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FIRST TRIMESTER BACTERIAL VAGINOSIS, INDIVIDUAL MICROORGANISM LEVELS AND RISK OF SECOND TRIMESTER PREGNANCY LOSS AMONG URBAN WOMEN

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Abstract

Objective—To examine the role of first trimester bacterial vaginosis (BV) and level of BV-associated microorganisms diagnosed using the Nugent's Gram stain criteria and the risk of second trimester pregnancy loss among urban women.

Design—Prospective cohort study.

Setting—Pregnant women seeking prenatal care in the first trimester.

Patients—Women presenting for their first prenatal care visit who had completed 12 weeks gestation or less and resided within Philadelphia PA.

Main Outcome Measures—Pregnancy Loss.

Results—1948 women enrolled at a mean gestational age of 10 weeks (range 7.4 to 12.6 weeks). Women with the highest level of BV-related vaginal flora alteration compared to women with normal vaginal flora had over a twofold increased risk of second trimester pregnancy loss after adjustment for confounders (adjusted HR: 2.49, 95% CI: 1.13 to 5.48). Low *Lactobacillus spp*, and the absence of *Lactobacillus spp.* were also significantly related to the risk of second trimester pregnancy loss (aHR: 1.32, 95% CI: 1.10–1.64 and aHR: 2.30, 95% CI: 1.09 – 4.85; respectively).

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No conflicts of interest.

Conclusions—Overall BV positivity was not related to second trimester pregnancy loss. Comparing the highest to lowest tertile of BV positivity in early pregnancy conferred a two-fold increased risk of second trimester pregnancy loss and low amounts or the absence of *Lactobacillus spp.* in the first trimester also significantly increased the risk of second trimester pregnancy loss.

Keywords

Bacterial Vaginosis; *Lactobacillus ssp.*; Second Trimester Pregnancy Loss

INTRODUCTION

Bacterial vaginosis (BV) has been prospectively linked to various adverse reproductive and pregnancy-related events including endometritis, pelvic inflammatory disease, preterm labor and preterm delivery, and postpartum endometritis (1–8). BV is a polymicrobial superficial vaginal condition characterized by a reduced number of hydrogen-peroxide producing (H_2O_2) *Lactobacillus* spp. and an overgrowth of gram negative and/or anaerobic bacteria the most common being *Bacteroides spp., Gardnerella vaginalis, Mobiluncus* spp., *Mycoplasma hominus* and *Ureaplasma urealyticum* (9).

The organisms characteristic of BV have been shown to ascend from the lower genital tract, through the cervix, resulting in deciduitis, chorioamnionitis and amniotic fluid and fetal infection (10). BV-associated microorganisms have been recovered from the amniotic fluid of women in premature labor with intact membranes(11) and early reports have suggested that among infants delivered prematurely, the most frequent microorganisms isolated from the placenta were *U. urealyticum* (47%) and *G. vaginalis* (26%) (6). The high concentration of these BV-associated organisms has been related to the reduction of leukocytes to halt localized infection, an increased level of endotoxins and protease enzymes stimulating proinflammatory cytokine production which can result in intrauterine death, the release of sialidases and mucinases promoting placental inflammation and weakening of the chorioamniotic membrane, and an increase in the ascension of other lower genital tract organisms (11–13).

Studies have shown an overall two-fold increased risk of spontaneous preterm birth before 37 weeks gestation (PTB) among women diagnosed with bacterial vaginosis (14–17) with over a sevenfold increased risk of PTB among women diagnosed with BV prior to 16 weeks and a fourfold increased risk of PTB among women diagnosed with BV before 20 weeks gestation (18). However, clinical trials examining the efficacy of BV treatment to reduce the risk of PTB among symptomatic and asymptomatic women have found no reduction in risk of PTL or PTB among treated BV positive pregnant women (19–20).

