MYCOPLASMA GENITALIUM: CLINICAL CHARACTERISTICS, RISK FACTORS AND ADVERSE PREGNANCY OUTCOME

by

Vanessa L. Short

BS, Pennsylvania State University, 2002

MPH, University of Pittsburgh, 2005

Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2009

UNIVERSITY OF PITTSBURGH

Graduate School of Public Health

This dissertation was presented

by

Vanessa L Short

It was defended on

April 17, 2009

And approved by

Dissertation Advisor:
Catherine Haggerty, PhD, MPH
Assistant Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Committee Member:
Sheryl Kelsey, PhD
Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Committee Member:
Pamela Murray, MD, MPH
Associate Professor
Department of Pediatrics
School of Medicine
University of Pittsburgh

Committee Member:
Roberta Ness, MD, MPH
Dean
The University of Texas School of Public Health

Catherine Haggerty, PhD, MPH

MYCOPLASMA GENITALIUM: CLINICAL CHARACTERISTICS, RISK FACTORS AND ADVERSE PREGNANCY OUTCOME

Vanessa L. Short, PhD

University of Pittsburgh, 2009

Mycoplasma genitalium (Mg), a sexually transmitted bacterium, may cause female reproductive morbidities, including pelvic inflammatory disease (PID). As the clinical and risk profile of women with Mg is not well understood, we examined the characteristics of Mg among women. Data from 586 women with clinically suspected PID enrolled in the PID Evaluation and Clinical Health Study were analyzed. Clinical, demographic, sexual and behavioral characteristics were compared between women positive and negative for Mg in the cervix and/or endometrium by polymerase chain reaction (PCR), and between Mg positive and *Chlamydia trachomatis* (Ct) and/or *Neisseria gonorrhoeae* (Gc) (Ct/Gc) positive women. Mg positive women had similar clinical characteristics as women without Mg and as women with Ct. Compared to women with Gc, women with Mg had lower pelvic pain scores (p=0.01), and were less likely to have cervicitis (p=0.001), erythrocyte sedimentation rate >15mm/hr (p=0.002), white blood cell count >10,000mm^3 (p=0.02), and oral temperature $\geq$38.3°C (p=0.08). Age <25 years (AOR 2.7, 95% CI 1.5-5.2), douching (AOR 2.3, 95% CI 1.3-4.1), and smoking (AOR 1.8, 95% CI 1.0-3.2) were associated with Mg. The demographic, sexual and behavioral characteristics were similar between Mg positive women and Ct/Gc positive women.

Since Mg is associated with PID, Mg may affect pregnancy, yet the consequences of prenatal Mg are unknown. Therefore, we next conducted a nested case-control study to examine the relationship between Mg and spontaneous abortion (SAB) among women enrolled in the Early Pregnancy Study, a study of violence and SAB among pregnant women presenting at an Emergency Department. Mg was

measured by PCR in urine from 82 women who experienced a SAB and 134 control women. Characteristics of cases and controls were compared and the relationship between Mg and SAB was evaluated. Mg was not associated with SAB but was associated with nulliparity (AOR 3.4, 95% CI, 1.0-11.6), self-reported difficulty conceiving (AOR 4.8, 95% CI 0.9-25.7), and history of PID (AOR 3.9, 95% CI 0.9-16.1) and Ct (AOR 3.0, 95% CI 0.8-10.5).

This dissertation yields significant public health findings by describing the clinical picture of Mg-PID, identifying women at risk, and examining the consequences of prenatal Mg.

TABLE OF CONTENTS

LIST OF TABLES

1.0 INTRODUCTION

1.1 SPECIFIC AIMS

M. genitalium has been associated with numerous female reproductive morbidities, including cervicitis, PID and tubal factor infertility. Still, *M. genitalium* has not been extensively studied in women. A gap exists in the research literature on the clinical features and presenting complaints of women with *M. genitalium* and the risk factors associated with *M. genitalium.* No studies have compared the clinical characteristics of and risk factors for lower and/or upper genital tract *M. genitalium* infection to other bacterial STDs. Another understudied area is the effect of *M. genitalium* in pregnancy. Therefore, the goal of the proposed research is to describe the clinical picture of *M. genitalium* female genital tract infection, identify women at high risk for *M. genitalium*, and examine the consequences of prenatal *M. genitalium* infection.

