PRETERM BIRTH & STI RISK ASSOCIATED WITH VAGINAL MICROBIOME DYSBIOSIS

FINAL PRESENTATION

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PROJECT TEAM

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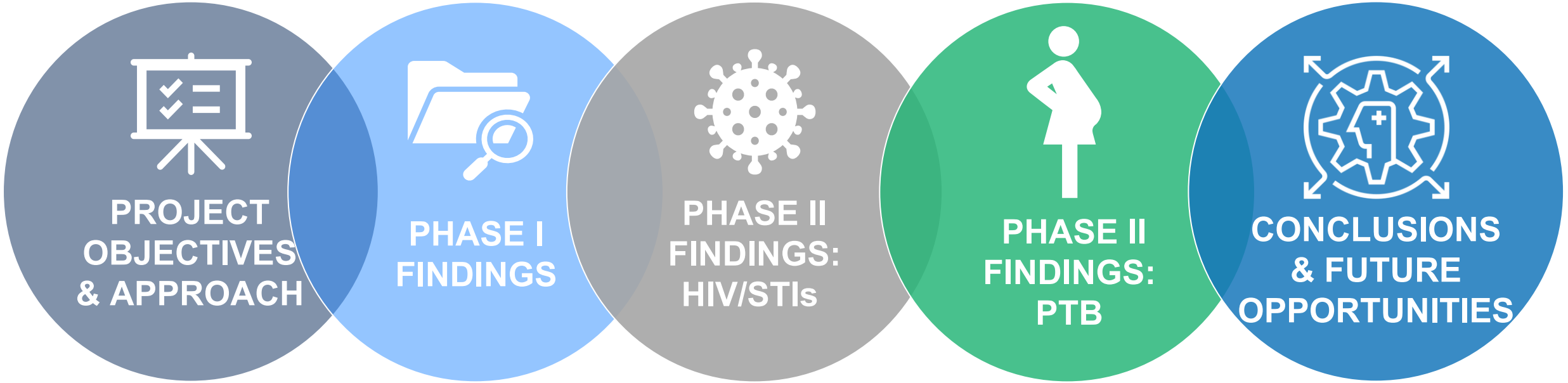
PRESENTATION OVERVIEW



KEY PROJECT OBJECTIVES

Summarize the evidence examining the causal relationship between VMB dysbiosis or BV and 1) acquisition/transmission of HIV/STIs; and 2) risk of PTB.

Take a deeper dive to assess how diagnostic method (Amsel's vs. Nugent) and symptom status modify the relationship between vaginal dysbiosis/BV and PTB or HIV/STI risk, particularly in intermediate VMB states.

Develop considerations for future trials for VMB interventions.

WORKFLOW: TWO PHASE APPROACH

Phase 1

- Tiered evidence rapid literature review exploring most rigorous and recent data on VMB/BV and PTB and STI/HIV acquisition/transmission
- Client meeting to discuss findings and align on next steps

Phase 2

- Deeper dive into literature on intermediate microbiota states and PTB and STI/HIV acquisition & review VMB-related clinical trials
- Hold 3 key informant interviews to validate key findings and inform considerations for future trial design

TIERED EVIDENCE RAPID LITERATURE REVIEW OVERVIEW

Step 1
Identified the most recent systematic review & meta-analysis

Step 2
Searched for strong studies published after the latest review/meta-analysis

Step 3
Shared high-level findings with content expert to gut check our understanding of current state of evidence and flag any additional important studies

FOCUS AREAS

- VMB & PTB: 12 studies
- VMB & HIV acquisition/transmission: 5 studies
- VMB & STI acquisition/transmission: 9 studies

Database: PubMed
Articles extracted: 26

PHASE I FINDINGS: Rapid Literature Review

A note on defining the strength & quality of the evidence

- Small sample size
- Low effect size (RR <1.5)
- Wide confidence intervals
- No adjustment for key confounders

- Large, multi-site studies or meta-analyses
- Consistent findings across populations
- Strong effect size (RR >2.0)
- Rigorous adjustment for confounding and bias

PHASE I SUMMARY: BV/VMB & PTB

BV is moderately associated with PTB: A pooled analysis (Mohanty et al. 2022) found a moderate association (RR 1.44), but findings were constrained by diagnostic variability, small sample sizes, and limited ability to control for confounding. Only two studies showed statistically significant results, and overall effects were smaller than in prior meta-analyses

***Lactobacillus* dominance is often associated with lower PTB risk (Ferrante et al. 2025):** *L. crispatus*-dominant microbiota (CST I) are consistently associated with lower PTB risk, while *Lactobacillus*-depleted communities (CST IV) are linked to higher risk. *L. iners*-dominant profiles (CST III) show mixed associations, likely reflecting transitional states.

VMB patterns vary by ancestry and shift over pregnancy: Fettweis et al. & Callahan et al. complicate this picture of a consistent VMB signature of PTB, reporting a spectrum of VMB states associated with PTB that differ between women of African ancestry and women of European ancestry.

To date, randomized controlled trials aimed at treating BV (including trials with live biotherapeutics), have not consistently reduced PTB risk (Wu et al. 2025).

PHASE I SUMMARY: BV/VMB & HIV

Consistent association (Low et al. 2011; Hilber et al. 2010): Women with BV (Nugent 7-10) have ~1.5–2× higher HIV acquisition risk; Amsel-based results are less consistent due to diagnostic variability.

Microbiota composition (Gosmann et al. 2017): High-diversity, low-Lactobacillus states (>4× risk) vs. *L. crispatus* dominance (protective). *L. iners* often linked to higher risk, though variable across ancestry groups.

Intermediate states (Low et al. 2011): Nugent 4–6 linked to ~1.5× higher HIV acquisition in some cohorts, suggesting these states are not benign.

Population differences (Eastment & McClelland, 2018): Strongest associations seen in African and African American women; weaker in European/Asian cohorts.

Limitations: Heterogeneity in diagnostics, confounding, and reliance on high-risk populations constrain causal inference; few RCTs directly test microbiome modification as HIV prevention.

PHASE I SUMMARY: BV/VMB & STIs

High-Level Summary

RCT evidence demonstrates that treating BV reduces STI incidence, supporting a causal link between BV and STI acquisition. Further trials are needed to track microbial shifts, clarify the protective role of Lactobacillus, and test stabilization strategies such as probiotics—while carefully accounting for condomless sex as a key confounder.

Chlamydia, Gonorrhea, Trichomonas	Consistently higher prevalence with BV (Nugent 7–10); the effect is strongest for CT (Carter et al. 2021) and TV (Carter et al.; Sethi et al. 2024).
M. genitalium	Strong evidence that recent BV increases M. gen susceptibility. Co-occurrence may worsen outcomes beyond either infection alone (Lokken et al. 2017)
Syphilis	No consistent association, very low prevalence; no variation across Nugent categories (Sethi et al. 2024).

