Title: A randomized controlled trial of treatment of bacterial vaginosis in late third trimester to prevent maternal peripartum infection

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Title: A randomized controlled trial of treatment of bacterial vaginosis in late third trimester to prevent maternal peripartum infection

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1. INTRODUCTION

1.1 Abstract

Bacterial vaginosis (BV) is the most common cause of vaginal discharge among reproductive aged women in the United States. It has an estimated prevalence of 30% or nearly 1 in 3 reproductive aged women. BV is an established risk factor for adverse outcomes in pregnancy, including preterm labor, preterm premature rupture of membranes, and chorioamnionitis, all of which increase the risk of postpartum complications including endometritis and surgical site infections (SSI). Infants born to women with BV have higher incidence of neonatal sepsis, respiratory distress, Neonatal Intensive Care Unit (NICU) admissions and low birth weight, as well as long-term adverse outcomes, irrespective of maternal peripartum infection. Prior studies have demonstrated that treating pregnant women with BV potentially reduces the risk of preterm labor. However, no studies have evaluated the impact of treating BV on the incidence of maternal peripartum infections, including chorioamnionitis, endometritis, and surgical site infection. The current standard of care is not to treat women with asymptomatic BV in pregnancy.

Our objective is to conduct a double-blinded placebo controlled, pragmatic randomized controlled trial of metronidazole (500 mg twice daily for 1 week) versus standard of care (i.e., no antibiotics, receipt of placebo) to prevent peripartum infection (primary composite inclusive of chorioamnionitis, endometritis, and surgical site infection, SSI) among pregnant non-laboring women enrolled after 34 weeks of gestation.

1.2 specific aims:

Primary aim 1: To determine if administration of a 7 days course of metronidazole 500 twice daily versus placebo to women with BV, regardless of symptoms, diagnosed clinically after 34 weeks reduces the incidence of peripartum infection inclusive of chorioamnionitis, endometritis, and SSI.

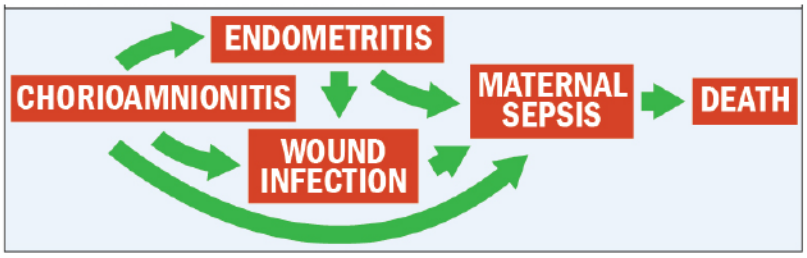
Secondary aims:
Secondary aim 1: To evaluate whether BV treatment affects the frequency of peripartum infection based on BV gram stain results at the time of randomization and labor admission.
Secondary aim 2: To evaluate whether BV treatment affects the frequency of intrapartum and postpartum fever, and individual infectious morbidities, as well as neonatal complications, including neonatal sepsis and NICU admission.
Secondary aim 3: To evaluate whether BV treatment affects the frequency of utilization of postpartum health care services, including maternal ER visits, postpartum clinic visits, OB triage visits, and postpartum hospital readmissions.

2. BACKGROUND

2.1 Bacterial vaginosis is a frequent condition in pregnancy. Bacterial vaginosis (BV) is the most common cause of vaginal discharge among reproductive aged women (1). In the United States, the prevalence of BV is estimated to be 30 %. Prevalence varies with ethnicity, including 50% in African Americans, 30% in Mexican Americans, and 24% in non-Hispanic whites. In obese women (BMI>30), its prevalence exceeds 35% (2). Even in women with an intermediate gram stain, the rate of BV is estimated to be 18% (4). Despite its high frequency in pregnancy and its known impact on adverse pregnant outcomes, it remains a complex, poorly understood clinical syndrome. BV is considered to result in an imbalance between naturally existing vaginal flora. There is a decrease in hydrogen peroxide secreting lactobacilli and an increase in pathogenic anaerobic bacteria including *Gardnerella vaginalis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*.

Several studies have evaluated the adverse effects of BV on maternal and infant outcomes. Gravett et al. reported that pregnant women with BV are at an increased risk of chorioamnionitis (OR 2.1), preterm labor (OR 2.2), premature rupture of membranes (PROM) (OR 2.4), and low birth weight (OR 1.7) (3). Similarly, Clark et al. evaluated 390 laboring women and found that pregnant with BV had a 2-fold frequency of chorioamnionitis and postpartum endometritis (16%) compared to women with a negative gram stain (8%). BV is also associated with adverse infant outcomes. Dingens et al. found that term infants born to women with BV were more likely to have respiratory distress (OR 1.28), suspected neonatal sepsis (OR 1.6) and NICU admission (OR 1.4), independent of maternal chorioamnionitis (5).