Prior studies, with small sample sizes and/or limited to high-risk women undergoing in vitro-fertilization, have suggested a role of BV with failed conception and increased risk of pregnancy loss (21–23). The ascent of these BV-related organisms during early pregnancy and/or the concurrent endometritis present prior to pregnancy due to chronic BV most likely leads to the complications of proper implantation and early placental development which increases the risk of SAB among BV positive women. We conducted a large prospective cohort study among urban pregnant women with a high prevalence of BV to examine the role of first trimester bacterial vaginosis and level of BV-related microorganisms, diagnosed using the Nugent's Gram stain criteria, and second trimester pregnancy loss. We assessed the role of overall BV positivity and degrees of BV-related vaginal flora alterations on the risk of second trimester pregnancy loss, as well as examined the relationship between each of the BV-associated morphotypes, *Bacteroides* spp., *G. vaginalis, Mobiluncus* spp, and *Lactobacillus* spp. related to the risk of second trimester pregnancy loss in this population.

METHODS

Study Design

During the period September 2001 through June 2004, 2249 pregnant women presenting for their first prenatal care visit to two obstetrical practices at the Hospital of the University of Pennsylvania (HUP) who had completed 12 weeks gestation or less based on self-reported last menstrual period and resided within the city of Philadelphia were eligible to participate in the project. To ensure the generalizability of the study results, one of the recruitment sites was a private obstetrical practice providing care to insured women, and the other recruitment site was a publicly-funded obstetric clinic. This study design allowed an assessment of first trimester exposures, including BV positivity, among a group of healthy, pregnancy women seeking routine low risk, first trimester prenatal care. Nurse interviewers specifically trained for the project screened each women for study eligibility, explained the purpose and practice of the study, and obtained informed consent. The nurse interviewers facilitated vaginal swab collection for BV assessment, obtained urine samples for toxicological confirmation of cigarette, marijuana, cocaine and alcohol use; collected contact information to inform follow-up activities; performed a comprehensive abstraction of the prenatal and ultrasound record; and completed the standardized baseline questionnaire. The baseline questionnaire contained information regarding maternal age at the time of conception, parity, gravidity, prior pregnancy outcomes, demographic information, substance use, and measures of psychosocial stress. Perceived stress in the prior one month was measured by the Cohen's perceived stress scale which measures the degree to which situations in the past month are appraised as stressful and has been validated among young women (24). Information on the presence and magnitude of vaginal bleeding was also collected as well as information on potential confounders, such as concurrent sexually transmitted diseases, history of BV, vaginal douching practices, history of incompetent cervix and sexual practices. The questionnaire was administered in a confidential location within the office by a female nurse interviewer. The remainder of prenatal care and BV screening and treatment was under the direction of the health care provider. The protocol and consent forms were approved by the Institutional Review Board of the University of Pennsylvania and Temple University.

Exposure Assessment

The exposure of interest was BV status at enrollment diagnosed using the Nugent's Gram stain criteria. At the enrollment visit, provider-collected (53%) or self-collected (47%) vaginal swabs were obtained for each woman participating in the study. We have previously documented excellent validity and intra-rater and inter-rater reliability of BV assessment, among this cohort, comparing these two methods of swab collection (25). Immediately after collection, the vaginal secretions were put on a glass slide, air dried and the slides were sent weekly to the Clinical Microbiology Laboratory at the HUP. Slides were Gram stained and blindly interpreted for BV status using the criteria described by Nugent et al which involves the quantification of the amount of *Lactobacillus* spp., *Mobiluncus* spp. and *Bacteroides* spp./*Gardnerella* spp (26).

According to the Nugent criteria, the amount of three morphotypes characteristic of BV were quantified and scored: *Lactobacillus spp., Mobiluncus species* and *Gardnerella vaginalis*. The range of scores for these morphotypes was used in the current analysis. For *Lactobacillus ssp.*, the scores range from 0 to 4, with 0 indicating that 30 or more organisms were found and 4 indicating that no organisms were found in the sample. In contrast, for *Gardnerella vaginalis*, a score of 0 indicates that no organisms were found and the highest score, 4, indicates that 30 or more organisms were found. For *Mobiluncus*, the scores ranged from 0 to 2, with a score of 2 indicating that 5 or more organisms were identified in the sample and 0 indicates no organisms were found. The Nugent criteria has been shown to have a high sensitivity and specificity compared to Amsel's criteria (89% and 83%, respectively) (27–28).