PHASE 2

PHASE 2 KEY QUESTIONS

INTERMEDIATE MICROBIOTA	How does the presence of intermediate Nugent scores relate to PTB and HIV/STI risk, and what are the implications for including or excluding this group in future trials?
SYMPTOM STATUS	How does vaginal symptom status influence PTB and HIV/STI risk, and how might this guide participant selection, screening criteria, or stratification in future trials?
KEY TRIAL DESIGN LEVERS	What are the critical parameters for an effective and feasible trial to modify the vaginal microbiome for PTB or HIV/STI prevention? • Exposure and outcome measurement • Sample size - achieving adequate power • Type of intervention – eliminating BV vs. achieving *L. crispatus* dominance, therapeutic regimen • Timing of intervention – optimal point(s) in pregnancy or reproductive life course • Study population characteristics – including geography, race/ethnicity, underlying risk factors, and care access

PHASE 2 METHODS

Deeper dive into literature on intermediate microbiota states and PTB and STI/HIV acquisition & review VMB-related clinical trials

Hold 3 key informant interviews to validate key findings and inform recommendations

Develop considerations for future trial design

KEY INFORMANT INTERVIEWS

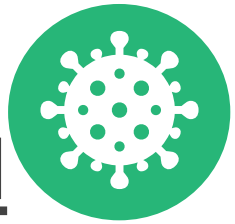
R. Scott McClelland, MD, MPH
Infectious Disease Physician & UW Professor in Epidemiology, Global Health, & Medicine
Expertise: *women's reproductive health, vaginal microbiome, STIs epidemiology, clinical and translational science*

Sharon Hillier, PhD
Microbiologist, Endowed Chair in Reproductive Infectious Disease & Vice Chair in Department of OB/GYN at University of Pittsburgh
Expertise: *microbiology, vaginal microbiome, reproductive health, co-developed Nugent scoring system*

Christina Muzny, MD
Professor of Medicine & Medical Director of the ID Vaginitis Clinic at University of Alabama at Birmingham
Expertise: *pathogenesis of BV, prevention of BV and T. vaginalis*

PHASE II FINDINGS: Considerations for HIV/STI prevention trial design

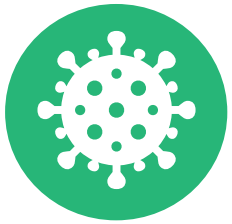
STUDY POPULATION CHARACTERISTICS: HIV/STI



Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Findings	• Reproductive-age, sexually active women recruited from high-prevalence and high-risk settings (e.g., sub-Saharan Africa, STI clinics, sex worker cohorts) showed positive associations between BV, vaginal dysbiosis, and HIV/STI acquisition (Julius et al. 2008) • Women with BV and other STIs (*T. vaginalis*, *M. genitalium*, *C. trachomatis*) vs those without (Chacra et al., 2023), and those with high-diversity, low-Lactobacillus (especially *L. iners*-dominant) vs *L. crispatus*-dominant microbiota were at higher risk of BV–HIV/STI associations (Gosmann et al., 2017)
Trial Design Considerations	• Recruiting women from high-risk settings, high-prevalence populations (e.g. sex workers, STI clinics) will maximize the number of events, but may not apply to women outside these groups • Screening for coinfections and stratifying participants by vaginal microbiota composition (e.g., *L. iners* vs. *L. crispatus*) are critical to understanding differential risks and intervention effects

INTERMEDIATE MICROBIOTA: HIV/STI



Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

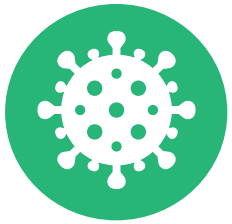
Key Findings

- Prospective studies suggest intermediate Nugent (4–6) and BV carry ~1.5-fold higher HIV risk (Low et al. 2011)
- No significant association with incident CT/GC/TV in Zambia (aOR 1.02, 95% CI 0.63–1.65) (Mulinganya 2021). Higher risk of *Mycoplasma genitalium* (OR 3.01, 95% CI 1.85–4.86) observed in African cohorts (Sethi 2024)
- Intermediate/BV flora associated with elevated IL-1β, IL-8, MIP-1α/β, increased activated CD4+ target cells, and mucosal barrier disruption, supporting biologic plausibility (Mitchell & Marrazzo 2014)
- Most studies do not disaggregate intermediate (4–6) from BV (7–10)

Trial Design Considerations

- Plan repeated sampling to capture transitions between Lactobacillus dominance, intermediate, and BV, testing whether risk gradients differ by state or persistence
- Power studies for stratified analyses with intermediate microbiota group separate from BV
- Consider inclusion of inflammatory or other immune markers as other trial endpoints in addition to STI or HIV acquisition

INTERVENTION TYPE: HIV/STI



Key Findings

- Periodic presumptive treatment trial (Balkus et al. 2016) showed that treating BV led to a lower incidence of CT, NG, and MG (IRR 0.54; 95% CI, .32–.91). Asymptomatic-BV RCT (Schwebke & Desmond, 2007): treating BV prolonged time to first STD (p=0.02) and reduced 6-mo STD rate (primarily CT)
- BV recurs in ~50–80% of women within 6–12 months (Bradshaw & Sobel, 2016). Lactin-V trial (Cohen et al., 2020): Metronidazole followed by *L. crispatus* CTV-05 reduced BV recurrence (RR 0.66 at 12 wk, RR 0.73 at 24 wk).
- Male-partner treatment trial (Vodstrcil et al. 2025) significantly reduced BV recurrence over 12 weeks (HR 0.37)

Trial Design Considerations

- Build protocols that include re-treatment or maintenance dosing and partner treatment, given the high recurrence rates of BV with standard care. Include longitudinal measurements to ensure that treatment was successful.
- Power trial for incident STIs (composite of CT/NG/MG), mirroring Balkus et al. (IRR≈0.54). Include time-to-first STI as a secondary endpoint (per Schwebke & Desmond), plus STI incidence rate over follow-up to capture both onset and burden
- Consider partner treatment (this could be through a factorial design) to improve feasibility and isolate the impact of partner treatment vs. standard of care on reducing HIV/STI acquisition

PHASE II FINDINGS: Considerations for trial design to reduce PTB

STUDY POPULATION CHARACTERISTICS: PTB

Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Findings	• Women of African ancestry vs European ancestry were more likely to have non-Lactobacillus communities (Fettweis et al., 2019) • In the US, African American women show higher *L. iners* and non-Lactobacillus species with reduced *L. crispatus*, (Dunlop et al., 2021) • African American women vs other ethnicities are 2× more likely to be diagnosed with BV and to experience PTB (Fettweis et al., 2019) • Risk behaviors (new partners, condom inconsistency, vaginal douching) disrupt protective communities, ↑ BV risk, and ↑ PTB risk (Muzny et al., 2016). • Parity and contraceptive use remain poorly studied in relation to VMB and PTB.
Trial Design Considerations	• Enrolling women with higher PTB risk ↑ PTB events but limits applicability to women with different baseline PTB risks • Enrolling women with high BV risk (e.g., multiple/new partners, douching) ↑ feasibility but limits applicability to women with lower behavioural risk profiles • Including diverse racial/ethnic groups ↑ generalizability but ↓ causal interpretation (due to microbial differences).