The following research aims and hypotheses will be used to achieve this goal:

1. To describe the clinical features of women with lower and/or upper genital tract *M. genitalium* infection in a population of women presenting with clinically suspected PID. *We hypothesize that presenting clinical characteristics, symptoms, and pelvic pain will be less frequent and severe among women with M. genitalium or C. trachomatis compared to women with gonococcal PID.*
2. To describe the risk factors of *M. genitalium* infection in the lower and/or upper genital tract in a cohort of women with clinically suspected PID. *We hypothesize that women with M. genitalium will be more likely to exhibit certain characteristics, such as younger*

age, black race, history of STDs or PID, young age at sexual debut, rare or inconsistent condom use, hormonal contraceptive use, douching, and drug, tobacco and alcohol use than women without M. genitalium.

3. To compare the risk factors of women with *M. genitalium* to women with *N. gonorrhoeae* and/or *C. trachomatis* female genital tract infections. *We hypothesize that the risk factors for M. genitalium will be similar to risk factors of C. trachomatis and N. gonorrhoeae.*
4. To compare early pregnancy *M. genitalium* urine PCR between pregnant women who subsequently experienced a SAB and pregnant women who maintained their pregnancies past 22 weeks gestation. *We hypothesize that women who experienced a SAB will be more likely test positive for M. genitalium than women who did not experience a SAB.*

1.2 BACKGROUND

1.2.1 *Mycoplasma genitalium*

1.2.1.1 Definition of *M. genitalium*

Mycoplasmas (class Mollicutes) are the smallest prokaryotic organisms known (1) and are distinguishable from other bacteria by their minute size and genome, and total lack of cell wall (2). Mycoplasmas have limited biosynthetic abilities and require sterols for growth and for membrane synthesis (1). Several mycoplasmal species, including 20 species in the genus Mycoplasma and two species in the genus Ureaplasma, have been isolated from humans, where they usually exhibit organ and tissue specificity (2, 3). The primary habitats in humans are the mucous surfaces of the respiratory and urogenital tracts, the eyes, alimentary canal, mammary glands and joints (2). Four mycoplasmas- *Mycoplasma genitalium*, *M. hominis, Ureaplasma urealyticum* (biovar 2) and *U. parvum* (biovar 1)- are associated with disease in the genitourinary tract of men and women (4).

M. genitalium has the smallest genome of the mycoplasmas; a size of only 580,073 base pairs of DNA encoding 517 genes (1, 4). In fact it is the smallest prokaryote capable of self-replication and its genome was the first of any microorganism to be fully sequenced (5). The pear-shaped *M. genitalium* cells are characterized by a complex terminal attachment organelle, known as a 'tip structure,' which mediates attachment to eukaryotic host cells and allows for motility across surfaces (5, 7). *M. genitalium* is distinguishable from other genitourinary mycoplasmas by its structure, energy source and its small genome size.

1.2.1.2 Pathogenesis of *M. genitalium*

The urogenital tract is the main site infected by *M. genitalium* (4), but it has been, in rare occasions, detected in rectal (6) and respiratory tract specimens by PCR and culture (7, 8). Adhesion of *M. genitalium* to host cells, accomplished using a specialized tip structure that contain proteins called adhesions which are most often encoded by the MgPa gene, is required for colonization and infection (9). This property of strong adherence to cells is the first step in bringing about cellular damage and invoking an inflammatory cell response (10). Evidence suggests that *M. genitalium* may also be able to enter host cells and reside intracellularly within epithelial cells using their tip structure (11).