A summary BV score was computed ranging from 0 to 10 and this score was dichotomized with values of 7 and over indicating a case of BV. This dichotomized BV scoring system has been used extensively in both clinical and epidemiologic studies and has been found to be a reliable method of detecting overall BV positivity (24). BV exposure was also classified as variations in BV-related vaginal flora alterations using the continuous BV score ranging from 0–10. Finally, three levels of overall BV-related vaginal flora alterations were created indicating normal vaginal flora (total BV score of 0–3), intermediate vaginal flora (total BV score of 4–6) and abnormal vaginal flora (total BV score of 7 and over) (26). These levels have been reported to be sensitive for characterizing alterations in vaginal flora, diagnosing BV and have been assessed for reliability elsewhere (27).

We were also interested in the contribution of each of the individual morphotypes on the risk of second trimester pregnancy loss and included the continuous scores for each of the three morphotypes (*Lactobacillus* spp., *Mobiluncus* spp. and *Bacteroides* spp./*Gardnerella* spp*)* to examine the individual contribution of these morphotypes to the risk of pregnancy loss. For this study, both the interviewer and obstetrician were blinded to the project-related Gram stain results; however, information on provider diagnosed and treated BV throughout the pregnancy was collected through medical record review.

Pregnancy Outcome Assessment

The outcome of interest, pregnancy loss, was identified through self-report and subsequent medical record confirmation. For this study, a pregnancy loss was classified as the loss of an intrauterine pregnancy at or before 20 weeks gestation. Any pregnancy loss occurring at enrollment was excluded from the study since we were interested in the prospective role of BV and pregnancy loss. Given the study enrollment criteria of healthy pregnant women seeking routine prenatal care prior to 12 weeks gestation, the majority of pregnancy losses captured during the study period were second trimester losses (15–18 weeks). Although recruitment of this study population reflects the majority of healthy pregnant women, many first trimester pregnancy losses occurring prior to seeking prenatal care may have been missed thus these study results primarily reflect the risk of second trimester pregnancy loss.

Women with a diagnosis of multiple gestations, hydatidiform mole, ectopic pregnancy (n=76) or induced abortion (n=74) during the follow-up period were excluded from the study. Through medical record/ultrasound review we found that 45 women participating in the study were over 12 weeks gestation at the time of enrollment, these women were also excluded leaving 1948 eligible, enrolled women. Almost sixty percent of pregnancy losses were confirmed through ultrasound and/or medical record review; however, the vast majority of ultrasounds conducted as part of this study were to confirm the pregnancy loss (98%) thus information on the presence of incompetent cervix or suspected congenital anomalies is not available.

Sample Size and Data Analysis

We estimated relative risk of second trimester pregnancy loss due to various classifications of BV as adjusted hazard ratios (with 95 percent confidence intervals) in Cox regression analysis with left-truncation and right-censoring. Days of gestations were used as the time variable with pregnancies entered at the time of enrollment and observed until the following events: pregnancy loss, induced abortion, lost-to-follow-up, or completion of 20 weeks gestation. As described above, separate models were created for BV positivity defined dichotomously (scores of 0–6 compared to 7–10), continuously with total BV score ranging from 0–10 (overall median value = 4.0, mean value = 4.9), and by tertile (normal, intermediate, or abnormal BV-related vaginal flora). In addition, the scores of *Lactobacillus* spp., *Mobiluncus* spp. and *Bacteroides* spp./*Gardnerella* spp. related to second trimester pregnancy loss were also independently assessed. An assessment of the role of the absence of *Lactobacillus spp*. in the

first trimester, compared to normal levels of *Lactobacillus ssp.*, and the risk of pregnancy loss was also conducted.

In the bivariate analysis to identify potential confounding factors, Pearson's X^2 or Fisher's exact tests were used to analyze categorical variables, and *t*-test were used for continuous comparisons. Initial models included the following covariates: maternal age at enrollment, history of two or more spontaneous abortions (y/n), reported vaginal bleeding (y/n), smoking confirmed through urinalysis (y/n), gestational age at enrollment, African American race (yes/other), history of incompetent cervix (y/n) and prior preterm premature rupture of the membranes (PPROM) (y/n). Education level and race were highly correlated thus we included race in the model. We used likelihood ratio tests to assess the interaction between gestational age at enrollment and BV status at enrollment to examine if the risk of second trimester pregnancy loss varied by time in pregnancy of BV diagnosis. Reduced models were developed which removed prior adverse pregnancy outcomes recognizing that chronic BV may affect prior pregnancy outcomes. There were no inconsistencies between the results reported in the full and reduced models, thus the results from the reduced models were reported in this manuscript. The goodness of fit of the final regression models were assessed using Pearson's X^2 and logistic regression diagnostics. The SAS statistical package version 8.0 (SAS Institute Inc., Cary, N.C.) was used. A 2-sided p value of <0.05 was considered statistically significant.