INTERMEDIATE MICROBIOTA: PTB

Key Findings

- Dynamic state; ~⅓ persist, ~⅓ → Lactobacillus-dominant (often L. iners), ~⅓ → BV (Hillier et al. 1992)
- Intermediate Nugent is a more opportunistic state for co-occurrence with T. Vaginalis and other STIs more than normal flora (Sethi et al. 2023) as well as other infections like Group B Streptococcus (Meyn, Krohn & Hillier 2009)
- Intermediate vaginal microbiota demonstrated a significantly higher risk of cervicitis compared to Nugent 0-3 and BV (Sesay Dissertation 2025).
- Evidence on PTB risk is null with wide CIs - meta-analysis (Leecurrent BV ↑ PTB risk (OR 1.66) (Blumenfeld et al., 2024); microbiome shifts across pregnancy (Waters et al., 2008).
- *"Intermediate Nugent scores are just so complex, especially with different Gardnerella morphotypes that we are just now beginning to appreciate. We would need more proof of concept and better understanding of the pathogenesis before including these women in a trial to reduce PTB.*" (Dr. Christina Muzny)

Trial Design Considerations

- Plan for opposing possibilities - intermediate may dilute PTB risk but could also increase risk through the pathway of cervicitis
- Enrich Nugent 4–6 group with cervicitis/inflammation or key taxa (Sneathia, BVAB1) to avoid diluting effects; stratify randomization by Nugent group and pre-specify subgroup analyses
- Include cervicitis/inflammation and opportunistic infections (*T. vaginalis*, GBS) as outcomes alongside PTB

INTERVENTION TYPE: PTB

Key Findings	• Network meta-analysis (Wu et al., 2025): Across 24 RCTs (11,584 women), treating BV in pregnancy with antibiotics, probiotics, or combined regimens did not reduce PTB overall (RR 1.00, 95% CI 0.80–1.24). IPD meta-analysis (Klebanoff et al., 2023): Similarly found no reduction in PTB overall, even in earlier treatment windows. Studies did not evaluate treatment efficacy. • Standard antibiotic regimens reduce concentrations of most BV-associated bacteria in pregnancy, but do not improve concentrations of *L. crispatus* (Mitchell et al. 2009) • Augmentation of antibiotic treatment with probiotic regimens has shown improved cure rates of BV (Martinez et al. 2009; Anukam et al. 2006), and reductions in genital inflammation and epithelial disruption biomarkers(Lactin-V immunology sub-study: Armstrong et al. 2022)
Trial Design Considerations	• Build trials that require demonstration of Nugent 0-3 (and, ideally, species-level Lacto dominance) at predefined timepoints, then stratify PTB by microbiologic responders vs. non-responders • Incorporate immune/inflammatory biomarkers (e.g., cytokines, sialidase, epithelial disruption) and vaginal microbiome sequencing as secondary outcomes to clarify why interventions may or may not reduce PTB.

SYMPTOM STATUS: PTB

Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Findings	• PTB risk is often driven by asymptomatic BV; symptomatic and asymptomatic cases are microbiologically similar, but trials often miss the asympt burden (Brown et al, 2023; Tachawatcharapunya et al, 2017;Afolabi et al, 2016). • Asymptomatic cohorts are reportedly small and underpowered, showing imprecise but suggestive PTB signals. In contrast, large meta-analyses consistently confirm that BV with mixed symptom status consistently show a modest association (OR 1.6–1.8), though with high heterogeneity (Krauss-Silva 2014) • Asymptomatic cohorts show suggestive but non-significant BV–PTB associations when sampled early (<20 wks) vs.late (28–32 wks), while mixed-symptom cohorts with later sampling report stronger, significant effects (Krauss-Silva et al, 2014; Afolabi et al, 2016)
Trial Design Considerations	• Deliberately enroll asymptomatic women early in pregnancy, to capture the risk-relevant BV burden and prevent diluting effects with symptom-driven care. • Pre-specify spontaneous PTB as the primary outcome; exclude medically indicated PTB. • Stratify by microbiota state e.g *L. crispatus*-dominant vs *L. iners* at baseline and longitudinally (disentangles biology from symptoms, sharpens causal inference, and clarifies which women should be targeted in trials)

OUTCOME MEASUREMENT: PTB

Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Findings	• PTB classification matters: Mixing spontaneous + medically indicated PTB dilutes infection-linked effects. (Mohanty 2022) • Earlier PTB effects are stronger: BV shows clearer associations when births occur <34 weeks. (Ng 2023) • Microbiome-related PTB also tends to occur much earlier (<32–34 weeks), making measurement difficult if studies lump all PTB <37 weeks. ○ *"There is just a ton of pre-term births that happen between 32 and 37 weeks that's just not infectious at all. So I think the relationship [between BV and PTB] just gets pretty muddy. Do I think that L. crispatus reduces the risk of some early pre-term births? Yes. But hardly any studies look at just those since there are so few*". (Sharon Hillier). • BV independently increases the risk of preterm, low-birth-weight infants (OR 1.4); the composite endpoint (PTB+LBW) captured effect more clearly than PTB alone (Hillier, 1995)
Trial Design Considerations	• Primary endpoint: Prioritize distinguishing spontaneous PTB from medically indicated PTB, using centralized review to ensure consistent classification across sites. Consider incorporating a composite outcome (e.g., PTB and/or low birthweight (PTB–LBW) to capture the broader spectrum of adverse neonatal outcomes. • Disaggregate GA thresholds (<32, <34, <37 wks) and analyze dose–response by gestational age. • Accurate GA dating (early ultrasound) mandatory; blinded adjudication committee.

