


Reproductive epidemiology

A prospective preconception cohort study of the association between *Mycoplasma genitalium* and fecundability in Kenyan women trying to conceive

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ABSTRACT

STUDY QUESTION: Is *Mycoplasma genitalium*-infection associated with reduced fecundability?

SUMMARY ANSWER: Preconception *M. genitalium*-infection was associated with 27% lower fecundability though confidence intervals were wide, and the association between *M. genitalium* and fecundability may be dependent on concurrent bacterial vaginosis (BV).

WHAT IS KNOWN ALREADY: *M. genitalium* has been associated with cervicitis, pelvic inflammatory disease, infertility, and preterm birth, but the extent to which *M. genitalium* is causally related to adverse reproductive sequelae in women is debated.

STUDY DESIGN, SIZE, DURATION: Kenyan women enrolled in a prospective preconception cohort provided vaginal fluid specimens and underwent monthly pregnancy testing. Stored samples from 407 women who had been trying to conceive for ≤ 6 months were tested for *M. genitalium* using a nucleic acid amplification test.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Data on first day of last menstrual period, sexual behavior, pregnancy status, and vaginal specimens were collected at monthly preconception visits. The association between *M. genitalium* detected at the visit prior to each pregnancy test and fecundability was estimated using discrete time proportional probabilities models. Secondary analyses explored the influence of concurrent BV on the association between *M. genitalium* and fecundability.

MAIN RESULTS AND THE ROLE OF CHANCE: The 407 participants experienced 1220 menstrual cycles and 213 pregnancies. The prevalence of *M. genitalium* at enrollment was 7.7%. After adjustment for age, frequency of condomless sex in the last 4 weeks, and study site, *M. genitalium* was associated with a 27% lower fecundability, but confidence intervals were wide (adjusted fecundability ratio (aFR) 0.73, 95% CI 0.44, 1.23). In secondary analyses, when compared to cycles without *M. genitalium* or BV at the visit prior, women with both *M. genitalium* and BV at the visit prior had a 51% lower fecundability (aFR = 0.49, 95% CI 0.22, 1.09) whereas there was no association of *M. genitalium* alone (aFR = 0.98 (95% CI 0.54, 1.76)), and a smaller reduction in fecundability for women with BV only (aFR = 0.80 (95% CI 0.60, 1.07)).

LIMITATIONS, REASONS FOR CAUTION: Results should be interpreted cautiously given the relatively low prevalence of *M. genitalium* and wide confidence intervals.

WIDER IMPLICATIONS OF THE FINDINGS: In this cohort of Kenyan women trying to conceive, the association between *M. genitalium* and fecundability was influenced by concurrent BV status, suggesting there may be a synergistic effect of *M. genitalium* and BV on fecundability.

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Introduction

Infertility affects ~15% of couples and can lead to significant financial and emotional burden (Evers, 2002). Public health approaches to preventing infertility emphasize preconception health, including screening and treating women for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (US Preventative Services Task Force, 2021). National *C. trachomatis* and *N. gonorrhoeae* screening programs were implemented in part due to evidence that these infections reduce reproductive potential (Paavonen et al., 2008).

Mycoplasma genitalium, a sexually transmitted bacterium, causes male urethritis (Taylor-Robinson and Jensen, 2011). Whether *M. genitalium* causes adverse reproductive sequelae in women is debated, despite studies demonstrating associations between *M. genitalium* and cervicitis, PID, infertility, and preterm birth (Lis et al., 2015; Workowski et al., 2021). A 2015 meta-analysis demonstrated >2-fold increased odds of PID (OR 2.14, 95% CI 1.31–3.49) and infertility (OR 2.43, 95% CI 0.93–6.24) among women with *M. genitalium* (Lis et al., 2015). However, few of the infertility studies were prospective and the effect size was imprecise. More recently, in a prospective cohort study of women discontinuing intrauterine devices (IUD) to conceive, lifetime exposure to *M. genitalium* detected via serology at enrollment was associated with a longer time-to-pregnancy (Hazard Ratio 0.76, 95% CI 0.58–0.99) (Peipert et al., 2021).