2.2 Peripartum infection increases the risk of adverse pregnancy outcomes. Peripartum infection is the 2nd leading cause of maternal mortality. Having a peripartum infection increases the risk for developing another peripartum infection, and ultimately, maternal sepsis and death (**Figure 1**).



Chorioamnionitis is an infectious and/or inflammatory disorder of chorion, amnion or both (6). Overall incidence in term pregnancies in an era of GBS prophylaxis is <5%, though the incidence increases up to 12% in laboring women who have a primary cesarean delivery. Multiple organisms have been isolated from chorioamnionitis patients. Notably, the most common organism found on PCR among women with chorioamnionitis is *Gardnerella vaginalis* followed by *Ureaplasma urealyticum* (10), the organisms that contribute to BV. Chorioamnionitis itself increases the risk of adverse maternal and fetal outcomes. Maternal complications of chorioamnionitis include: uterine atony (RR 2.5), pelvic abscess (RR 3.7), septic thrombophlebitis (RR 2.7), thromboembolism (RR 1.49), hysterectomy (RR 1.57) and need for blood transfusion (RR 2.25). Neonatal complications include: confirmed neonatal sepsis (RR 2.9), low Apgar score at 1 minute < 3 (RR 2.09) and neonatal seizures (2.7).(7, 8) Additionally, longer term infant outcomes, including cerebral palsy, are higher with chorioamnionitis (OR 2.4).(9)

Endometritis is defined as postpartum infection of the endometrial lining that can extend to the myometrium and parametrium. It remains one of the most common infectious complication in the peripartum period estimated to affect nearly a tenth of all pregnancies (11). Notably, endometrial cultures on patients diagnosed with postpartum endometritis have found that one of the most common organisms is *Gardnerella vaginalis* (12).

SSI is defined as infection occurring within 30 days of surgery occurring at the incision site site superficially or deep or affecting an organ that was manipulated during surgery. BV microorganisms are commonly isolated from surgical sites in women undergoing gynecologic procedures. Addition of metronidazole to preoperative antibiotic regimen in patients with BV has shown in many studies to reduce SSI, with a significant reduction ranging from 2 to 3 fold (18).

2.3 Diagnosis of BV

The complex etiology of BV can contribute to an inaccurate diagnosis, especially in asymptomatic individuals in late pregnancy.(20) Microscopy of vaginal fluid has been a mainstay of office-based testing for vaginitis. A clinical diagnosis using Amsel criteria allows for a diagnosis at the bedside with prompt treatment. In addition, microscopic testing with Gram stain using Nugent score is considered the gold standard.(21) However, both methods require skilled staff and time and suffer from reduced sensitivity and specificity. Inaccurate BV results may lead to misdiagnosis and delays in treatment. NAATs can identify and differentiate between organisms associated with vaginitis and be a convenient point of care test, but remains to be validated in pregnancy.

Nucleic acid amplification tests (NAATs) to diagnose BV have been developed and its performance in comparison to gram stain and microscopy has been assessed outside of pregnancy among symptomatic and asymptomatic women. The Labcorp NAATs test includes Atrobium vaginae, BVAV-2, and Megasphaera-1, and in recent study of 100 non-pregnant individuals with and without vulvovaginal symptoms in gynecologic care, this test had a lower sensitivity but similar specificity (80 and 98%) compared to Amsel (99 and 99%) and gram stain (83 and 96%).(22) These studies have generally been conducted among samples of women seeking gynecologic care, family planning services, or testing for sexually transmitted diseases.

Because the performance of a diagnostic test varies according to the characteristics of the study population, studying the performance of a NAAT test in pregnancy is warranted. The prevalence of BV is known to decrease in late pregnancy in the setting of a changing hormonal milieu. The ideal test for women presenting with symptoms of vaginitis would have high sensitivity and specificity and would be available at the point of care.

2.4 Treatment of BV and obstetrical and surgical outcomes. The benefits of treating BV in pregnancy have been evaluated, but have been done in the setting of preterm birth prevention, and not to prevent peripartum infection. Hauth et al reported 18% decrease in the rate of preterm labor following treatment of BV in the 2nd and early 3rd trimesters in women with previous

history of preterm labor (14). However, another randomized controlled trial failed to demonstrate benefit in low-risk women.(19) In the setting of scheduled hysterectomy for benign indications, pre-procedure treatment of BV been associated with 3-fold decrease in the incidence of post-hysterectomy cuff cellulitis (11% vs 34%). Persson et al. noted a decrease in wound infections in patients with BV from 8% to 25% (18).