SAMPLE SIZE: PTB

Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Assumptions

- Baseline PTB risk: 11% or 20% (varies by study population; 20% assumes high risk cohort)
  - Infection-mediated fraction**:** 40% of PTB cases attributed to infectious/inflammatory pathways
- BV suppression efficacy**:** Lactin-V + 5-day vaginal metronidazole reduces BV recurrence by 34% (RR BV = 0.66; Cohen etal. 2020, Phase 2b trial)
- Preventable share of infection-mediated PTB (g): 0.5, 0.75, 1.0 (1.0 assumes *all* infection-mediated PTB directly arises fromBV/dysbiosis or its inflammatory sequelae – purely theoretical)
- Implied PTB effect from BV suppression:
  RR PTB = 1 − (0.40 × 0.34 × g) →
  - g=0.50: RR PTB ≈ 0.93 (6.8%↓)
  - g=0.75: RR PTB ≈ 0.90 (10.2%↓)
  - g=1.00: RR PTB ≈ 0.86 (13.6%↓)
- Design parameters: Two-arm superiority, α=0.05 (two-sided), 80–90% power; inflate totals for attrition (10%)

SAMPLE SIZE: PTB

Baseline PTB Risk	G=0.50 (RR=0.93) (Realistic)	G=0.75 (RR=0.90) (Optimistic)	G=1.0 (RR=0.86) (Theoretical Ceiling)
11% (global average)	8,800 (80% power) 11,800 (90% power)	3,400 (80%) 4,600 (90%)	2,000 (80%) 2,600 (90%)
20% (high-risk cohort)	3,600 (80%) 4,800 (90%)	1,400 (80%) 1,800 (90%)	900 (80%) 1,200 (90%)

Note: these sample size calculations are for a general population. To sufficiently power a trial with stratified analyses, sample size would likely increase by ~30-50%

CONCLUSIONS & OPPORTUNITIES

CONCLUSIONS

1. BV is modestly associated with PTB, but evidence of reduction in PTB with treatment of BV from RCTs is limited
2. Evidence linking BV and intermediate microbiota to HIV/STI acquisition is stronger and more consistent
3. Intermediate microbiota group is rarely analyzed separately from those with BV, however, current evidence does not suggest an increased risk of PTB
4. Standard antibiotic regimens achieve short-term cure, but recurrence is high; live biotherapeutic approaches show greater durability

OPPORTUNITIES

Feasible trial designs

- PTB as a single endpoint would require large sample size, especially with stratified populations
- STI prevention would require fewer participants

Efficiency gains

- Consider composite endpoints (PTB-LBW, multiple STIs)
- Recruit from high-incidence populations to maximize event accrual

Mechanistic insights

- Strengthen causal inference through collection of inflammatory and immune markers to uncover mechanisms of protection or risk

Bundled interventions

- Pair VMB intervention with other strategies addressing other modifiable risk factors for PTB or STI/HIV acquisition to maximize impact

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APPENDICES

APPENDICES

- **A** Detailed Study Findings
- **B** Directed Acyclic Graph
- **C** Additional Trial Design Considerations
- **D** Literature Extraction Sheet

APPENDIX A:
Detailed Study Findings

DETAILED STUDY FINDINGS - BV/VMB & PTB

High-level outcome summary (Mohanty et al.): A significant association between BV and PTB, with a pooled RR of 1.44 (95% CI: 1.19–1.73) was observed. The analysis included 20 studies contributing 26 effect estimates, though only 6 studies were included in the RR meta-analysis. While the overall association was statistically significant, substantial heterogeneity was noted across studies in terms of BV diagnostic methods (e.g., Nugent score, Amsel's criteria, self-report), timing of diagnosis during pregnancy, and study geography. Importantly, only two individual studies contributing to the RR estimate demonstrated statistically significant results, one of which relied on self-reported BV. Despite these methodological differences, the findings reinforce a modest but consistent association between BV and increased PTB risk, particularly when BV is diagnosed later in pregnancy.

Geography: Global (studies included from multiple continents including South America, Sub-Saharan Africa, South Asia, and North America)
No. of studies: 22, 6 included in pooled RR
Key participant characteristics: varied asymptomatic/symptomatic, limited stratification by SES
BV/VMB classification & diagnostic method: Nugent (5 studies), Self-report (1 study)
Specific info on intermediate microbiota: Nugent 4-6 category acknowledged but not analyzed separately
Main finding: Significant association between BV and PTB - RR 1.44 (95% CI: 1.19-1.73)
Key limitations: inability to stratify by intermediate Nugent score vs. BV, heterogeneity in geography of studies, wide confidence intervals/small sample sizes in included studies.

DETAILED STUDY FINDINGS: BV/VMB & PTB

High-level outcome summary (Ferrante et al.): This systematic review of 38 studies evaluated the relationship between the VMB and risk of PTB. Although the review did not include a meta-analysis, consistent patterns emerged across studies: *Lactobacillus crispatus*-dominant communities (CST I) were associated with reduced risk of sPTB, while *Lactobacillus*-depleted communities (CST IV) were more commonly observed among those who delivered preterm. *L. iners*-dominant microbiota (CST III) showed inconsistent associations and may represent a transitional state. The review highlighted substantial heterogeneity in methods used to characterize the vaginal microbiota (including sequencing region, bioinformatics pipelines, and CST definitions), as well as differences in sampling time and population risk profiles. Few studies stratified results by key modifiers such as race, parity, or prior preterm birth, limiting conclusions about differential effects. Nevertheless, findings support the hypothesis that vaginal microbiota composition plays a role in sPTB risk, particularly the protective role of *L. crispatus* and the potential adverse role of *Lactobacillus*-depleted states.

Geography: Global (North America, Europe, Sub-Saharan Africa, Asia)
No. of studies: 38
Key participant characteristics: diverse risk profiles, limited reporting on race/ethnicity, prior PTB, parity, or treatment
BV/VMB classification & diagnostic method: CST classifications; 16S rRNA gene sequencing
Specific info on intermediate microbiota: Nugent scores not used; intermediate flora not explicitly defined; CSTs used instead to capture gradations in community structure
Main finding: CST I (*L. crispatus*) protective; CST IV (diverse anaerobes) associated with elevated sPTB risk; CST III (*L. iners*) showed inconsistent associations
Key limitations: heterogeneity in sequencing and CST definitions, poor stratification by key modifiers

DETAILED STUDY FINDINGS: BV/VMB & PTB

High-level outcome summary (Fettweis et al.): This large prospective U.S. cohort study of 1,572 pregnancies (597 with longitudinal vaginal microbiome sampling), found that PTB was associated with distinct vaginal microbiome dynamics, particularly among women of African ancestry. Women who delivered at term were more likely to have *Lactobacillus crispatus*-dominant microbiomes. In contrast, PTB was linked to increases in specific bacterial taxa (*A. vaginae, BVAB1, G. vaginalis, P. amnii, S. amnii, TM7-H1*), especially in African ancestry participants. European ancestry participants showed generally stable microbiomes, with only a modest increase in G. vaginalis among those with PTB. Overall, the study highlights ancestry-specific microbial patterns and suggests that shifts in vaginal microbial composition over pregnancy are more pronounced in African ancestry women who go on to deliver preterm.