Given a prevalence of bacterial vaginosis (y/n) of 40% and an incidence of SAB among BV negative women of 5%, a sample size of 1900 pregnant women is needed to detect a relative risk of spontaneous abortion of 1.65 or higher with 80% power and 5% two-sided significance.

RESULTS

Over 95% of pregnant women presenting for prenatal care were screened for study eligibility. The study enrolled 1,948 women and 1,916 women completed a baseline questionnaire, had a Gram stain result, follow-up information and were included in this analysis (98.4%). We successfully classified 98% of women enrolled in the study for pregnancy outcome at 20 weeks gestation and prenatal records and/or ultrasound reports were reviewed for 94% of participants. Forty percent of pregnant women screened positive for BV at the time of enrollment in the study (n=757) and over 20% reported at least one prior case of BV. 130 women (6.8%) reported a pregnancy loss during the follow-up period with the majority of pregnancy losses occurred between 15 and 18 weeks gestation with a mean follow-up time of 4 weeks (range 2.8 to 6 weeks).

Almost three quarters of the women were African-American (72%), the average maternal age at enrollment was 25 ± 6.1 years, and the average gestational age at enrollment and at the time of BV assessment was 10 ± 2.6 weeks. BV positive women were more likely to be African American (RR=1.42, 95% CI: 1.33 – 1.51), report a lower socioeconomic status and earlier sexual activity, report higher levels of psychosocial stress (p=0.01), were more likely to have documented current cigarette use (RR=2.14, 95% CI: 1.55–2.96), and more likely to report a history of vaginal douching (RR=1.4, 95% CI: 1.24–1.45).

The risk of pregnancy loss during the follow-up period was 6.8/100 women (n=130). Table 1 describes the demographic characteristics, health behaviors and reproductive characteristics according to pregnancy status at 20 weeks gestation. Women experiencing a pregnancy loss were older (mean maternal age 27.7 years vs. 25.5 years; p<0.001), and less likely to be African American (64.3% vs. 72.7%; p=0.01). The women experiencing a pregnancy loss were enrolled, on average, 5 days earlier in gestation compared to women maintaining their pregnancy (9.1 weeks vs. 10.3 weeks; p= 0.006) and reported more vaginal bleeding (41.9% vs. 23.1%; p<0.0001, respectively). Women experiencing a pregnancy loss were also more

likely to report at least one prior case of BV and had a higher prevalence of prior adverse pregnancy outcomes including prior miscarriages, ectopic pregnancies and preterm premature rupture of the membranes (PPROM).

The overall risk of pregnancy loss comparing BV positive (BV scores of 7–10) to BV negative women (BV scores of 0–6) was elevated but not significantly related to pregnancy loss (crude HR= 1.17, 95% CI: 0.78 – 1.75) after adjusting for the following confounding factors gestational age at enrollment, maternal age, cigarette use, race, history of incompetent cervix, and vaginal bleeding (adjusted hazard ratio (HR) = 0.84, 95% confidence interval (CI): 0.38 – 1.87) (Table 2). However, examining the BV scores as a continuous variable indicating an increase in BV-related vaginal flora alterations, the risk of second trimester pregnancy loss was significant after adjustment for confounding factors (adjusted HR=1.24, 95% CI: 1.02–1.64). In addition, we found that women at the highest level of BV (BV scores of 7–10), compared to women with normal vaginal flora (BV scores of 0–3), had a twofold increased risk of second trimester pregnancy loss (crude HR= 1.98, 95% CI: 1.13 – 3.48) and after adjustment for confounding factors, the risk still remained significant (adjusted HR: 2.49, 95% CI: 1.13 to 5.48). We did not find a significant increased risk of pregnancy loss among pregnant women classified in the intermediate stage of vaginal flora (BV scores of 4–6) compared to women with normal vaginal flora (HR = 1.72, 95% CI: 0.99 – 2.99). We also found no statistically significant interaction between gestational age at BV assessment and BV status, but vaginal bleeding during pregnancy was consistently related to a two-fold increased risk of second trimester pregnancy loss in all models. A small proportion of women reported significant vaginal bleeding but adjusting for the magnitude of bleeding did not change the results. No other confounders were significantly related to risk of pregnancy loss in the multivariate models.