Following *M. genitalium* infection, most of the damage to host cells is caused by the inflammatory response of the host immune system, and only partially caused by toxins and by-products produced by *M. genitalium* (12). Further, *M. genitalium* infections may follow a chronic course, as demonstrated with primate and human studies. Taylor-Robinson et al showed that all 4 female chimpanzees inoculated urogenitally with *M. genitalium* shed for 12 to 15 weeks (13). Tosh et al used PCR to test vaginal and urine samples from 383 young women aged 14-17 years and urine samples from their 117 male partners for *M. genitalium* (14). Samples were collected and tested weekly following a positive *M. genitalium* test result. The authors found that *M. genitalium* shedding may persist longer than 8-12 weeks, as 31.3% of untreated *M. genitalium* infections lasted over 8 weeks and 21.9% (7/32) of infections lasted over 12 or more weeks (14). Cohen et al collected and tested cervical samples every 2

months for *M. genitalium* by PCR from 258 Kenyan female sex workers (15). At enrollment, 16% of the women tested positive for *M. genitalium* (15). After 3, 5 and 7 months, 17%, 9%, and 21% of the *M. genitalium* infections persisted despite the high prevalence of antibiotic use, including doxycycline and ciprofloxacin, among this study population (15). In fact, 96% (104/107) of incident *M. genitalium* infections occurred in women who were taking antibiotics at some point during follow up (15). Although re-infection might have accounted for these findings, in an analysis of 7 participants who were persistently infected with *M. genitalium* for up to 21 months, all cervical specimens tested both early and late during infection from the same woman contained *M. genitalium* of the same strain type (15). This suggests that women had persistent infection and were not simply getting re-infected.

The chronic, persistent course of *M. genitalium* infection seems to be similar to infections caused by *C. trachomatis,* a sexually transmitted obligate intracellular bacterium that grows in eukaryotic epithelial cells. Chlamydial infections are self-limiting, acute, low-grade persistent infections, and infections in women tend to be asymptomatic or nonspecific. (16). The clinical manifestations of chlamydial infections represent the combined effects of tissue damage from chlamydial replication and inflammatory responses to *C. trachomatis* (16). The number of infecting organisms influences the magnitude of the initial inflammatory response and the likelihood of repeated infection (17). There is also potential for self-induced immune damage to the host arising from chlamydial heat shock proteins or other antigens that are similar to host components (17). A frequent pathologic end point of *C. trachomatis* infection is scarring of affected mucous membranes, eventually causing infertility and ectopic pregnancy (16). *C. trachomatis* infection may result in severe inflammation, particularly with chronic infection. Further, repeat infections may contribute to the development of a delayed hyper-sensitivity response, which may play an important role in chlamydial-related sequelae (18). Recurrent infections may provide repetitive injury to the pelvic organs and provide more opportunities for adhesion formation, and thus, adverse sequelae.

On the other hand, *M. genitalium* infections are less like those caused by *N. gonorrhoeae*, a gram-negative diplococcus which produces a variety of extracellular products that damage host cells at the time

of infection. In humans, mucous membranes lined by columnar epithelial cells are susceptible to gonococcal infections, as *N. gonorrhoeae* adheres to mucosal cells in a process mediated by surface proteins (19). Ciliary motility is impaired and destruction of ciliary cells is possible, which may promote further attachment of additional organisms. *N. gonorrhoeae* may also cause sloughing of ciliated mucosal cells (20).

1.2.1.3 Transmission of *M. genitalium*

Evidence suggests that *M. genitalium* is sexually transmitted. First, detection of the *M. genitalium* has been associated with a history of sexual intercourse and with increasing number of sexual partners (21, 22). Second, the concordance rate among female sexual partners of *M. genitalium* infected males has been high, ranging from 46 to 63% (23-25). Third, sequence-based typing of *M. genitalium* has revealed sexual transmission. In a study of 19 couples conducted by Hjorth et al, sequence-based typing of *M. genitalium* revealed sexual transmission, for the sequence type found in specimens from the female partner was identical to that found in the male partner in all the couples studied (26).