Geography: US
Sample size: 597
Key participant characteristics: predominantly African ancestry,
BV/VMB classification & diagnostic method: relative abundance of relevant vaginal bacteria taxa; 16S rRNA gene sequencing
Specific info on intermediate microbiota: Nugent scores not used; intermediate flora not explicitly defined
Main finding: women who went onto deliver term were more likely to have *L. crispatus* dominant VMB (p=0.014); variations in relative abundance of specific taxa over the gestational period and by ancestry
Key limitations: very strong study with large sample size; no notable limitations

DETAILED STUDY FINDINGS: BV/VMB & HIV ACQUISITION/TRANSMISSION

High-level outcome summary: *Disrupted vaginal microbiota, including BV and intermediate states, is linked to increased HIV acquisition and transmission risk. Associations vary by classification method, with limited generalizability across settings.*

Geography:	-Systematic reviews and metanalysis majorly included studies from sub-Saharan Africa[1,2] -The cohort studies reviewed were conducted in Africa, particularly in Southern and Eastern African countries[4,5]
Sample size:	-Number of women enrolled in the meta-analysis of individual participant data from 13 prospective cohort study was 14,874[2] -Number of women enrolled in the primary studies of the systematic review and meta-analysis ranged from 154-4531[3] -Number of women enrolled in other cohort studies reviewed ranged from 236-2236
Key participant characteristics:	-Majorly reproductive age group, with median age 30 years in the systematic reviews, and <25 years in the cohort studies -Pregnancy status of participants were not explicitly mentioned in the studies -Participants in the systematic reviews self-identified as sex workers, work in bars, live with HIV partners
BV/VMB classification & diagnostic method:	-Majorly Nugent scores. Some studies used CST and CT dominance framework via 16S rRNA gene sequencing
Info on intermediate microbiota	-Intermediate vaginal microbiota (Nugent 4–6) was associated with increased risk of HIV acquisition in one study (pooled aHR 1.54 (95% CI: 1.20–1.97) [2] -In contrast, no significant association was found between intermediate vaginal microbiota (Nugent 4–6) with HIV transmission ([aHR: 1.63 (95% CI: 0.62–4.26)] [4]
Main finding:	-BV (Nugent 7–10) is consistently associated with increased HIV acquisition and transmission: aHRs range from 1.53–1.69 for acquisition[2,3]; aHR 3.17 (95% CI: 1.37–7.33) for female-to-male transmission[4] -High-diversity, low-Lactobacillus communities (CT3/CT4) showed >4-fold increased HIV acquisition risk compared to *L. crispatus*-dominant: [CT4: HR 4.03 (95% CI: 1.14–14.27); CT3: HR 4.22 (95% CI: 1.06–16.88)] [5] -No HIV acquisition occurred among women with *L. crispatus*-dominant communities[5]
Key limitations:	-Variability in BV/VMB classification and lack of standardized reporting hindered comparison across studies -Most data came from specific high-risk groups (e.g., HIV-serodiscordant couples), which may not reflect broader populations -Unclear timing between microbiota sampling and HIV infection

[1]Carter et al. 2023, DOI: 10.1097/OLQ.0000000000001744 (Systematic review and metanalysis)
[2]Low et al. 2011, DOI: 10.1371/JOURNAL.PMED.1000416 (Meta-analysis of individual participant data from 13 prospective cohort study)
[3]Hilber et al. 2010, DOI: 10.1371/JOURNAL.PONE.0009119 (Systematic review and metanalysis)
[4]Cohen et al. 2012, DOI: 10.1371/JOURNAL.PMED.1001251 (Prospective cohort study)
[5]Gosmann et al. 2017, DOI: 10.1016/j.immuni.2016.12.013 (Prospective cohort study)

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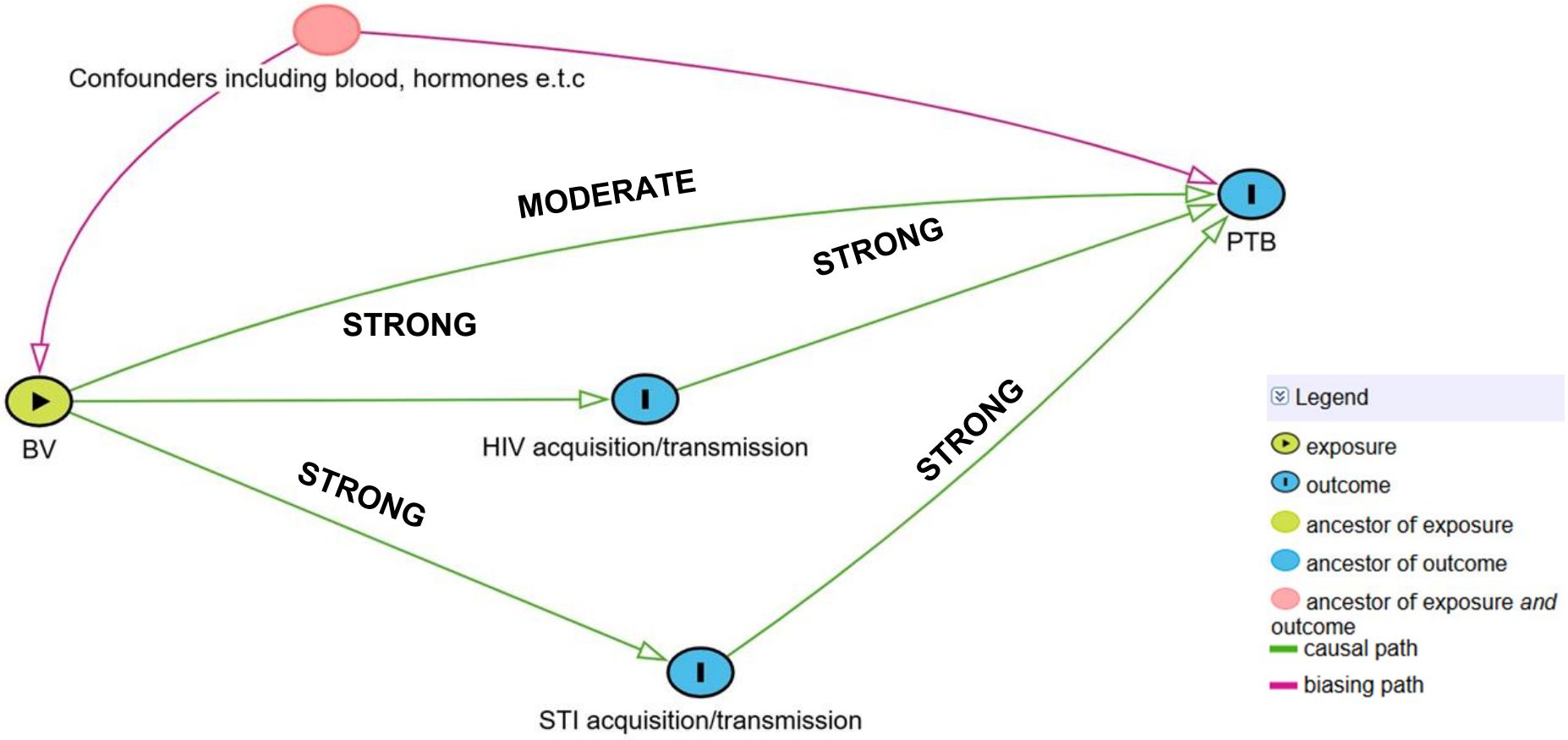
BV/VMB & STI ACQUISITION/TRANSMISSION: KEY FINDINGS