The objective of this study was to assess the association between *M. genitalium* infection and fecundability, the per-menstrual cycle probability of pregnancy, using data from a prospective preconception cohort of Kenyan women.

Materials and methods

Study design and population

Data from the Microbiota and Preterm Birth (MPTB) Study, which enrolled women planning pregnancies in Nairobi and Mombasa, Kenya between 18 April 2017 and 18 March 2020, were used. The MPTB study was designed to assess the association between peri-conceptual vaginal microbiota and spontaneous preterm birth (Lokken et al., 2020). Women enrolled in the MPTB study were trying to conceive, HIV-seronegative, 18–45 years old, had menstruated in the 3 months before enrollment or recently discontinued hormonal contraception that can influence menstruation. Women who reported medical history potentially increasing preterm birth risk, had used antibiotics in the last 4 weeks, or had ever sought care for infertility were excluded. Participants recently using depot-medroxyprogesterone acetate (DMPA) injection were not eligible until 3 months after their last injection.

Women eligible for the fecundability sub-cohort were participants contributing ≥ 1 menstrual cycle, without reproductive co-morbidities (ectopic pregnancy, polycystic ovary syndrome, endometriosis, in-hospital treatment of PID), and trying to conceive for ≤ 6 menstrual cycles prior to enrollment. For this analysis, women who conceived or were lost to follow-up prior to the initiation of *M. genitalium* sampling, which started in 2018 (1 year after study launch), were excluded as they did not have

M. genitalium results for analysis. Women whose first *M. genitalium* sample was available at >6 menstrual cycles of total conception attempt time were also excluded to reduce potential bias associated with unobserved cycles (Weinberg et al., 1994; Schisterman et al., 2013).

Ethical approval

The study procedures were approved by the institutional review boards of Kenyatta National Hospital-University of Nairobi and the University of Washington. Participants provided written informed consent.

Clinical and laboratory procedures

Study procedures have been described (Lokken et al., 2020). At enrollment, participants completed an interview on demographics, medical history, sexual behavior, and reproductive history including recent contraceptive use, first day of last menstrual period (LMP), and the number of menstrual cycles attempting to conceive before enrollment ('pre-enrollment conception attempt time'). Clinicians collected vaginal swabs during a pelvic examination. Participants returned for up to 6 monthly preconception visits, where they completed an interim interview, underwent urine pregnancy testing (One Step Urine Pregnancy Test, EROVITA; Eros Ventures Ltd, Nairobi, Kenya), self-collected vaginal swabs, and reported the first day of their most recent LMP. Beginning in July 2018, participants who missed a preconception visit reported all first days of menstrual cycles occurring between their prior and current attended preconception visit ('reported interim menstrual cycles'). Due to a potential for delayed return to fertility with DMPA use, participants who discontinued DMPA 3–6 months prior to enrollment were eligible for an additional 3 months of preconception follow-up (9 months total) (Yland et al., 2020). If participants had genital symptoms suggestive of vaginal infections, including BV, they were treated per Kenyan syndromic management guidelines (National AIDS & STI Control Programme of Kenya, 2018).

Enrollment specimens were tested for *N. gonorrhoeae*, *C. trachomatis*, and *T. vaginalis* (Aptima Combo-2 CT/NG Detection System, Aptima *T. vaginalis* assay; Hologic, Inc). Treatment for these infections was provided at the participant's next visit if they were not covered by syndromic treatment at enrollment. Bacterial vaginosis (BV) was assessed at enrollment and preconception visits by Gram staining and scoring of vaginal fluid samples using the criteria of Nugent and Hillier (Nugent et al., 1991).