On the contrary, *M. genitalium* has been detected in women who denied ever having any sexual contact. In the Manhart et al study, 2 of the 34 (0.05%) women who tested positive for *M. genitalium* denied sexually activity (22), while Tosh reported that only one of the 65 (1.5%) adolescent women who tested positive for *M. genitalium* denied sexually activity (14). However, very few women have reported no sexual experience, and these small numbers do not support the concept that *M. genitalium* is not sexually transmitted. These data could be explained by false positive tests or intentional underreporting of sexual activity.

1.2.1.4 Detection of *M. genitalium*

There is no routinely used method to detect *M. genitalium* and no commercial detection method is currently available. Historically, because of its small size and growth requirements, detection of *M. genitalium* has been difficult. It is extremely hard to culture, due to the fastidiousness and slow growth of

the microorganism (14). Serology can produce false positives due to the cross-reactions between *M. genitalium* and *M. pneumoniae* (27) and is not routinely used for epidemiological studies. Currently, in-house nucleic acid amplification tests (NAAT) are the most widely used method for detecting *M. genitalium*, due to their sensitivity, specificity and rapidity. Recent developments in PCR-based *M. genitalium* assays have allowed for more research. These tests use amplification of portions of the DNA which are highly conserved in *M. genitalium* but not found in other species. Assays can differ in their target DNA sequences, with most targeting the MgPa adhesion gene (the first gene to be sequenced) or the rDNA gene (there is only one copy in the *M. genitalium* genome) (28, 29). PCR is inexpensive and faster than culture and requires only a small amount of DNA. In addition to being less time consuming than culture, studies have shown that PCR is more sensitive and is better at detecting *M. genitalium* specifically (30).

The optimal specimen type to used for *M. genitalium* PCR has not been thoroughly assessed, and differential detection may reflect varying bacterial loads between samples (28). Wroblewski et al found that self-obtained vaginal swab specimens (91%) were more sensitive than urine (65%) or cervical swab (53%) for the detection of *M. genitalium* by PCR in samples from a population of 284 symptomatic women presenting at a Seattle STD clinic (28). In contrast, Jensen et al compared the efficacy of first void urine (FVU) with cervical and urethral swab specimens from 776 women attending an outpatient STD clinic for detection of *M. genitalium* and *C. trachomatis* using in-house inhibitor-controlled PCR assays and found urine to be the most sensitive (31). The urine detected more infections (88%) than cervical (71%) and urethral swab specimens (57%), and urine specimen was significantly more efficient than both the urethral ($p=0.0009$) and the cervical swab specimen ($p=0.049$) (31). The number of infections detected with the cervical swab was not significantly different than the number of infections detected with the urethral swab ($p=0.21$) (31). However, as only 88% of infections were diagnosed using urine samples, urine specimens could be supplemented with a cervical swab specimen to increase sensitivity (31). When cervical and urine samples were analyzed together, 96% of infections were identified (31). Similar results were found for *C. trachomatis.*

1.2.1.5 Prevalence of *M. genitalium* in women

The prevalence of *M. genitalium* in women has varied among studies and has been estimated between less than 1% and 38%, depending on the study population. In the lower genital tract, *M. genitalium* tends to be more prevalent among STD clinic attendees (4.5% to 38%) (15, 23, 32-38) compared to the general population of asymptomatic women (0% to 2.8%) (14, 21, 22, 33, 38, 39) (Table 1). Further, case-control studies have reported a significant difference between the prevalence of *M. genitalium* among STD clinic attendees compared to asymptomatic women (33, 38). Tsunoe et al conducted a case-control study among 174 female commercial sex workers attending a Japanese STD clinic and 90 asymptomatic pregnant women (38). The prevalence rate of *M. genitalium* detected by PCR in endocervical samples was significantly higher in commercial sex workers compared to the pregnant women (12.6% vs. 1.1%, p= 0.0016) (38). Falk et al used PCR to test urine from 465 women attending a Swedish STD clinic and 59 women in a cervical cancer screening program for *M. genitalium* (33). They found that 5.6% (26/461) of the women in the STD group tested positive for *M. genitalium* compared to none of the women in the cancer screening group (p=not given) (33).