STI	Sample Size & Geography	BV/VMB classification & diagnostic method	Specific info on intermediate microbiota	Main findings	Key limitations
Chlamydia trachomatis[1]	N>402 across all 7 studies (Systematic Review and Meta analysis), Global	L. iners vs L. crispatus dominance via community state types	Classified by dominant taxa classification; using **16S rRNA gene sequencing** to determine which bacterial species is most abundant	**L. iners–dominated vaginal communities** were consistently associated with a **higher prevalence or odds of CT infection** compared to **L. crispatus–dominated communities.** Women with **L. iners dominance had 4.2 times higher adjusted odds of CT** (aOR 4.20, 95% CI: 1.20–15.40) - most significant finding	Cross-sectional; confounding by sexual behavior/contraception
Neisseria gonorrhoeae[2]	N = 3531 (Cross sectional), India	Nugent Score mostly used	No consistent change in NG prevalence across 4- 6 category	**Increased NG prevalence in Nugent 7-10** (11.1%) **vs 0 –3** (0.3%). BV associated with increased GC prevalence; intermediate flora not significantly elevated	No behavioral confounder adjustment
Trichomonas vaginalis[1,2]	N= 394, United States[1], N = 3531, (Cross sectional) India[2]	Nugent Score (0-3, 4-6, 7-10)	Intermediate flora (4-6) showed elevated risk compared to normal flora	PR ~1.56 (95% CI 0.08–93.14); extremely wide CI; inconclusive[1]. **Prev increases with Nugent score: 1.0% (0-3) to 3.8% (4-6),to 5.2% (7-10)**[2]. BV and intermediate flora both associated with increased TV prevalence	Cross-sectional; cannot infer causality
M. gen[3]	N = 280 (Prospective cohort), Kenya	Nugent Score (0-3, 4-6, 7-10)	Women with intermediate microbiota had a **1.70-fold increased odds** of acquiring *M. genitalium* compared to those with normal microbiota[3]	**Women with BV, defined by a Nugent score ≥7, had a 3.5-fold higher odds of acquiring M. gen**	Potential exposure misclassification
Syphilis[2]	N = 3531 (Cross sectional), India	Nugent Score (0-3, 4-6, 7-10)	No significant difference across Nugent groups	Syphilis **prevalence remained consistent across all Nugent categories** (~1.3%) with no significant association (p=0.993)	Low event rate; underpowered to detect associations

1. Carter KA, Fischer MD, Petrova MI, Balkus JE. Epidemiologic Evidence on the Role of Lactobacillus iners in Sexually Transmitted Infections and Bacterial Vaginosis: A Series of Systematic Reviews and Meta-Analyses. Sex Transm Dis. 2023 Apr 1;50(4):224-235. doi: 10.1097/OLQ.0000000000001744. Epub 2022 Dec 1. PMID: 36729966; PMCID: PMC10006306.
2. Sethi S, Yadav R, Sharma N, Dadwal R, Chaudary H, Kaur K, et al. Association of intermediate Nugent Score and bacterial vaginosis with sexually transmitted infections and vulvovaginal candidiasis. Indian J Dermatol Venereol Leprol. 2024;90:296-301. doi: 10.25259/IJDVL_775_2022
3. Lokken EM, Balkus JE, Kiarie J, Hughes JP, Jaoko W, Totten PA, McClelland RS, Manhart LE. Association of Recent Bacterial Vaginosis With Acquisition of Mycoplasma genitalium. Am J Epidemiol. 2017 Jul 15;186(2):194-201. doi: 10.1093/aje/kwx043. PMID: 28472225; PMCID: PMC5860020.

APPPENDIX B: DIRECTED ACYCLIC GRAPH (DAG) DEPICTING THE CAUSAL RELATIONSHIP BETWEEN BV/VMB & PTB, HIV ACQUISITION/ TRANSMISSION, & STI ACQUISITION/TRANSMISSION



APPENDIX C:
Additional Trial Design Considerations

EXPOSURE MEASUREMENT: HIV/STI

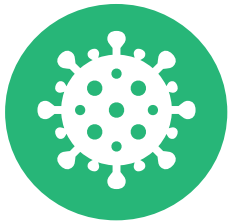
Key Findings

- Nugent score (≥7) shows strongest associations (HIV risk ↑ ~1.6–2.0 fold [Atashili et al., 2008]; *T. vaginalis* aHR 2.4, 95% CI: 1.9–3.0 [Balkus et al., 2015]). Amsel (≥3/4) shows weaker, less consistent association (~1.3–1.5 fold ↑ risk [Myer et al., 2005]).
- CST IV (low-Lactobacillus, anaerobe-rich) → ~4-fold ↑ HIV risk (Anahtar et al., 2015; Gosmann et al., 2017). *L. crispatus* (CST I) is protective, whereas *L. iners* (CST III) ↑ HIV/STI susceptibility (e.g., 3.4-fold ↑ odds of C. trachomatis [Carter et al., 2023]).
- BV commonly co-occurs with STIs (e.g., *T. vaginalis*, *C. trachomatis*, *N. gonorrhoeae*), with coinfections more prevalent in HIV-positive women, suggesting synergistic effects on transmission (Nava-Memije et al., 2021).