2.5 Current standard of care for BV management in pregnancy. Current standard of care does not involve screening for or treating BV without symptoms of vaginitis in pregnancy. Similarly, the standard of care at OSUWMC does not involve screening for or treating BV in pregnancy. The current practice at our center is to screen for BV in women presenting with preterm contractions as part of a preterm labor evaluation or for those women presenting with symptoms consistent with vaginitis. We use the Amsel criteria to screen for BV in this population and if they test positive, and the decision is made to discharge the patient, a 7-day course of metronidazole is prescribed, 500 mg twice daily. Stated otherwise, we do not routinely screen for or treat BV in late 3rd trimester as a prophylaxis prior to induction or spontaneous labor, unless the patient reports vaginal discharge or other symptoms suggestive of BV.

2.6 Treatment regimens for BV in pregnancy. Multiple regimens have been suggested for treatment of BV in pregnancy including:

1) metronidazole 500 mg twice daily for 7 days,
2) metronidazole 2 g once, and
3) clindamycin 300 twice daily for 7 days.

Metronidazole is classified as FDA category B, and current data does not suggest an increased risk of congenital anomalies with metronidazole use in pregnancy (1). Pharmacokinetics of metronidazole are not affected by pregnancy (13). Koss et al did not find any association between metronidazole use and preterm birth, low birth weight or congenital anomalies (2). Given the high prevalence of BV in our population and the potential of BV treatment to decrease the incidence of peripartum infection as well as other maternal and neonatal complications, there is public health benefit in proceeding with investigating the effects of treatment of BV in early labor. While prior studies have focused on screening and treating BV in pregnancy to prevent preterm birth, no studies have been performed to evaluate the benefits of treating BV in the late 3rd trimester prior to delivery to prevent peripartum infection.

3. CONCISE SUMMARY OF PROJECT

3.1 Primary Research Question:

Does treatment of BV with 7-day course of metronidazole >34 weeks decrease the incidence of peripartum infection?

Hypothesis: Treatment of BV will reduce the incidence of peripartum infection, namely chorioamnionitis, endometritis, and SSI.

3.2 Secondary Research Questions

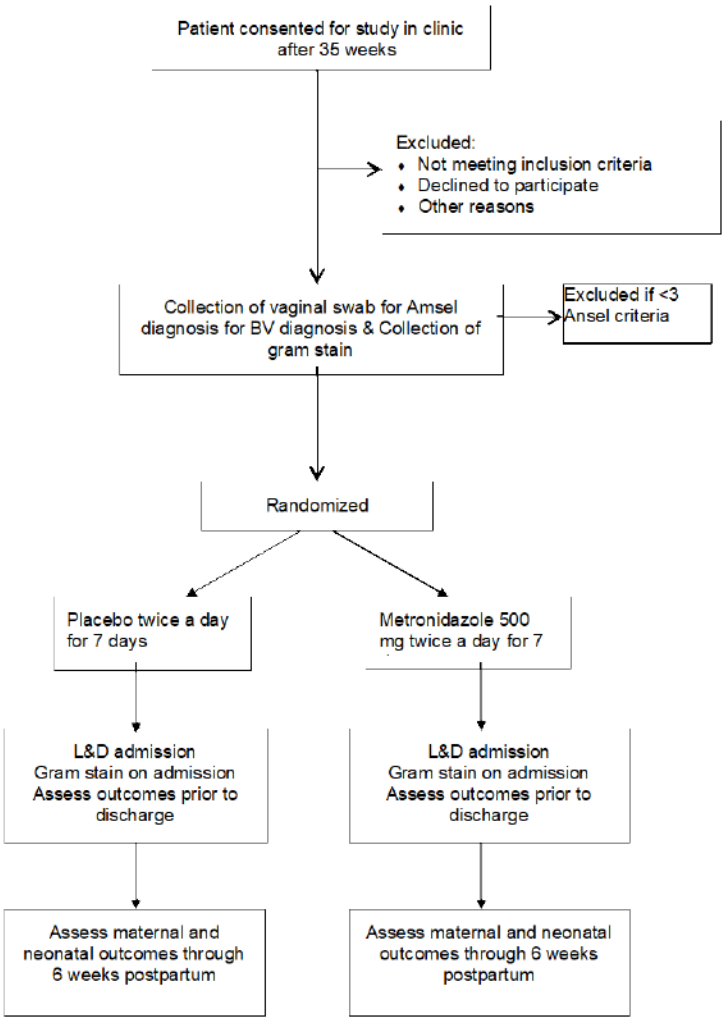
3.2.1: Does treatment of BV with 7-day course of metronidazole >34 weeks decrease the incidence of Gram stain +ve for BV at time of admission for labor?

