on preterm birth in women with bacterial vaginosis flora (Gardnerella vaginalis): a randomised, placebo controlled trial.* Br J Obstet Gynaecol, 1997. **104**(12): p. 1391-7.

40. Larsson, P.G., L. Fahraeus, B. Carlsson, et al., *Late miscarriage and preterm birth after treatment with clindamycin: a randomised consent design study according to Zelen.* Bjog, 2006. **113**(6): p. 629-37.

41. Guaschino, S., E. Ricci, M. Franchi, et al., *Treatment of asymptomatic bacterial vaginosis to prevent pre-term delivery: a randomised trial.* Eur J Obstet Gynecol Reprod Biol, 2003. **110**(2): p. 149-52.

42. Kiss, H., L. Petricevic, and P. Husslein, *Prospective randomised controlled trial of an infection screening programme to reduce the rate of preterm delivery.* Bmj, 2004. **329**(7462): p. 371.

43. Andrews, W.W., B.M. Sibai, E.A. Thom, et al., *Randomized clinical trial of metronidazole plus erythromycin to prevent spontaneous preterm delivery in fetal fibronectin-positive women.* Obstet Gynecol, 2003. **101**(5 Pt 1): p. 847-55.

44. Lamont, R.F., S.L. Duncan, D. Mandal, et al., *Intravaginal clindamycin to reduce preterm birth in women with abnormal genital tract flora.* Obstet Gynecol, 2003. **101**(3): p. 516-22.

45. Kekki, M., T. Kurki, J. Pelkonen, et al., *Vaginal clindamycin in preventing preterm birth and peripartal infections in asymptomatic women with bacterial vaginosis: a randomized, controlled trial.* Obstet Gynecol, 2001. **97**(5 Pt 1): p. 643-8.

46. Klebanoff, M.A., J.C. Carey, J.C. Hauth, et al., *Failure of metronidazole to prevent preterm delivery among pregnant women with asymptomatic Trichomonas vaginalis infection.* N Engl J Med, 2001. **345**(7): p. 487-93.

47. Carey, J.C., M.A. Klebanoff, J.C. Hauth, et al., *Metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units.* N Engl J Med, 2000. **342**(8): p. 534-40.

48. Leitich, H., M. Brunbauer, B. Bodner-Adler, et al., *Antibiotic treatment of bacterial vaginosis in pregnancy: a meta-analysis.* Am J Obstet Gynecol, 2003. **188**(3): p. 752-8.

49. McDonald, H., P. Brocklehurst, and J. Parsons, *Antibiotics for treating bacterial vaginosis in pregnancy.* Cochrane Database Syst Rev, 2005(1): p. CD000262.

50. Goldenberg, R.L., A. Mwatha, J.S. Read, et al., *The HPTN 024 Study: the efficacy of antibiotics to prevent chorioamnionitis and preterm birth.* Am J Obstet Gynecol, 2006. **194**(3): p. 650-61.

51. Rogan, M.P., P. Geraghty, C.M. Greene, et al., *Antimicrobial proteins and polypeptides in pulmonary innate defence.* Respir Res, 2006. **7**: p. 29.

52. Selsted, M.E., D. Szklarek, and R.I. Lehrer, *Purification and antibacterial activity of antimicrobial peptides of rabbit granulocytes.* Infect Immun, 1984. **45**(1): p. 150-4.

53. Ganz, T., M.E. Selsted, D. Szklarek, et al., *Defensins. Natural peptide antibiotics of human neutrophils.* J Clin Invest, 1985. **76**(4): p. 1427-35.

54. Selsted, M.E., D. Szklarek, T. Ganz, et al., *Activity of rabbit leukocyte peptides against Candida albicans.* Infect Immun, 1985. **49**(1): p. 202-6.

55. Aley, S.B., M. Zimmerman, M. Hetsko, et al., *Killing of Giardia lamblia by cryptdins and cationic neutrophil peptides.* Infect Immun, 1994. **62**(12): p. 5397-403.

56. Lehrer, R.I., K. Daher, T. Ganz, et al., *Direct inactivation of viruses by MCP-1 and MCP-2, natural peptide antibiotics from rabbit leukocytes.* J Virol, 1985. **54**(2): p. 467-72.