Table 1. Prevalence of *M. genitalium* in the female lower genital tract among the general population and STD clinic attendees

Study	Location	N	Sample[1]	Percentage of women testing positive for *M. genitalium* %
General Population				
Hamasuna, 2008	Japan	298	Urine	2.8
Andersen, 2007	Denmark	921	Vaginal	2.3
Manhart, 2007	U.S.	1714	Urine	0.8
Tosh, 2007	U.S.	383	Vaginal Urine	0.8
Falk, 2005	Sweden	59	Urine	0
Tsunoe, 2000	Japan	90	Endocervical	1.1
STD Clinic Attendees				
Moi, 2009	Norway	7646	Cervical Urethral	4.5
Cohen, 2007	Kenya	299	Cervical	16
Anagrius, 2005	Sweden	445	Endocervix	6.3
Falk, 2005	Sweden	465	Urine	5.6
Pepin, 2005	Sub-Saharan Africa	826	Cervical	26.3
Manhart, 2003	U.S.	719	Cervical	7
Casin, 2002	France	170	Cervical Vaginal Urethral	38
Tsunoe, 2000	Japan	174	Endocervical	12.6
Palmer, 1991	England	57	Cervical Vaginal Urethral	17.5

[1] All samples assessed using PCR assays

Fewer studies have examined at the prevalence of *M. genitalium* upper genital tract infections. The prevalence of *M. genitalium* in samples from women with PID has ranged from 7-16% (40-43) (Table 2), and PID cases are more likely to test positive for *M. genitalium* than women without PID (13% vs. 0%, $p<0.001$) (43). Further, the correlation between *M. genitalium* lower genital tract infections and

M. genitalium upper genital tract infections has been high. In the PID Evaluation and Health (PEACH) Study, among women with mild to moderate clinically suspected pelvic inflammatory disease, infections of the cervix and infection of the endometrium were highly correlated (Phi correlation 0.63, $p<0.0001$) (42). In fact, 60% of women who tested positive in the cervix also tested positive in the endometrium and 74% of women who tested positive in the endometrium also tested positive in the cervix (42). In another study conducted among a cohort of 299 female sex workers aged 18 to 35 years in Kenya, 52% (24/46) of the subjects in which *M. genitalium* was detected in a cervical specimen and who had an endometrial biopsy collected at the same visit for *M. genitalium* PCR testing were also positive in the endometrium. No women were positive in endometrial and not in cervical specimens (15).

Table 2. Prevalence of *M. genitalium* in women with upper genital tract infections

Study	Location	Study Population	Sample[1]	Percentage of women testing positive for *M. genitalium* %
Haggerty, 2008	U.S.	586 women with clinically suspected mild to moderate PID	Cervical Endometrial	15% cervix and/or endometrium 12% cervix 8% endometrium
Cohen, 2005	Kenya	123 women with laparoscopically confirmed acute salpingitis	Cervical Endometrial Fallopian tube	7% overall 3% cervix 1% endometrium 1% fallopian tube
Simms, 2003	England	45 PID cases and 37 controls	Endocervical	13% of PID cases 0% of controls
Cohen, 2002	Kenya	58 women with and 57 women without endometritis	Cervix Endometrium	16% with endometritis 2% without endometritis