Hypothesis : Treatment of BV will reduce the incidence of +ve Gram stain for BV at time of admission for labor.

3.2.2: Does treatment of BV with 7-day course of metronidazole >34 weeks decrease the incidence of neonatal complications including neonatal sepsis and NICU admission?

Hypothesis : Treatment of BV will reduce the incidence of neonatal complications.
3.2.3: Hypothesis : Does treatment of BV decrease utilization of health care services including maternal ER visits, postpartum clinic visits, OB triage visits, and postpartum hospital readmissions?

Hypothesis: Treatment of BV will reduce health care services utilization



4. STUDY DESIGN:

4.1 Study enrollment: We propose a double-blinded, placebo controlled, multi-center randomized trial of 482 pregnant women who are diagnosed with BV in late 3rd trimester (>34 weeks). During routine clinic visit after 34 weeks, prospective patients will be counseled about the study. Patients who agree to be enrolled, will sign informed consent.

Following enrollment, patients will be screened for BV. Those patients who are BV positive, will be randomized to receive either metronidazole 500 mg BID orally for 7 days or identically appearing placebo. The PI, Co-Investigators or study coordinators will be responsible for the informed consent.
1500 participants will be enrolled for screening to randomize 482 participants.

4.2 Study sites: This will be a multi-center, double-blinded randomized clinical trial. The primary site will be OSUWMC. The secondary center will be University of Texas Medical Branch, Galveston, Texas, and may include additional medical centers over time
The study period will be between November 2020 to September 2022. The number of subjects studied will be 482 patients. If a patient wishes to withdraw from study after randomization and receipt of study medications, no additional interventions will be performed, but we will collect study outcomes with patient consent as this is intent to treat study and patients' outcomes will be analyzed in the group they were assigned to.

Neither the patient nor provider will be aware of treatment assignment. No significant adverse effects are expected with the use of antibiotics in the study population. As with any other instance in which antibiotics are administered, there is a chance of an allergic or other adverse reaction. The patient will receive standard inpatient monitoring during the labor course and in the postpartum period, and so any immediate adverse reaction can be promptly detected. The study period will be November 2020 to September 2022. The number of subjects studied will be 482 patients. If a patient wishes to withdraw from study after randomization and receipt of study medications, no additional interventions will be performed, but we will collect study outcomes as this is intent to treat study and patients' outcomes will be analyzed in the group they were assigned to.

5. INCLUSION CRITERIA:

- Pregnant women 18 to ≤50 years with the ability to give informed consent.
- Patients expected to have a vaginal delivery with no obstetric contraindication for vaginal delivery at time of screening.
- Gestational age ≥ 34 weeks

6. EXCLUSION CRITERIA:

- Plan for elective cesarean delivery
- Allergy or contraindications to metronidazole
- Receipt of metronidazole or clindamycin on admission for delivery for other indications.
- Hemodialysis
- Severe liver dysfunction
- Patient reports BV to nurse or clinician provider at current clinic visit or has been treated for BV within the past 3 months.

7. STUDY PROCEDURES:

7.1 Randomization and enrollment in clinic at or after 34 weeks: After obtaining informed consent, patients with a plan for vaginal delivery will be screened for BV by self-collecting 2 vaginal swabs for Gram stain, and Amsel criteria. A 3rd swab will be collected and stored at -80C for future microbiome analysis at the end of the study. The 3rd swab for future microbiome analysis is associated only with this study. When all study is complete, we will run the analysis to see if there is an association between microbiome and peripartum infections. The reason for storing the specimens and running them at the end is to decrease the cost. Patients with positive Amsel criteria will be eligible for randomization. Randomization and providing the patient with medications will be done at the same visit.

7.2 Clinical diagnosis of BV:

Amsel criteria are:

- Homogenous thin grayish-whitish discharge that coats vaginal mucosa
- Vaginal PH >4.5
- Positive whiff-amine test, presence of fishy odor on adding a drop of 10% KOH
- Clue cells on wet mount

Patients who have at least 3 out of 4 of Amsel criteria will be considered positive for BV and will be randomized into either a control group or study group. Patient will be asked to characterize their vaginal discharge. Nitrazine paper will be used to assess PH. Saline will be added and light microscopy will be used to assess for clue cells and 10 % KOH will be added for whiff test.

Gram stain for BV: A gram stain will be collected from participants in clinic at time of screening and will be sent to the lab.