Beginning in May 2018, an additional vaginal specimen was collected at enrollment and self-collected at each monthly preconception visit for *M. genitalium* testing. Because antimicrobial resistance is selected for during treatment and the link between *M. genitalium* and adverse reproductive health outcomes in women remains debated, screening for and treating asymptomatic *M. genitalium* infections is not recommended (Australian Sexual Health Alliance, 2018; Soni et al., 2019; Workowski et al., 2021; Jensen et al., 2022). Therefore, vaginal samples were stored at -80°C after collection and tested for *M. genitalium* in 2020 and 2021 (Aptima *M. genitalium* assay, Hologic, Inc). Women whose

last sample in the study was positive were offered immediate re-testing and treatment if the re-test was positive.

Data analysis

First days of LMP reported at preconception visits and urine pregnancy test results were used to calculate the number of menstrual cycles for participants (Lokken et al., 2021). Because pregnancy testing occurred at different times in the menstrual cycle based on participants' visit schedules, cycles were considered negative for pregnancy only if the pregnancy test was negative and the participant reported a subsequent LMP at their next visit. Median time-to-pregnancy and cumulative incidence of pregnancy were estimated using Kaplan–Meier methods.

To estimate the association between *M. genitalium*-infection and fecundability, we used proportional probabilities models (log-binomial generalized linear models with robust standard errors) to generate fecundability ratios (FR) and 95% CI (Weinberg et al., 1989, 1994). The models included an indicator variable for menstrual cycle at risk and delayed entry for prior trying time (Weinberg et al., 1994; Schisterman et al., 2013), defined for this analysis as the total number of menstrual cycles of trying time at the first *M. genitalium* result for this analysis (number of pre-enrollment cycles trying to conceive + prospective cycles during follow-up prior to first sample collected for *M. genitalium* testing). Censoring criteria included self-reported initiation of biomedical infertility treatment ($n=3$), participant withdrawal or loss to follow-up ($n=76$), no conception by end of eligible preconception follow-up ($n=79$), and end of in-person study visits due to the COVID-19 pandemic ($n=36$). We imputed missing *M. genitalium* and time-varying covariate data (i.e. frequency of condomless sex, BV) due to missed preconception visits ($n=103$) and missingness for time-varying covariates at attended preconception visits ($<0.03\%$ missing) using data from the last visit carried forward.

The primary exposure was *M. genitalium* detected at the visit prior to each pregnancy test, modeled as a time-varying exposure. In secondary analysis, we assessed *M. genitalium* status at enrollment as a time-independent measure to evaluate whether a single measure of *M. genitalium* during preconception was associated with reduced fecundability. We also evaluated the potential synergistic effect of *M. genitalium* and BV at the visit prior to pregnancy with a four-level categorical variable: neither *M. genitalium* nor BV, *M. genitalium* positive only, BV positive only, positive for both *M. genitalium* and BV. To accompany this analysis, we estimated the relative excess risk due to interaction (RERI). Because FRs <1 are interpreted as reduced fecundability and >1 as increased fecundability, we have interpreted the RERI similarly with an RERI <1 demonstrating positive interaction/more than additivity.

We adjusted *a priori* for participants' age (<25 , 25–29, 30–34, 35–39, 40–45), frequency of condomless sex in the prior 4 weeks (continuous), and study site based on existing literature, site-specific differences in demographic characteristics, and consideration of potential causal associations between variables. Because only a small proportion of participants were prescribed antibiotics with activity against *M. genitalium* or BV (doxycycline: 2.6% of visits, azithromycin: 1.3% of visits, metronidazole: 2.1% of visits), we did not adjust for antibiotic use.

We conducted sensitivity analyses excluding participants with reproductive co-morbidities or history that may be suggestive of potential sub-fecundity. First, we conducted the primary analysis excluding women with *N. gonorrhoeae* or *C. trachomatis* at enrollment. Second, we additionally excluded those with *T. vaginalis* or PID diagnosis at enrollment, any history of treatment for *N.*

gonorrhoeae, *C. trachomatis*, *T. vaginalis*, syphilis, or PID, self-report of fibroids or unknown uterine abnormality, any DMPA use within 6 months of enrollment, or a partner living with HIV. We conducted two additional sensitivity analyses of the primary analysis including (i) additional adjustment for BV and (ii) excluding the participant-reported interim menstrual cycles to assess whether their inclusion influenced results.