[1] all samples assessed using PCR assays

1.2.1.6 Prevalence of *M. genitalium* compared to other STDs

Several studies have found that lower genital tract *M. genitalium* infections are as prevalent as *C. trachomatis* or *N. gonorrhoeae* (15, 22, 23, 33, 36, 38, 43); the two most frequently reported bacterial STDs in the U.S. (Table 3). However, not all results have been consistent (33, 35, 38). In women with PID or salpingitis, the prevalence of *M. genitalium* has been found to be similar or greater than that of *C.*

trachomatis and *N. gonorrhoeae.* In the PEACH study, *M. genitalium* was as prevalent as *C. trachomatis* and *N. gonorrhoeae* (42). At baseline, approximately 15% of women were infected with *M. genitalium*, 14% were infected with *C. trachomatis* and 15% were infected with *N. gonorrhoeae* (42). In Kenyan women with laparoscopically diagnosed acute salpingitis, *M. genitalium* (7%) was more prevalent than *C. trachomatis* (6%), but less prevalent than *N. gonorrhoeae* (15%) (41). The low prevalence of other STDs in some of these populations may be due to effective STD screening and treatment programs in these regions, which would not include *M. genitalium*.

Table 3. Prevalence of *M. genitalium* compared to the prevalence of *C. trachomatis* and *N. gonorrhoeae* in select populations of women

Study	Location	Study Population	*M. genitalium* prevalence %	*C. trachomatis* prevalence %	*N. gonorrhoeae* prevalence %
Women with evidence of lower genital tract infections					
Moi, 2009	Norway	STD clinic attendees	4.5	9.5	Not assessed
Hamasuna, 2008	Japan	General population	2.8	8.8	Not assessed
Cohen, 2007	Kenya	Commercial sex workers	22.7[1]	14[1]	8[1]
Manhart, 2007	U.S.	General population	1.0	4.2	0.4
Tosh, 2007	U.S.	Asymptomatic adolescents	0.8	10.2	3.8
Anagrius, 2005	Sweden	STD clinic attendees	6.3	4.0	Not assessed
Falk, 2005	France	STD clinic attendees	6	10	0
Simms, 2003	England	Women with and without PID	13.3	26.7	2.2
Tsunoe, 2000	Japan	Asymptomatic pregnant women	1.1	5.6	0.0
Tsunoe, 2000	Japan	Commercial sex workers	6.3	19	33
Palmer, 1991	England	STD clinic attendees	19	16	Not assessed
Women with evidence of upper genital tract infections					
Haggerty, 2008	U.S.	Women with PID	15	14	15
Cohen, 2005	Kenya	Women with salpingitis	7	6	17

[1] incidence, n per 100-person years

1.2.1.7 Co-infections of *M. genitalium* and *C. trachomatis*

Several studies have reported dual infections of *M. genitalium* and *C. trachomatis* in women (14, 23, 32, 33, 35, 36, 39, 41, 43, 44). However, in the lower genital tract, the co-infection rate has generally been low (≤5% in most studies), indicating that *M. genitalium* is most likely transmitted alone (Table 4). The co-infection rate of *M. genitalium* and *C. trachomatis* is comparable to that of *C. trachomatis* and *N. gonorrhoeae,* which was about 4% in a large, multi-centered study conducted among 1,701 women in the U.S. (45). Compared to the studies that found a low rate of co-infection, Casin et al found a higher rate of dual infections of *M. genitalium* and *C. trachomatis* in women attending a STD clinic in France (32). The prevalence of *M. genitalium* in the lower genital tract of women also positive for *C. trachomatis* was high (43%), but did not differ significantly from that among women negative for *C. trachomatis* (36%) (p = 0.7) (32). However, it may be possible that the higher incidence of *M. genitalium* in this population compared to that of *C. trachomatis* (38% vs. 8%) actually indicates sensitivity issues with the PCR method used to detect *M. genitalium* and not true differences.