7.3 Confirmation of therapy success

Following completion of therapy, patients admitted to labor and delivery will have 1 additional swab. The swabs can be collected by the provider during performing normal labor exam or it can be self-collected by the patient. This swab will be sent to the lab for another gram stain. However, results will not be used for clinical decision guidance. It will be used for analysis later.

7.4 Clinical care: The care of patients in the remaining antepartum period, intrapartum period, including the method of induction or augmentation of labor will be according to standard of care at the participating site, and per the managing clinical team. After a subject who meets inclusion criteria agrees to participation in our study, the obstetric team will contact one of the investigators. Signed informed consent will be obtained by the PI, Co-Investigators or study coordinators. The total participation time in the study will be considered terminated at 6 weeks postpartum. No additional antepartum or postpartum will be required for the study, only routine visits will be recommended.

7.5 Data collection: The data collected will be kept on a password-secured Ohio State Wexner Medical Center (OSUWMC) computer. An encrypted USB flash drive will be used to transfer data. The data will be linked to the patient by patient's MRN number. This identifier is needed to access and analyze demographic data. During analysis of the data, all identifiers will be deleted.

7.6 Informed consent. Under the direction of the PI, trained research staff will be available 24/7 to screen and consent patients according to study protocol. Patients will be enrolled in clinic at or after 34 weeks gestation.

A written informed consent will be obtained to screen patients for BV via 3 self-collected vaginal swabs and testing for Amsel criteria and gram stain , in-addition to storage for later microbiome analysis. Randomization and treatment will then follow if positive by Amsel criteria. Vaginal swab for Gram stain will also be collected at time of admission to L&D for Labor for later analysis, and not for clinical decision-making. Full disclosure of the nature and potential risks of participating in the trial are to be made. Women who are not fluent in English will be enrolled by a person fluent in their language. Both verbal and written informed consent and authorization will be obtained in that language; if this is not possible, the patient will not be included. A copy of the signed consent form will be provided to the patient.

7.7 Screening and consent procedures. Under the direction of the study team members, we will enroll patients in clinic at or after 34 weeks gestation.
Medical records of all potential patients will be reviewed and those who satisfy inclusion and exclusion criteria will be approached for informed consent. If the potential subjects are not patients of the investigators then the permission of the treating provider will be obtained before approaching the patients to invite them to participate in research.

Written consent will be obtained by direct person-to-person contact. The principal investigator, co-Investigator, or study coordinators will be responsible for the informed consent. Non-English speaking subjects are anticipated to be part of the study population and informed consent will be provided in their primary language. The data collected will not be used for clinical management. Subjects will be reassured that participation in the study is voluntary and will not interfere with diagnose or treatment of her condition or management of her labor. The subjects will receive the same care and expertise as any other patient treated in our unit.

Informed consent will be obtained to screen for BV, and if BV positive, participate in the study. A screening log will be used to track all patients approached for the study, according to local guidelines.

7.8 Randomization and Masking: After obtaining informed consent, women with BV will be randomized to either control or study group according to a randomization sequence that is computer generated and maintained by the investigational drug pharmacy. A study enrollment log with a study ID number, subject name and medical record number, will be used to track enrolled patients. The investigational pharmacy at OSUMC will be notified after randomization and study drugs or placebos will be sent as dictated by patient's randomization group.

7.9 Drug Administration and Dispensation. Once a subject is consented randomization will be performed by the OSUWMC's investigational drug pharmacy (IDS). The appropriate study drug according to the group assignment will then be dispensed by OSUWMC's investigational drug services (IDS) pharmacy as well. Patients will receive 7 days of metronidazole 500 BID oral or similarly appearing placebo. The 14 pills will be provided in a container. The IDS pharmacy will dispense the drugs, account for dispensing and stock number. Funding provided by the department will cover the cost of the medications, and the patient's insurance company will not be billed. Subjects will be instructed to bring the container to clinic next visit or on admission to labor and delivery to count remaining pills and assess adherence.

The subject will be included in the analysis by intent-to-treat.

7.10 Baseline Procedures. Routine Ante, intra- and postpartum care will be provided to patients according to the local standard of care. Patients' clinical providers who will be blinded to the study interventions. Trained and experienced research staff (also blinded to group assignment) will be responsible for screening patients in clinic for eligibility and for all research data abstraction. All data will be de-identified after collection and tracked only with a study ID number.

The PI and co-PI will review and validate the diagnosis for all patients identified to have the primary composite outcome, applying current CDC and other standard criteria. These reviews

will be conducted masked to treatment group. If there is uncertainty, a second investigator will review the chart, discuss with the PI as needed, and make a final determination regarding the outcome. Maternal and neonatal outcomes will be assessed in the hospital following delivery (on an ongoing basis until discharge) and at the routinely scheduled postpartum follow-up visits up to 6 weeks postpartum.