The individual assessment of the contribution of each of the BV-associated morphotypes and the risk of second trimester pregnancy loss found that low scores of *Lactobacillus* spp. early in pregnancy were significantly related to the risk of second trimester pregnancy loss (HR: 1.16; 95% CI: 1.01 – 1.33) after adjusting for confounding factors (adjusted HR: 1.32, 95% CI: 1.10–1.64). An assessment of the absence of *Lactobacillus ssp*. in the first trimester of pregnancy compared to normal levels of *Lactobacillus spp*. found a significant increased risk of second trimester pregnancy loss among women with an absence of *Lactobacillus ssp.* (adjusted HR: 2.30; 95% CI: 1.09 – 4.85). Levels of *Mobiluncus* spp. (adjusted HR: 0.73; 95% CI: 0.47 – 1.17) and *Bacteroides* spp./*Gardnerella* spp. (adjusted HR: 0.92; 95% CI: 0.72–1.17) measured in early pregnancy were not related to second trimester pregnancy loss after adjusting for confounding factors (Table 2).

DISCUSSION

Although overall BV positivity was not related to the risk of second trimester pregnancy loss, we did find an elevated risk of second trimester pregnancy loss according to the magnitude of BV measured in the first trimester of pregnancy, with women categorized with the highest level of BV-related vaginal flora alterations experiencing a two-fold increased risk of second trimester pregnancy loss independent of confounding factors. These findings were consistent when classifying BV status continuously or by tertiles of abnormal vaginal flora. The risk of pregnancy loss among women with intermediate vaginal flora compared to women with normal vaginal flora was slightly elevated suggesting that as the concentration of BV-associated microorganisms increases the risk of second trimester pregnancy loss increases. Consistent with our findings, a recent large community-based study of low risk pregnant women linked BV to a four fold increased risk of pregnancy loss in the early second trimester, between 13 and 15 weeks gestation (RR=3.5, 95% CI: 1.2–10.3)(29).

We also found that low *Lactobacillus spp*. scores, corresponding to low levels of *Lactobacillus* spp., and the absence of *Lactobacillus spp.* in the first trimester of pregnancy were independently related to the risk of second trimester pregnancy. The levels of *Mobiluncus* spp. or *Bacteroides* spp./*Gardnerella* spp. were not related to second trimester pregnancy loss in our study. The relationship between low *Lactobacilli* and pregnancy loss is interesting since low levels of *Lactobacillus* spp. have been related to decreased implantation and increased pregnancy loss among women undergoing in-vitro fertilization (22). Our study is one of the few studies relating low *Lactobacillus* spp. or the absence of *Lactobacillus spp.* to second trimester pregnancy loss among a population of women without a history of recurrent pregnancy loss and without a history of early pregnancy management.

Some but not all studies have reported a similar association between BV and pregnancy loss (6,21,23,29–30); however, we are one of the few to measure BV so early in pregnancy. Measuring BV in the first trimester may be the most accurate assessment of the role of BV in early placental development and risk of subsequent pregnancy loss. Many prior studies measured BV in the late second trimester, 18–20 weeks gestation, and found a threefold increased risk of second trimester pregnancy loss among BV positive women (6,31). Given the later measurement of BV in these prior reports, pregnancy losses occurring prior to 18 weeks gestation were generally missed. We specifically implemented a protocol to identify BV status in early pregnancy, an average of 10 weeks gestation, and capture pregnancy losses in the early second trimester.