Results

Of the 701 participants enrolled, 98 (14.0%) were excluded from this fecundability analysis due to reproductive co-morbidities or more than six cycles of pre-enrollment conception attempt time and 92 (13%) were excluded solely for contributing no follow-up time. An additional 104 (14.8%) were excluded because they conceived or were lost to follow-up before *M. genitalium* sampling was initiated, leaving 407 (58.1%) eligible participants (Supplementary Fig. S1). While there were some minor differences in characteristics comparing those eligible to those excluded for missing *M. genitalium* results (Supplementary Table S1), time-to-pregnancy was similar (logrank $P=0.51$). Eligible participants attended 1117 study visits and experienced 1220 menstrual cycles, of which 103 (8.4%) were reported after missed preconception visits. There were 213 incident first pregnancies. The median time-to-pregnancy was four cycles (IQR 2, 10) with a cumulative six-cycle pregnancy rate of 67.4% (95% CI 61.8–72.9) and a 12-cycle pregnancy rate of 82.8% (95% CI 73.7–90.1).

Participants' median age was 29 years (IQR 25, 34). Most had finished secondary school (57.7%, $n=235$), experienced a prior pregnancy (92.9%, $n=378$), and reported zero pre-enrollment cycles trying to conceive (71.0%, $n=289$) (Table 1). At enrollment, 36.4% (148/407) had BV, 8.7% (35/407) had *C. trachomatis* infection, 1.0% (4/403) had *N. gonorrhoeae* infection, 1.2% (5/403) had *T. vaginalis* infection, and 7.7% (30/389) had *M. genitalium* infection. During follow-up, *M. genitalium* was detected at 7.8% (87/1114) of visits.

Eight percent (98/1220) of menstrual cycles were considered exposed to *M. genitalium*. *Mycoplasma genitalium* infection at the visit prior to pregnancy testing was associated with a 19% lower fecundability in unadjusted analysis (FR 0.81, 95% CI 0.48–1.36) (Table 2). After adjustment for age, frequency of condomless sex in the last 4 weeks, and study site, *M. genitalium* was associated with a 27% lower fecundability, but confidence intervals remained wide (adjusted FR (aFR) 0.73, 95% CI 0.44–1.23). Results were similar for sensitivity analyses that additionally adjusted for BV; excluded participant-reported interim menstrual cycles; and excluded additional participants with potential sub-fecundity (Supplementary Table S2). When considering *M. genitalium* at the first visit only, *M. genitalium* was associated with a 24% lower fecundability ((aFR) 0.76, 95% CI 0.47–1.22).

In the analysis assessing potential synergy between *M. genitalium* and BV at the visit prior to pregnancy, compared to cycles without *M. genitalium* or BV, the aFR was 0.98 (95% CI 0.54, 1.76) for women with *M. genitalium* only, 0.80 (95% CI 0.60, 1.07) for women with BV only, and 0.49 (95% CI 0.22, 1.09) for women with both *M. genitalium* and BV (Table 2). The RERI estimate was -0.29 (95% CI -1.05 , 0.47).

Discussion

In this cohort of Kenyan women trying to conceive, *M. genitalium* infection at the visit prior to pregnancy testing was associated with a non-significant 27% lower per-menstrual cycle probability

Table 1. Enrollment characteristics for 407 Kenyan women planning pregnancies and eligible for the *M. genitalium* and fecundability analysis.