7.11. Study visits / Follow-up. Part of the standard of care, all postpartum patients are followed after delivery to assess any postpartum complications. Before the subject leaves the hospital, patients are usually given their follow up appointment. This will be documented in the datasheet, in order to ascertain follow up. Patients will be educated about the signs and symptoms of infection and other study outcomes and encouraged to call the study research team with any concerns. In addition, records of visits (to any hospital clinic or ER) prior to the routine postpartum visit will be obtained and reviewed to ascertain study outcomes (treating providers may also be called if clarifications are needed). Finally, if patient does not present to routine postpartum care, a research staff will call all patients at 6 weeks postpartum to inquire about occurrence of study outcomes. Newborn outcomes will be ascertained through initial discharge from hospital or up to 90 days or death, whichever occurs first. Readmissions and related diagnoses for infants will also be ascertained through medical records.

7.12. Withdrawals. Patients who withdraw from the study after randomization will be followed up to ascertain outcomes. Outcomes ascertained will be reported in intent to treat fashion.

7.13. Primary Outcome #1: Composite of chorioamnionitis, postpartum endometritis, SSI, wound infection, or other post-cesarean infections (occurring within 6 weeks after delivery) defined as follows. These outcomes will be defined and assessed by trained research staff and reviewed and validated by the investigators, all blinded to group assignment.

1) **Chorioamnionitis:** this will be diagnosed if patient develops: Fever (defined as either 2 readings of ≥ 38.0 °C / 100.4 ° F 30 minutes apart or one reading ≥ 39.0 °C /102.2 °F) in the absence of any cause for fever PLUS one of the following: 1) fetal tachycardia (>160 for 30 minutes or longer), 2) Leukocytosis (> 15000 in absence of corticosteroids), 3) Purulent discharge from cervical os, 4) biochemical or microbiological changes of the amniotic fluid (positive gram stain, positive culture, low glucose, high WBC).(1)

2) Endometritis: If the patient develops two of the following: 1) Oral body temperature of 101 °F at any time, or a temperature of 100.4 °F 24 hours after delivery, 2) maternal tachycardia that parallels the temperature, 3) Uterine tenderness, 4) purulent vaginal discharge, or 5) Associated findings with advanced endometritis (dynamic ileus, pelvic peritonitis, pelvic abscess, bowel obstruction, necrosis of the lower uterine segment) (1)

3) SSI: We will also be following the criteria set by the CDC's National Healthcare Safety Network (formerly the National Nosocomial Infection Surveillance System) for hospital-acquired SSI*:*
A **superficial incisional SSI** must meet the following criteria:
Date of event for infection occurs within 30 days after the operative procedure (where day 1 = the procedure date) **AND** involves only skin and subcutaneous tissue of the incision **AND** patient has **<u>at least *one*</u>** of the following:

a) Purulent drainage from the superficial incision.
b) Organisms identified from an aseptically-obtained specimen from the superficial incision or subcutaneous tissue by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment (e.g., not Active Surveillance Culture/Testing (ASC/AST).
c) Superficial incision that is deliberately opened by a surgeon, attending physician or other designee and culture or non-culture based testing is not performed. **AND** Patient has ***at least one*** of the following signs or symptoms:
    a. pain or tenderness;
    b. localized swelling;
    c. erythema;
    d. or heat.

d. Diagnosis of a superficial incisional SSI by the surgeon or attending physician or other designee.

A **deep incisional (or wound) SSI** must meet the following criteria:
The date of event for infection occurs within 30 after the operative procedure (where day 1 = the procedure date) **AND** involves deep soft tissues of the incision (e.g., fascial and muscle layers) **AND** patient has ***at least one*** of the following:

a) Purulent drainage from the deep incision.
b) A deep incision that spontaneously dehisces, or is deliberately opened or aspirated by a surgeon, attending physician or other designee and organism is identified by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment (e.g., not Active Surveillance Culture/Testing (ASC/AST) or culture or non-culture based microbiologic testing method is not performed **AND** patient has ***at least one*** of the following signs or symptoms:
    a. Fever (>38°C); localized pain or tenderness.
    b. A culture or non-culture based test that has a negative finding does not meet this criterion.
c) An abscess or other evidence of infection involving the deep incision that is detected on gross anatomical or histopathologic exam, or imaging test.