Table 4. Lower genital tract co-infections of *M. genitalium* and *C. trachomatis* in select populations of women

Study	Location	Study Population	N	Percentage of women with *M. genitalium* and *C. trachomatis* %
Moi, 2009	Norway	Women attending a STD clinic	7646	0.6%
Hamasuna, 2008	Japan	Asymptomatic female students	298	1%
Tosh, 2007	U.S.	Asymptomatic female adolescents	383	5.2%
Kataoka, 2006	Japan	Pregnant women	877	7.1 %
Angarius, 2005	Sweden	Women attending a STD clinic	445	3.5%
Falk, 2005	Sweden	Women attending a STD clinic	465	1%
Cohen, 2005	Kenya	Women with salpingitis	123	< 1%
Simms, 2003	England	Women with and without PID	82	2%
Casin, 2002	France	Women attending a STD clinic	170	43%
Palmer, 1991	England	Women attending STD clinic	57	5.3%

Co-infections of *M. genitalium* and other STDs in women with upper genital tract infections have not been thoroughly examined. Co-infection was very common in the PEACH Study. Approximately 66.2% (43/63) of *M. genitalium* positive women were also co-infected with *N. gonorrhoeae* and/or *C trachomatis* in the cervix and/or endometrium and women with *M. genitalium* were significantly more likely to be co-infected with *N. gonorrhoeae* and/or *C trachomatis* than women who tested negative for *M. genitalium* ($p< 0.0001$) (42). In a case-control study conducted in Sweden, Jurstrand et al analyzed sera obtained from patients with clinical PID and sera from healthy pregnant women using lipid-associated membrane protein (LAMP) enzyme immunoassay assays (EIA) (46). Nearly 12% of the women with PID were seropositive for both *M. genitalium* and *C. trachomatis*, while 5% were *M. genitalium* antigen seropositive only (p=not given) (46). However, as serology represents both current and past infections, women may or may not have been infected with *C. trachomatis* and *M. genitalium* simultaneously. Further, co-infection may have been common in these populations of women because all women had clinically suspected PID, and hence, were women with a high burden of STDs in general.

In contrast to these two studies, Simms et al found a low co-infection rate among English women with PID; only 2.2 % (1/45) of women tested positive for both *M. genitalium* and *C. trachomatis* in the lower genital tract, and none of the women had *M. genitalium-N. gonorrhoeae* co-infections (43). Cohen et al found that among Kenyan women with acute salpingitis, none were co-infected with *M. genitalium* and *N. gonorrhoeae*, while *C. trachomatis* was identified in less than 1% (1/123) of women with cervical *M. genitalium* (41). Co-infection in this population may have been low due to the *C. trachomatis* and *N. gonorrhoeae* screening and treatment programs available to this population.

1.2.1.8 Risk factors of *M. genitalium*

Given that data suggests *M. genitalium* is sexually transmitted, the risk factor profile of women with *M. genitalium* should be similar to women with other bacterial STDs. Risk factors for STD acquisition and PID are generally similar. Women who are younger, black, non-married, less educated, of lower socioeconomic status or abuse drugs or alcohol have a greater risk of STDs and PID. Further, women

who report a young age at sexual debut, a greater number of sexual partners, a prior history of STD or PID may have an increased risk (47, 48). Thinning of the cervical mucus plug during the estrogen phase of the menstrual cycle (49), surgical procedures that disrupt the cervical barrier (50), douching (51-54), smoking (55, 56), and using intrauterine contraceptive device (IUCD) (47) also may increase the risk of PID by promoting the ascension of organisms from the lower to the upper genital tract. Oral contraceptives have been positively associated with STDs but negatively correlated with PID (57), as oral contraceptives may mask the symptoms of PID symptoms in women with endometritis (58).

Young age is a risk factor for STDs, especially for females. Biologically, younger females are more susceptible to bacterial infections than older females due to cervical ectopy. During adolescence, endocervical columnar epithelial cells extend to the vaginal surface, increasing the surface area and increasing the number of receptive cells which may favor the growth of some mucosal pathogens (59). Younger women may also be at a greater risk for STDs because they may be more likely to engage in risky sexual behaviors, such as unprotected intercourse and multiple sexual partners.