Characteristic	N	Total (N = 407)
Demographic and partnership characteristics		
Cohort	407	
Nairobi	286	(70.3%)
Mombasa	121	(29.7%)
Age (years)	407	
<25	84	(20.6%)
25–29	136	(33.4%)
30–34	105	(25.8%)
35–39	69	(17.0%)
40–45	13	(3.2%)
Education level	407	
<8 years	31	(7.6%)
8–11 years	141	(34.6%)
12–15 years	173	(42.5%)
≥16 years	62	(15.2%)
Monthly household income (KSh)	404	
<2500	17	(4.2%)
2500–10 000	138	(34.2%)
10 000–30 000	180	(44.6%)
30 000–75 000	45	(11.1%)
>75 000	24	(5.9%)
Partner's HIV-serostatus	406	
HIV-seronegative	317	(78.1%)
HIV-seropositive	18	(4.4%)
Unknown	71	(17.5%)
Reproductive history		
Most recent contraceptive method ^a	407	
None	118	(29.0%)
Condoms	19	(4.7%)
Oral contraceptive pill	7	(1.7%)
DMPA injectable	16	(3.9%)
Copper intrauterine device	97	(23.8%)
Implant	147	(36.1%)
Other	3	(0.7%)
Ever pregnant	407	378 (92.9%)
History of miscarriage	378	76 (20.1%)
Parous	407	366 (89.9%)
Number of menstrual cycles of prior conception attempt time at enrollment ^b	407	
0	289	(71.0%)
1–3	70	(17.2%)
4–6	48	(11.8%)
Sexual behavior and vaginal washing in last 4 weeks		
Any vaginal washing	407	149 (36.6%)
Frequency of condomless sex	407	
No condomless sex	29	(7.1%)
1–4	132	(32.4%)
5–8	97	(23.8%)
≥9	149	(36.6%)
Median number of condomless sex acts (Q1–Q3)	6	(3–12)
STI, BV, and vaginal symptoms		
History of STI ^c	407	4 (1.0%)
<i>N. gonorrhoeae</i>	403	4 (1.0%)
<i>C. trachomatis</i>	403	35 (8.7%)
<i>T. vaginalis</i>	403	5 (1.2%)
<i>M. genitalium</i> ^d	389	30 (7.7%)
BV (Nugent ≥7)	407	148 (36.4%)
Abnormal vaginal discharge ^e	407	37 (9.1%)
Vaginal/vulvar itching ^e	407	37 (9.1%)

^a Women reporting OCP, contraceptive implant, or copper IUD discontinuation >2 months before enrollment or a last DMPA injection >6 months before enrollment were included in the 'none' category.

^b Women reporting >6 cycles of conception attempt time prior to enrollment were excluded for this analysis.

^c Self-reported history of syphilis, chlamydia, gonorrhea, and/or trichomoniasis.

^d N = 18 participants had their first *M. genitalium* sample collected at a subsequent preconception follow-up visit.

^e Self-report.

KSh, Kenyan shillings; DMPA, depot-medroxyprogesterone acetate; (Q1–Q3) Quartile 1–Quartile 3; STI, sexually transmitted infection; BV, bacterial vaginosis.

of pregnancy and this association appeared to be influenced by concurrent BV status. In exploratory analysis, *M. genitalium* alone showed no association with fecundability and BV alone showed a modest association with fecundability, but women with concurrent BV and *M. genitalium* had 51% lower fecundability compared to cycles with neither condition. While this suggests a synergistic relationship between *M. genitalium* and BV, results should be interpreted cautiously given the wide confidence intervals (aFR 0.49, 95% CI 0.22–1.09).