An **organ/space SSI (e.g. endometritis or abscess)** involves any part of the body, excluding the skin incision, fascia, or muscle layers, that is opened or manipulated during the operative procedure. An **organ/space SSI (endometritis or abscess)** must meet one of the following criteria:
Infection occurs within 30 days after the operative procedure **AND** infection involves any part of the body, excluding the skin incision, fascia, or muscle layers, that is opened or manipulated during the operative procedure **AND** patient has ***at least one*** of the following:

a) Purulent drainage from a drain that is placed through a stab wound into the organ/space;
b) Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space;

c) An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination;
d) Diagnosis of an organ/space SSI by a surgeon or attending physician.

4) Wound infection: Presence of either superficial or deep incisional SSI described as cellulitis/erythema and induration around the incision or purulent discharge from the incision site, with or without fever, such as necrotizing fasciitis (diagnosed based on necrotizing wound infection). Wound hematoma, seroma, or breakdown alone in the absence of the above signs does not constitute infection.

5) Other infections: Pelvic septic thrombosis (diagnosis based on persisting signs of endometritis with or without radiological confirmation), abdominal or pelvic abscess based on radiological diagnosis.

- Primary outcome #2 Validation of NAAT in diagnoses and treatment of BV in pregnant women in the third trimester

7.14 Secondary Outcomes

Maternal secondary outcomes:

- Individual infections: chorioamnionitis, endometritis, wound infection (including necrotizing fascitis), other infections including abscess, septic thrombosis, pneumonia, pyelonephritis and breast infection
- Maternal Death
- Puerperal fever: Temperature > 100.4 F at least twice 30 minutes apart, or once with the use of antipyretic, or ≥101 F once. This will be analyzed for intrapartum and postpartum fever.
- Use of resources: Hospital stay, postpartum clinic or emergency room visit within 4 weeks of delivery, need for imaging or other invasive procedures, postpartum antibiotic use.
- Adverse events: Allergic reactions (anaphylaxis, angioedema, skin rashes including Stevens Johnson and Toxic Epidermal necrolysis), GI symptoms (nausea, vomiting, diarrhea, constipation, ileus, etc)

Neonatal secondary outcomes:

- Suspected sepsis (need for sepsis work-up): Presence of clinical signs/symptoms (hypothermia, fever, irritability, poor feeding, hypotonia, etc) causing the clinician to perform a sepsis work-up (blood, urine and/or cerebrospinal fluid, or chest X-ray), within 7 days of delivery- excludes routine work-up solely for positive maternal GBS status.
- Confirmed sepsis: Findings indicating positive cultures of blood, cerebrospinal fluid or urine obtained by catheterization or suprapubic aspiration, or cardiovascular collapse, or an unequivocal X-ray confirming infection in a clinically septic neonate, within 7 days of delivery.
- NICU admission and duration

- Neonatal morbidities: Respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC), periventricular leucomalacia (PVL), intraventricular hemorrhage (IVH), and BPD. RDS is presence of any one of i) oxygen requirement at 6-24 hours of life in a preterm infant, ii) abnormal chest radiograph consistent with RDS, or iii) need for surfactant; NEC is based on the clinical diagnosis of the neonatologist supported by findings on radiological imaging, surgical intervention and/or autopsy; IVH and PVL are diagnosed by an attending neonatologist based on clinical indications leading to a confirmatory ultrasound interpreted by a certified radiologist; BPD (or chronic lung disease) is defined as infant oxygen requirement at 28 days, at 36 weeks post-conceptional age or at time of hospital discharge

8. SOURCES OF RESEARCH MATERIAL: Electronic Medical chart/records.

9. POTENTIAL RISKS:

9.1. Randomization risk

Since antibiotics versus placebo will be randomized, it is possible that the patient may be in a group with higher adverse outcomes.

9.2. Loss of Confidentiality

Any time information is collected; there is a potential risk for loss of confidentiality. Every effort will be made to keep patient's information confidential; however, this cannot be guaranteed.

9.3 Risks from intervention

Most common adverse effects associated with metronidazole use are gastrointestinal, nausea, vomiting, anorexia, cramping, constipation and diarrhea, others adverse effects include headache. Metronidazole is categorized as pregnancy category B by FDA. GI adverse effects are common. Nausea affects about 12% of patients, diarrhea and abdominal pain affects 4%. Headache affects 18 % of patients. Hypersensitivity affects 5%. However, severe allergic reactions are very rare.

There are rare risks of serious peripheral and central nervous system issues, hematopoiesis, and cardiovascular and renal adverse reactions. Additionally, there are risks of drug interactions, including with alcohol. There are risk of fungal overgrowth/yeast infection and the risk of experiencing an unpleasant metallic taste. Metronidazole crosses the placental barrier. Though there is no evidence of harm to the fetus, it is present in breast milk for up to 24 hours after treatment ends.