In prior reports, Donders et al. found that levels of *Mycoplasma hominis* (RR=12.5, 95% CI: 3.0–52), *G. vaginalis* (RR=5.8, 95% CI:2.1–16) and *U. urealyticum* (RR=5.8, 95% 2.1 – 16) were independently associated with an increased risk of any pregnancy loss prior to 20 weeks (21). *Lactobacillus spp.* levels were not related to the risk of pregnancy loss in this study. In the Donders study, the group of women experiencing a pregnancy loss contained a higher proportion of women with a prior history of pregnancy loss compared to the group of women with evolving pregnancies. We did attempt to recognize that prior adverse pregnancy events, (i.e. prior pregnancy loss, prior preterm premature rupture of the membranes (PPROM) and prior PTB) may be due to chronic BV across multiple pregnancies (32). This may be particularly important in our study population of primarily African-American women since BV is known to be more prevalent and perhaps more likely to be a chronic condition among African American women (2). In fact, we did find that BV positive pregnant women in this study were more likely to report a history of PPROM, PTB and a prior case of BV. Thus, we also examined models which excluded prior pregnancy outcomes. In both sets of models, adjusting for prior adverse pregnancy outcomes and not adjusting for these outcomes, we continued to find that more severe BV-related vaginal flora alterations in the first trimester and low *Lactobacillus spp.* increased the risk of second trimester pregnancy loss by over twofold.

Given the nature of enrolling a cohort of healthy pregnant women seeking prenatal care, we recognize that we may have missed very early, first trimester pregnancy loss thus these results do not suggest that first trimester BV is related to first trimester pregnancy loss. However, given that the majority of pregnancy losses occurring during the first trimester are chromosomally abnormal, the results from this study primarily assess the role of first trimester BV and chromosomally normal, second trimester pregnancy loss (33–35).

There are some limitations to recognize as part of this study. We only assessed overall *Lactobacillus* spp. There are many species of *Lactobacilli* with H_2O_2-producing *Lactobacillus* spp. being the most prevalent and low H_2O_2-producing *Lactobacillus* spp. has been linked to numerous adverse events including BV development (2,36). Thus, we are most likely measuring H_20_2-producing *Lactobacilli* and relating low H_20_2-producing *Lactobacillus* spp. to the increased risk of second trimester pregnancy loss in this study. In this

assessment, we did examine both low *Lactobacilli spp* and an absences of *Lactobacilli spp.* in early pregnancy and continued to find an increased risk of second trimester pregnancy loss given low levels and, in particular, absence of *Lactobacilli spp* in early pregnancy. Second, we did not measure vaginal pH in early pregnancy. High pH levels, which may indicate heightened inflammation, in conjunction with BV positivity has been linked to an increased risk of PTB in other studies(37) In addition, high pH levels in the second trimester of pregnancy may be a useful criteria for identifying pregnant women at risk for future PTB (38). Third, we measured BV status using the Nugent criteria which allowed an assessment of *Lactobacillus* spp., *Mobiluncus* spp. and *Bacteroides* spp./*Gardnerella* spp. but we did not measure the level or contribution of other micro-organisms which commonly coexist with BV such as *Prevotella spp., Peptostreptococcus spp., M. hominis* and *Ureaplasma urealyticum* (39). Fourth, consistent with many other studies conducted to date, we captured only a one time assessment of BV during early pregnancy and included self-reported cases of prior BV without confirming prior cases of BV through medical review. Using this strategy, we may have underestimated the cases of prior BV since we have previously found that almost 50% of BV positive pregnant women in this cohort are asymptomatic (25).

Unfortunately limited information is available regarding alternative medical risk of the pregnancy loss (i.e. congenital fetal anomalies or chorioamnionitis) given the nature of the study population (i.e. a low risk obstetrics population) and the study design (i.e. collecting pregnancy loss data at the time of the 20 week follow-up visit). However, these findings are generalizable to the majority of pregnant women not seeking early management of pregnancy. In addition, given that is was a community sample of healthy pregnant women, routine first trimester ultrasounds were not conducted thus we relied on self-reported prior incompetent cervix to adjust for the role of current incompetent cervix on the risk of subsequent pregnancy loss. We feel that a history of incompetent cervix is a fair proxy for current incompetent cervix and any misclassification would be nondifferential and bias the estimates toward the null. Finally, we recognized the characteristics of this population may limit generalizability since low income, African American women are more likely to douche and experience a higher prevalence of chronic co-morbidities, such as diabetes and hypertension. However, even after adjusting for these factors, the results remained the same.