There are limited data on *M. genitalium* or BV and fecundability. A recent prospective cohort study of women discontinuing contraception to conceive in the US assessed lifetime exposure to *M. genitalium* and time-to-pregnancy (Peipert et al., 2021). In a multivariable piecewise exponential proportional hazards model assessing IUD use and time-to-pregnancy that included *M. genitalium* seropositivity and sociodemographics as covariates, lifetime exposure to *M. genitalium* was associated with a 24% longer time-to-pregnancy (adjusted Hazard Ratio 0.76, 95% CI 0.58–1.00). *Chlamydia trachomatis*, in comparison, was not associated with time-to-pregnancy. This effect size was similar to the 27% reduced fecundability for *M. genitalium*-infection measured by NAAT in our primary analysis. Prior analyses of this Kenyan cohort demonstrated 17% lower fecundability among women with BV at the visit prior to pregnancy testing and 43% lower fecundability associated with persistent BV (Lokken et al., 2021). Given the delay in launching sample collection for *M. genitalium* testing, this analysis includes a slightly different sub-cohort. Nonetheless, the 20% lower fecundability associated with recent BV alone here is consistent with the original result (Lokken et al., 2021).

Studies of *M. genitalium* and reproductive outcomes in females have focused on PID and infertility. Although most of these were limited by cross-sectional designs, inclusion of potentially sub-fertile women, and unclear timing of *M. genitalium* infection (Taylor et al., 2013; Lis et al., 2015), the limited prospective data suggest that both BV and *M. genitalium* infection are linked to female infertility. Sero-epidemiologic studies that adjusted for antibody to *C. trachomatis* observed 4- to 5-fold increased risk of infertility associated with prior *M. genitalium* infection (Clausen et al., 2001; Svenstrup et al., 2008). In a prospective study of women with clinically suspected PID, cervical or endometrial *M. genitalium* infection was associated with infertility (adjusted relative risk 1.4, 95% CI 0.6–2.9), though confidence intervals were wide (Haggerty et al., 2008). In the same study, women without *N. gonorrhoeae* or *C. trachomatis* who had four BV-associated bacteria (*Sneathia sanguinegens*, *Sneathia amnii*, *Fannyhessea vaginae* (previously known as *Atopobium vaginae*), and BVAB1) at the cervix or endometrium at baseline had a 3.4-fold increased risk of infertility compared to participants without these bacteria (Haggerty et al., 2016). As the effects of infections on fertility likely represent a continuum ranging from reduced fecundability to clinically defined infertility (e.g. tubal factor infertility) these earlier studies of infertility provide useful background for consideration when interpreting our fecundability results.

Our observation that the greatest reduction in fecundability occurred in women with both *M. genitalium* and BV is consistent with studies suggesting potential synergy. BV is often present in women with *M. genitalium* infection (Nye et al., 2020), BV may enhance susceptibility to *M. genitalium* (Lokken et al., 2017), and periodic presumptive therapy active against BV was associated with decreased incidence of *M. genitalium* (Balkus et al., 2016). These data suggest that *M. genitalium* and BV bacteria may enhance one another's growth, survival, or pathogenicity. Furthermore,

Table 2. Unadjusted and adjusted associations between *M. genitalium* (MG) and fecundability among 407 Kenyan women trying to conceive.

Exposure	Menstrual cycles		Pregnancies		Unadjusted		Adjusted ^a	
	N	n (%) exposed	N	n (%) exposed	FR (95% CI)	FR (95% CI)		
Primary analysis								
MG at visit prior ^b	1220	98 (8.0)	213	14 (6.6)	0.81 (0.48, 1.36)	0.73 (0.44, 1.23)		
Secondary analyses								
MG status at enrollment ^c	1193	101 (8.5)	205	14 (6.8)	0.81 (0.50, 1.30)	0.76 (0.47, 1.22)		
MG and BV status at visit prior ^b	1220		213					
MG–, BV–		768 (63.0)		143 (67.1)	REF	REF		
MG+, BV–		43 (3.5)		8 (3.8)	1.04 (0.57, 1.92)	0.98 (0.54, 1.76)		
MG–, BV+		354 (29.0)		56 (26.3)	0.84 (0.63, 1.11)	0.80 (0.60, 1.07)		
MG+, BV+		55 (4.5)		6 (2.8)	0.57 (0.25, 1.27)	0.49 (0.22, 1.09)		

^a A priori inclusion of age, study site, and frequency of condomless sex.