Trial Design Considerations

- Combine Nugent scoring with Amsel criteria to enhance robustness and cross-study comparability.
- Use 16S rRNA sequencing to classify CSTs and differentiate species (e.g., L. crispatus vs. L. iners), illuminating mechanistic pathways and therapeutic targets.
- Test for common STIs (CT, NG, TV, MG, HSV-2, syphilis) to capture synergistic effects on HIV/STI risk.

OUTCOME MEASUREMENT: HIV/STI



Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Findings

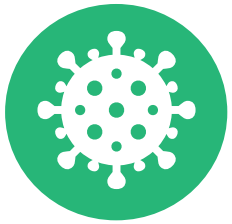
Frequent follow-up is essential for accurate endpoint measurement

- **HIV**: Very frequent testing (twice-weekly HIV RNA, plus quarterly clinical visits allowed for precise capture of incidentinfections and directly associates them to baseline microbiota states. (Gosmann 2017)
- **MG:** Monthly BV assessments and bimonthly MG NAATs allowed detection of new MG infections shortly after BVepisodes, showing recent BV as a strong risk factor. (Lokken 2017)
- **CT:** Quarterly testing over a full year enabled persistence versus clearance tracking, showing that BV/intermediate floraimpaired spontaneous clearance (Brown 2023)
- **TV:** Multiple longitudinal cohorts pooled in a meta-analysis consistently demonstrated ~2× higher incidence of TV in BV-positive women. (Seña 2021)

Trial Design Considerations

- Must decide whether the endpoint is acquisition, persistence, or transmission.
- For acquisition, follow-up needs to be ≥12 months with regular STI testing (increases trial cost/logistics; considerself-collected swabs, pooled NAAT, or point-of-care platforms to reduce burden).
- For persistence, shorter (3–6 months) follow-up with microbiota monitoring may be sufficient.

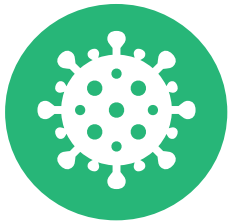
INTERVENTION TIMING: HIV/STI



Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Findings	• Recent BV at the preceding visit predicts MG acquisition; OR 3.48. (Lokken 2017) • Dysbiosis impedes CT clearance over quarterly follow-up. (Brown 2023) • Observational studies show dysbiosis increases acquisition risk in real-time follow-up; interventions must sustain vaginal health over the long term rather than focus on a fixed “pregnancy” window(Lokken, 2017, and Gosmann 2017).
Trial Design Considerations	• Use longer follow-up (≥12 months) with maintenance strategies (e.g., probiotic/live biotherapeutic sequences after antibiotics) to keep communities in a protective state. • Consider long-acting or periodic delivery forms (e.g., rings, scheduled dosing) to maintain colonization and reduce relapse.

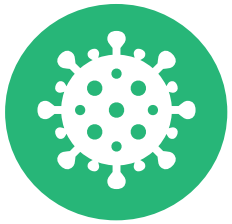
SYMPTOM STATUS: HIV/STI



Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Findings	• BV, even when asymptomatic, increases risk of HIV/STI acquisition, especially *for M. gen*, *CT*, and *TV* (Lokken et al, 2017) • Intermediate microbiota is usually asymptomatic and is associated with elevated HIV/STI risk compared to healthy microbiota (Sethi et al, 2024) • Symptoms alone are not reliable predictors of HIV/STI risk (Brown et al, 2023)
Trial Design Considerations	• Screen regardless of symptoms: Don't rely on clinical presentation alone, include asymptomatic women since risk is elevated. • Track persistence/clearance: Use microbiologic endpoints (e.g., CT non-clearance, MG persistence) instead of symptom resolution alone.

SAMPLE SIZE: HIV/STI



Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Assumptions

- Primary endpoint: Incident composite bacterial STI (CT/NG/MG), NAAT-confirmed; incidence rate as primary measure; time-to-first STI as key secondary
- Baseline incidence
  - General high-risk clinic population: 10–20 per 100 person-years (PY).
  - Very high-risk cohorts: ≥20 per 100 PY
- Effect size
  - Anchored to presumptive partner treatment trial (Balkus et al. 2016): IRR=0.54
  - Conservative IRR=0.65
  - Optimistic IRR=0.44
- 12 months of follow-up per participant
- Design parameters: Two-arm superiority, α=0.05 (two-sided), 80–90% power; inflate totals for attrition (10%)

SAMPLE SIZE: STI ACQUISITION

Baseline STI Incidence (per 100 PY)	Conservative (IRR=0.65)	Anchored (IRR=0.54)	Optimistic (IRR=0.44)
10	2,148 (80% power) 2,876 (90% power)	1,180 (80%) 1,580 (90%)	764 (80%) 1,022 (90%)
20%	1,074 (80%) 1,438 (90%)	590 (80%) 790 (90%)	382 (80%) 512 (90%)

EXPOSURE MEASUREMENT: PTB

Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Findings	• Nugent-defined BV: Strongest association with PTB when Nugent 7–10; ~2-fold higher risk (Leitich et al., 2003). • Amsel's criteria: Sensitivity 100% with high Nugent (9–10) and 81% with low Nugent (7–8); few missed cases (Rajni et al., 2022). • Molecular CSTs: "Low lactobacilli" (↑ anaerobic diversity) ↑ PTB risk vs. *L. crispatus* dominance (OR 1.69) (Gudnadottir et al., 2022). • First-trimester BV in women with prior PTB ↑ risk <36 wks (OR 4.56) (Guerra et al., 2006); recurrent BV ↑ PTB risk (OR 1.66) (Blumenfeld et al., 2024); microbiome shifts across pregnancy (Waters et al., 2008).
Trial Design Considerations	• Use Nugent scoring (≥7) and/or Amsel criteria (≥3/4) as primary methods; combining both improves robustness and cross-trial comparability. • Consider refined microbiota profiling with 16S rRNA sequencing to offer insight into potential intervention targets. • Use repeated measures across pregnancy to capture BV persistence or resolution; early detection in the 1st–2nd trimester is a key window for intervention to reduce PTB risk.

INTERMEDIATE MICROBIOTA: PTB

Deeper Dive

- Krauss-Silva et al., 2014 (Rio de Janeiro; prospective cohort, n=1,699). Compared BV (7–10) vs intermediate (4–6) for sPTB: <34 wk RR 0.81 (95% CI 0.13–11.8); <37 wk RR 1.86 (0.41–8.47) → imprecise, no significant differences; BV-negative women were excluded, limiting interpretability.

- Tachawatcharapunya et al., 2017 (Thailand; prospective cohort, n=270; asymptomatic, early 3rd trimester). Intermediate (4–6) vs normal: aRR 1.3 (0.3–5.1) (ns). BV vs normal: aRR 2.3 (0.6–9.4) (ns). Small sample, wide CIs.