Thus among this large group of urban, primarily African American women with a high prevalence of BV, we found that low levels or the absence of *Lactobacillus* spp. and high degrees of BV-related vaginal alterations are significantly related to an increased risk of second trimester pregnancy loss. In this study, we did not find that BV positive women compared to BV negative women were at increased risk of second trimester pregnancy loss but that women with the most severe BV-related alterations, or the absence of *Lactobacillus spp.* are the most likely to experience of a second trimester pregnancy loss. Future studies should assess the benefits of antenatal and/or early first trimester prenatal screening and treatment for BV to reduce the degree and duration of inflammation in early pregnancy in order to decrease the risk of pregnancy loss in this potentially high risk group of pregnant women. In addition, an examination of the systemic and localized immunologic response among BV positive pregnant women and women with low *Lactobacillus spp.* in early pregnancy would be helpful in understanding the role of these microorganisms in early placental development, inflammation and pregnancy loss.

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Table 1

Demographic, Health and Reproductive Characteristics According to Pregnancy Status at 20 Weeks Gestation

	Women experiencing a SAB (n=130)	Women maintaining their pregnancy (n=1786)	Unadjusted HR	95% CI
Demographic Characteristics				
Maternal Age (mean) *	27.7 years	25.5 years		
African American race *	64.3%	72.7%	0.69	0.49 – 0.99
≥ High School Education	81.5%	80.1%	1.00	0.87 – 1.15
Social Support (mean number of close friends)	3.2	2.7		
Stress (mean)	5.7	5.2		
Health Behaviors				
Ever report vaginal douching	57.7%	61.3%	0.87	0.62 – 1.21
Douche, since LMP *	16.9%	19.1%	0.87	0.49 – 1.55
Cigarette use	11.9%	10.7%	1.11	0.64 – 1.95
Marijuana use	11.8%	13.9%	0.84	0.48 – 1.47
Reproductive Health Characteristics				
Gestational age at enrollment *	9.1 weeks	10.3 weeks		
Vaginal bleeding *	41.9%	23.1%	2.23	1.59 – 3.11
Nulliparous	23.1%	29.5%	1.39	0.92 – 2.12
Ever had a STD	74.6%	78.8%	0.81	0.55 – 1.18
STD, since LMP*	—	2.9%		
Prior incompetent cervix	3.1%	1.2%	2.44	0.98 – 6.06
2+ prior miscarriages	17.8%	10.2%	1.79	1.02 – 3.15
Prior ectopic pregnancy	7.7%	2.4%	2.97	1.66 – 5.32
Prior PPROM *	10.0%	4.1%	2.33	1.37 – 3.9
Ever had bacterial vaginosis	26.4%	22.8%	1.60	0.88 – 2.89

Stress measured using the Cohen perceived stress scale (summary scores range from 0 to 16; mean = 5.21 and median = 5.0). Cigarette and marijuana use confirmed through toxicology. PPROM = preterm, premature rupture of the membranes.

Table 2

Crude and adjusted hazard ratios for the relation between spontaneous abortion and BV status during early pregnancy

BV Status	Crude HR	95% CI	Adjusted HR	95% CI
BV status				
BV status (yes/no)	1.17	0.78 – 1.75	0.84	0.38 – 1.87
Continuous BV Score (0–10)	1.04	0.98 – 1.10	1.24	1.02 – 1.64
Abnormal BV-related flora (BV scores 7–10) vs. normal flora (BV scores 0–3)	1.98	1.13 – 3.48	2.49	1.13 – 5.48
Individual morphotypes				
Decreasing *Lactobacillus spp.*	1.16	1.01 – 1.33	1.32	1.10 – 1.64
Increasing *Gardnerella/Bacteroides spp.*	1.02	0.92 – 1.14	0.92	0.72 – 1.17
Increasing *Mobiluncus spp.*	0.97	0.69 – 1.34	0.73	0.47 – 1.14

● HR = hazard ratio.

● Adjusted for gestational age at enrollment, maternal age at enrollment, cigarette use, African American race, vaginal bleeding, history of incompetent cervix, and BV*gestational age interaction term