^b *Mycoplasma genitalium* at the visit prior and BV at the visit prior were time-varying.

^c N = 389 participants with MG sample at enrollment.

BV, bacterial vaginosis.

successful metronidazole-mediated treatment of BV may facilitate *M. genitalium* clearance. A recent randomized trial assessed the effect of adding metronidazole, which targets anaerobes commonly present in women with BV, to a standard PID treatment regimen. After 30 days, the proportion of cervical *M. genitalium* infections was lower in the treatment arm with metronidazole versus without (4.4% versus 14.4%, $P=0.04$) and endometrial *M. genitalium* was less common in the metronidazole arm compared to the standard therapy arm (1.2% versus 4.4%, $P=0.4$) (Wiesenfeld et al., 2021).

There are several potential mechanisms for a synergistic effect of concurrent BV and *M. genitalium* on fecundity. First, inflammation occurs with both BV and *M. genitalium* and their co-occurrence may have an additive effect. Two studies have demonstrated increased concentrations of inflammatory mediators associated with *M. genitalium* in the lower reproductive tract, although a third study did not (Dehon et al., 2016; Garza et al., 2021; Wang et al., 2022). *In vitro*, an endocervical epithelial cell-model demonstrated host defense activation and pro-inflammatory cytokine secretion with *M. genitalium* infection (McGowin et al., 2013). While BV is not associated with vaginal neutrophils and does not often present with clinically apparent inflammation, BV has been associated with elevated levels of the pro-inflammatory cytokines IL-1 β , IL-8, IL-6, and CXCL10 (Mitchell and Marrazzo, 2014; Sabo et al., 2020; Dabee et al., 2021). It is possible that when both BV and *M. genitalium* occur together, inflammation induced by one condition may enhance inflammation from the other. The inflammatory response to these infections could reduce fecundability by damaging ciliated cells in Fallopian tubes, disrupting fertilization, reducing endometrial receptivity and implantation, and disrupting embryonic/fetal-maternal immune interactions (Granot et al., 2012; Mor et al., 2017; D'Ippolito et al., 2018).

Given that *M. genitalium* can induce salpingitis and damage Fallopian tube epithelial and ciliated cells as soon as 6 days of infection (Møller et al., 1985; Cohen et al., 2005; Baczynska et al., 2007), the absence of an association between *M. genitalium* infection alone and reduced fecundability in sub-analysis was surprising. This supports a second possible mechanism, whereby BV may facilitate the ascension of *M. genitalium* to the upper reproductive tract. Many BV-associated bacteria produce sialidases and mucinases, which can degrade cervical mucus, reducing the effectiveness of this barrier to ascending pathogens (Wiggins et al., 2001). In the absence of BV, it may be more difficult for *M. genitalium* to ascend to the upper reproductive tract. We did not

sample the endometrium, so were unable to evaluate whether women with BV had *M. genitalium* in the upper reproductive tract more often than women without BV. A third potential mechanism for reduced fecundability is a direct influence of *M. genitalium* on sperm. *In vitro*, *M. genitalium* can attach to spermatozoa and may make them less efficient at ascending to the uterus (Svenstrup et al., 2003).

The major strengths of this analysis were its prospective design, which is the gold standard of fecundability studies, and monthly sampling for *M. genitalium* and BV, enabling cycle-specific estimates. In addition, this study included general population women trying to conceive early in their conception attempt and most had been pregnant before, reducing biases associated with underlying primary fertility problems. Lastly, *M. genitalium* was assessed using a highly sensitive and specific diagnostic test and BV was evaluated using a research gold standard, the Nugent score.