- Mulinganya et al., 2021 (DRC; prospective cohort, n=533; 2nd-trimester assessment). Intermediate vs healthy for PTB: aOR 1.02 (0.50–2.07) for combined PTB/LBW 2.78 (0.86 - 9.95) (ns). BV vs healthy: aOR 1.20 (0.66–2.22) for PTB (ns). Combined outcome and limited events.

- Cauci et al., 2002 (Seattle; cross-sectional, n=218; women in preterm labor). BV or intermediate (grouped) showed more deliveries ≤34 wk than normal; early PTB associated with enzymatic markers (e.g., sialidase). Design limits causal inference and does not isolate intermediate

- Leitich, 2007 (meta-analysis; 32 studies; n≈30,518). Intermediate flora was not significantly associated with PTD, late miscarriage, or infectious outcomes.

- Sesay, 2025 (Dissertation: *Advancing Sexual Health for Cisgender Women: A Series of Studies Addressing Doxycycline Post-Exposure Prophylaxis, Vaginal Health, and Antimicrobial Resistance in Kenyan Women).* The intermediate vaginal microbiota demonstrated a significantly higher risk of cervicitis (adjusted RR=1.32, 95% CI: 1.18-1.48) compared to normal microbiota, a risk surpassing that associated with BV (adjusted RR=1.12, 95% CI: 1.01-1.25).

INTERVENTION TIMING: PTB

Study Population Characteristics	Exposure Measurement	Intermediate Microbiota	Intervention Type	Symptom Status	Intervention Timing	Outcome Measurement	Sample Size

Key Findings	• Earlier sampling (<20 wks) shows stronger or more significant informative BV–PTB signals; later sampling yields weaker or null effects. (Krauss-Silva 2014; Tachawatcharapunya 2017; Afolabi 2016) • Observational studies found positive associations when BV is detected earlier; first trimester, <14weeks *(*Mulinganya et al*, 2021)*. • Systematic reviews suggest that early pregnancy or preconception interventions are required for meaningful PTB reduction (Wu et al, 2025).
Trial Design Considerations	• Enroll early (<12–14 wks) and sample longitudinally; consider preconception subcohorts or pre-pregnancy interventions where feasible.

APPENDIX D: LITERATURE EXTRACTION SHEET*

ID	Title	Author	Year Publishe	Years of Data/ Search Period for Systematic Reviews	DOI	Study Design	Geography	Sample Size	Participant age range	Participant race/ethnic	Pregnancy Status	Number of previous pregnancies	Other key participant characteristics	Participant SES	Participant menstrual health product use	Participant vaginal washing statu
1	The vagin	Margherit	2025	2014-2024	10.101	Systematic revie	Global: 6 stud	2,194 initia	reproductive age (1	2 US studies st	Currently preg	Not reported	Excluded studies v	Not reporte	Not reported	Reported; one
2	Reassessir	Sawsan M	2025	1990-2019	10.101	Systematic revie	Global: Ameri	1116 initia	reproductive age (1	Not reported	Currently preg	Not reported	Excluded studies v	Not reporte	Not reported	Not reported
2	Reassessir	Sawsan M	2025	1990-2019	10.101	Systematic revie	Global: Ameri	19 studies	reproductive age (1	Not reported	Currently preg	Not reported	Excluded studies v	Not reporte	Not reported	Not reported
2	Reassessir	Sawsan M	2025	1990-2019	10.101	Systematic revie	Global: Ameri	9 studies u	reproductive age (1	Not reported	Currently preg	Not reported	Excluded studies v	Not reporte	Not reported	Not reported
3	Systemati	Kenfack-S	2023	1986-2021	10.101	Systematic revie	Global: 53.9%	3047 for P	reproductive age (1	Not reported	Currently preg	Not reported	None	Not reporte	Not reported	Not reported
4	Prevalenc	Neha Seth	2025	2013-2023	10.118	Systematic revie	Global; major	2337 initia	reproductive age (1	Not reported	Currently preg	Not reported	None	Available fo	Not reported	Not reported
5	Basic vagi	Leticia Kra	2014	2006-2008	10.118	Prospective coh	Rio De Janeirc	1699	reproductive age(1	24.4% black; 3	Currently preg	Not reported	Excluded women	Low SES - n	Not reported	Not reported
5	Basic vagi	Leticia Kra	2014	2006-2008	10.118	Prospective coh	Rio De Janeirc	1699	reproductive age(1	24.4% black; 3	Currently preg	Not reported	Excluded women	Low SES - n	Not reported	Not reported
6	The Preva	Suphapho	2017	2014	Not ava	Prospective coh	Thailand	270	reproductive age(1	Not reported	Currently preg	50.6% primiparo	None	99.6% educ	Not reported	Not reported
6	The Preva	Suphapho	2017	2014	Not ava	Prospective coh	Thailand	270	reproductive age(1	Not reported	Currently preg	50.6% primiparo	None	99.6% educ	Not reported	Not reported
7	Bacterial \	Bosede B	2016	2016	10.109	Prospective coh	Nigeria	246	reproductive age(1	Not reported	Currently preg	37.4% nulliparou	None	most comm	Not reported	Not reported
8	Maternal	Beng Kwa	2023	2014-2015	10.338	Prospective coh	Malaysia	237	reproductive age(1	Ethnicity captu	Currently preg	42.6% nulliparou	Pregnant women	52.7% recei	Not reported	Reported; exc
9	Prevalenc	Guy Mulir	2021	2017-2018	10.137	Prospective coh	DRC	533	reproductive age (1	Tribe reported	Currently preg	Did not report th	Excluded women	72.6% had '	Not reported	Found that wo
9	Prevalenc	Guy Mulir	2021	2017-2018	10.137	Prospective coh	DRC	533	reproductive age (1	Tribe reported	Currently preg	Did not report th	Excluded women	72.6% had '	Not reported	Found that wo
10	Effect of b	Trishna M	2022	2008-2022	10.100	Systematic revie	Global	370 initial	reproductive age (1	Not reported	Currently preg	Not reported	None	Not reporte	Not reported	Not reported
11	The vagin	Unnur Gu	2022	2014-2021	10.103	Systematic revie	Global; subgr	4321 initia	reproductive age (1	Reported for e	Currently preg	Not reported	Excluded studies i	Not reporte	Not reported	Not reported
11	The vagin	Unnur Gu	2022	2014-2021	10.103	Systematic revie	Global; subgr	4321 initia	reproductive age (1	Reported for e	Currently preg	Not reported	Excluded studies i	Not reporte	Not reported	Not reported

*Complete extraction workbook (.xlsx) shared separately. The screenshot above serves only as an illustrative example of the captured inputs.