10. SUBJECT SAFETY AND DATA MONITORING

The PI, Co-Investigators and study coordinators will be responsible for monitoring the safety of this study. The report will include participant demographics, expected versus actual recruitment rates, summary of any quality assurance or regulatory issues, summary of adverse events (AEs) or serious adverse events (SAEs) which may have occurred, and any changes in the protocol as a result of these issues.

The PI, Co-Investigators and study coordinators will ensure all aspects of data quality, including monitoring for adherence to consent procedures, inclusion and exclusion criteria, valid abstraction, correct entry, timeliness and responsiveness to data queries.

Data will be collected and stored with the participant ID code only. The master enrollment log linking patient identifiers with study ID numbers will be kept in a password-protected database

on the Ob/Gyn Department's internal server separate from the data. Several data collection forms will be used. Data on these forms devoid of personal identifiers will be securely stored at our perinatal research division. The research coordinator will be available to monitor the data and correct any discrepancies based on source documents if needed.

11. PROCEDURES TO MAINTAIN CONFIDENTIALITY:

- The data collected will be indirectly linked to the subject by patient's MRN number.
- When the subject accepts to participate in the study, a number will be attributed to the patient - from BVO1 to BVO482 - and this number will be entered on the data collection sheet.
- The data collected will be transferred to the PI's password-secured computer that is stored in a locked room using an USB flash drive.
- The study PI will be the only person to have a list with the number designated to each subject and the corresponding patient's MRN number. This list will be kept in a locked cabinet of the PI's locked office. This identifier is needed to access and analyze demographic data. During analysis of the data, all identifiers will be deleted.
- Data will not be disclosed to outside persons or entities.

12. POTENTIAL BENEFITS:

Subjects receiving the metronidazole may have a decreased risk of peripartum infection. If this is the case, our study stands to benefit women with BV who may undergo induction of labor or spontaneous labor in the future.

13. STATISTICAL APPROACH:

Analysis will be performed by intent to treat. Univariable and multivariable analysis will be used to describe the population in the study and to identify potential confounding variable. For this analysis normality will be tested using Shapiro-Wilk method. Demographics and descriptive statistics such as t test, Pearson's chi-square, and Mann-Whitney tests will be used as indicated. Incidence rates between the study groups will be compared using Chi-square or Fisher Exact tests as appropriate. Data will be either shown as median [IQR], mean ± SD, or n (%). Given this is a RCT, multivariate logistic regression may be used to adjust for confounding variables, should there be evidence of unbalanced distribution of these variables despite randomization

Sample size was calculated using a superiority trial design. The primary outcome will be the proportion of subjects having the composite infectious outcome. Clark et al evaluated 390 laboring patients and reported the incidence of chorioamnionitis and postpartum endometritis in patients with BV to be 16.4 % in contrast to 8.1 % among those without BV. This is an underestimate since it did not include the surgical site infections and other infectious morbidity in the composite primary outcome. Assuming the rate of composite infectious morbidities in women with BV to be at least 17% and estimating treatment effect to be 50%, we estimate that a sample size of 482 patients will need to be enrolled to have sufficient power of 80%, at an alpha 0.05. Given estimated incidence of BV, enrolling 1500 patients will be needed, given that only 30% will be randomized.

Rate in Placebo	Effect size	Number per group for power		
		80%	85%	90%
17%	50%	241	275	321

Secondary analysis: Given our estimated baseline composite primary outcome is about 10% regardless of BV, assuming that in patients with BV, the outcome might be higher up to 20%. Analysis will be performed to assess if a sample size of 398 patients (199 per group) will be sufficient to demonstrate a similar difference in primary outcome.

Sample size calculation using baselines primary outcome in our institution.

Rate in Placebo	Effect size	Number per group for power		
		80%	85%	90%
20%	50%	199	227	266

STATA 14 (College Station, TX) will be used for statistical analysis. This trial will be registered with Clinical Trials Register (Clinicaltrials.gov), before recruitment is initiated and after IRB approval.

14. FEASABILITY

The rate of BV in our study population is at least 30%. Therefore we will need to screen and enroll close to 1500 patients. Assuming 30% of patients will not agree to be screened for BV and decline the study, we will need to approach 2,000 patients. We are assuming that 482 BV positive patients will be eligible to participate in the full study at OSUWMC, Miami Valley Hospital (MVH) and Good Samaritan Hospital (GSH). 50 % of patients will be enrolled at OSUWMC and 50% of patients at MVH and GSH together. Based on our historical data regarding delivery volume at OSUWMC MVH and GSH, we expect the study can be completed in 12-18 months.

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