There were also limitations. First, this study had limited statistical power leading to reduced precision of the results. However, the direction and magnitude of the results point to biologically plausible associations between *M. genitalium* and fecundability that may be driven by concurrent BV. This question deserves further attention. Second, the lack of molecular testing of the vaginal microbiota means we have limited understanding of the underlying microbiota and their relationship to *M. genitalium* and how that may be driving the observed association. Third, 13% of women enrolled in the parent study were excluded for this analysis solely because they did not contribute any follow-up time and another 15% were excluded because they lacked *M. genitalium* data due to a 1-year delay in sample collection. For the former, while most enrollment characteristics were similar between those eligible and those excluded because they lacked follow-up data, the women excluded for having no follow-up time may have had different underlying fertility potential as they were less likely have ever been pregnant, were marginally younger, and had a slightly higher *M. genitalium* prevalence at baseline. Characteristics were also similar when comparing those eligible versus those excluded for only lacking *M. genitalium* data. Most notably rates of other STIs and BV were similar suggesting that *M. genitalium* test positivity would not be differential. However, it is possible these exclusions could have biased effect estimates.

Among Kenyan women attempting to conceive, there was a non-significant 27% reduction in fecundability associated with *M. genitalium* detected at the visit prior to each pregnancy test.

Fecundability was further reduced (~50%) among women with both BV and *M. genitalium*. While these results should be interpreted cautiously given the small number of *M. genitalium* cases and reduced precision, they raise important questions about how *M. genitalium* and BV-associated bacteria may interact to influence fecundability. Further exploration of the extent to which *M. genitalium* causes a pro-inflammatory response *in vivo*, whether and how BV-associated bacteria influence *M. genitalium* acquisition, persistence, and ascension to the upper reproductive tract, and whether *M. genitalium* may itself enhance the role of non-optimal reproductive tract microbiota on reproductive function is warranted. Ultimately, the emotional and financial burden associated with delayed time-to-pregnancy and infertility are immense (Ethics Committee of the ASRM, 2015; Luk and Loke, 2015). BV is present in approximately one-third of reproductive-age women (Koumans *et al.*, 2007), and *M. genitalium* is as, or more, common as *C. trachomatis* infection (Seña *et al.*, 2018; Torrone *et al.*, 2021). Both conditions can persist for long periods, yet both are treatable. If larger studies confirm these findings, preconception screening for and treatment of BV and *M. genitalium* may be a low-cost intervention to reduce impaired fertility.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

This study was conducted with approval from the Kenyatta National Hospital—University of Nairobi Ethics and Research Committee (KNH-UON ERC), which requires their written approval for additional analyses and release of data (including de-identified data) from Kenyan studies. Therefore, data for this study will be available from the authors upon request, with written approval for the proposed analysis from the KNH/UON ERC. Application forms and guidelines can be accessed at <http://erc.uonbi.ac>. To request these data, please contact KRTC Administrator at kenyares@uw.edu.

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Authors' roles

R.S.M. is the principal investigator of the parent study and supervised study protocol development and implementation. J.K. and W.J. were site principal investigators and oversaw study staff and implementation at Kenyatta National Hospital and Ganjoni Health Center. R.S.M., E.M.L., J.K., W.J., and K.M. participated in designing the parent study, protocol, data collection tools, and staff training. W.J. and K.M. oversaw laboratory protocols and methods. E.K. conducted all APTIMA testing and managed

laboratory quality control. B.O. and M.N. oversaw clinical data collection and interpreted data. E.M.L., R.S.M., and L.E.M. designed this analysis and developed the statistical analysis plan for the study. E.M.L. directed the study, conceived of the analysis, conducted the statistical analyses, and wrote the first draft of the manuscript. All authors reviewed and approved the final manuscript.

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Conflict of interest

R.S.M. receives research funding, paid to the University of Washington, from Hologic Corporation and consulting fees from Lupin Pharmaceuticals. L.E.M. receives research funding and material for research studies, paid to the University of Washington, from Hologic Corporation and Nabriva Therapeutics, travel support from Hologic, and consulting fees from Health Advances. E.M.L.'s contributions to this study primarily occurred while affiliated with the University of Washington; at the time of submission, E.M.L. was an employee of and holds stock or stock grants for AbbVie, Inc. The other authors have no conflicts of interest.

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