57. Heine, R.P., H. Wiesenfeld, L. Mortimer, et al., *Amniotic fluid defensins: potential markers of subclinical intrauterine infection.* Clin Infect Dis, 1998. **27**(3): p. 513-8.

58. Balu, R.B., D.A. Savitz, C.V. Ananth, et al., *Bacterial vaginosis, vaginal fluid neutrophil defensins, and preterm birth.* Obstet Gynecol, 2003. **101**(5 Pt 1): p. 862-8.

59. Balu, R.B., D.A. Savitz, C.V. Ananth, et al., *Bacterial vaginosis and vaginal fluid defensins during pregnancy.* Am J Obstet Gynecol, 2002. **187**(5): p. 1267-71.

60. Holzman, C., X. Lin, P. Senagore, et al., *Histologic Chorioamnionitis and Preterm Delivery.* Am J Epidemiol, 2007.

61. Kistka, Z.A., L. Palomar, K.A. Lee, et al., *Racial disparity in the frequency of recurrence of preterm birth.* Am J Obstet Gynecol, 2007. **196**(2): p. 131 e1-6.

62. Simhan, H.N. and L.M. Bodnar, *Prepregnancy body mass index, vaginal inflammation, and the racial disparity in preterm birth.* Am J Epidemiol, 2006. **163**(5): p. 459-66.

63. Menon, R., M. Merialdi, S.J. Lombardi, et al., *Differences in the placental membrane cytokine response: a possible explanation for the racial disparity in preterm birth.* Am J Reprod Immunol, 2006. **56**(2): p. 112-8.

64. Holzman, C., B. Bullen, R. Fisher, et al., *Pregnancy outcomes and community health: the POUCH study of preterm delivery.* Paediatr Perinat Epidemiol, 2001. **15 Suppl 2**: p. 136-58.

65. Grable, I.A. and R.P. Heine, *Neutrophil granule products: can they identify subclinical chorioamnionitis in patients with preterm premature rupture of membranes?* Am J Obstet Gynecol, 2003. **189**(3): p. 808-12.

66. Espinoza, J., T. Chaiworapongsa, R. Romero, et al., *Antimicrobial peptides in amniotic fluid: defensins, calprotectin and bacterial/permeability-increasing protein in patients with microbial invasion of the amniotic cavity, intra-amniotic inflammation, preterm labor and premature rupture of membranes.* J Matern Fetal Neonatal Med, 2003. **13**(1): p. 2-21.

67. Newton, E.R., J.M. Piper, R.N. Shain, et al., *Predictors of the vaginal microflora.* Am J Obstet Gynecol, 2001. **184**(5): p. 845-53; discussion 853-5.

68. Simhan, H.N., B.L. Anderson, M.A. Krohn, et al., *Host immune consequences of asymptomatic Trichomonas vaginalis infection in pregnancy.* Am J Obstet Gynecol, 2007. **196**(1): p. 59 e1-5.

69. Chen, H., Z. Xu, L. Peng, et al., *Recent advances in the research and development of human defensins.* Peptides, 2006. **27**(4): p. 931-40.

70. Porter, E., H. Yang, S. Yavagal, et al., *Distinct defensin profiles in Neisseria gonorrhoeae and Chlamydia trachomatis urethritis reveal novel epithelial cell-neutrophil interactions.* Infect Immun, 2005. **73**(8): p. 4823-33.

71. Jenssen, H., P. Hamill, and R.E. Hancock, *Peptide antimicrobial agents.* Clin Microbiol Rev, 2006. **19**(3): p. 491-511.

72. Andrews, W.W., R.L. Goldenberg, B. Mercer, et al., *The Preterm Prediction Study: association of second-trimester genitourinary chlamydia infection with subsequent spontaneous preterm birth.* Am J Obstet Gynecol, 2000. **183**(3): p. 662-8.

73. Goepfert, A.R., R.L. Goldenberg, B. Mercer, et al., *The preterm prediction study: quantitative fetal fibronectin values and the prediction of spontaneous preterm birth. The National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network.* Am J Obstet Gynecol, 2000. **183**(6): p. 1480-3.