Non-white race is also associated with STDs (60)and BV (61). In 2007, the rate of chlamydia among blacks was over 8 times higher than that of whites (1,398.7 and 162.3 cases per 100,000, respectively) (60) . The rates among American Indian/Alaska Natives (732.9) and Hispanics (473.2) were also higher than that of whites (4.5 and 2.9 times higher, respectively) (60). The rate of gonorrhea among blacks was 19.1 times greater than the rate among whites (34.7 cases per 100,000 population) (60). Gonorrhea rates were 3.1 times greater among American Indian/Alaska Natives (107.1 cases per 100,000 population), and 2.0 times greater among Hispanics (69.2 cases per 100,000 population) than among whites in 2007 (60). In the U.S., BV is more common among non-Hispanic black women (61, 62).

There is no clear explanation as to why Blacks experience higher rates of STDs and BV. Blacks may have an increased risk due to behavioral factors, such as douching, multiple sexual partners and condom use, or demographic factors, such as socio-economic status, which may limit access to health care services. However, a study conducted by Ness et al among 900 black and 235 white women from

five U.S. sites to determine whether racial differences in known BV risk factors can explain why blacks are more likely to have BV found that black race was associated with BV, independent of demographic and lifestyle factors (62). After adjustment for demographic and lifestyle factors, blacks remained at elevated risk for BV/intermediate flora (OR 2.2, 95% CI 1.5-3.1) (62). Blacks were also more likely to have specific gonococcal or chlamydial cervicitis (OR 2.2, 95% CI 1.2-3.8) after adjustment for age, education, history of trichomonas, gravidity, smoking, sex with menses, hormonal contraceptive use and douching (62). The authors concluded that the risk factor differences did not explain the observed racial disparity in the occurrence of BV or gonococcal or chlamydial cervicitis.

Numerous behaviors are associated with an increased STD and PID risk. Douching can create an environment favorable to facultative aerobes and anaerobes over the usually predominant hydrogen-peroxide-producing lactobacilli (63). Douching can alter the vaginal microflora, remove protective components from the vagina or cervix, and/or promote the ascension of microorganisms from the lower to the upper genital tract, all increasing a woman's susceptibility to infection (64). Substance use may increase the risk of STDs by promoting riskier behaviors, such as unprotective sex or multiple sexual partners. Such behaviors can increase the chance of being exposed to an infected partner. Smoking is thought to exert a biologic effect on the genital tract via compromised immunity or altered estrogen status (55, 65), while alternatively, smoking may mark poor health-seeking behavior. Using oral contraceptives (OC) use may increase risk by promoting cervical ectopy (66) or by promoting inconsistent or no use of barrier methods. However, OCs may also decrease the risk of STDs because those that use OCs may be more likely to have better health behaviors in general. Clinical studies indicate that correct and consistent condom use can help reduce the risk of bacterial and viral STD acquisition (67). However, it can be difficult to measure the true effectiveness of condom use due to several biases often introduced into epidemiological studies, most importantly over-reporting of correct and consistent use.

Some studies have looked at the risk factors for *M. genitalium* genital tract infection, but results have been inconsistent. However, the high background level of many risk behaviors among STD clinic patients or commercial sex workers may mask or diminish the association of such factors with *M.*

genitalium (15, 34, 37). Still, infection of the lower genital tract is a risk factor for progression to PID, so a better understanding of correlates associated with risk of lower genital tract infection may help understand the factors associated with of upper genital tract infection. Several population-based studies conducted in the U.S. have examined the risk factors associated with *M. genitalium* lower genital tract infections (14, 22, 68) (Tables 5 and 6). Manhart et al used data from a subsample of participants in Wave III of the National Longitudinal Study of Adolescent Health (Add Health) to examine the potential risk factors of *M. genitalium* (22). PCR was used to test the urine of 1714 women and 1218 men aged 18 to 27 years (22). *M genitalium* infection was strongly associated with ever having engaged in vaginal intercourse (Prevalence Ratio (PR) 22.5, 95% CI 4.3-116.6), and in multivariate analyses the prevalence of *M. genitalium* increased by 10% with each additional vaginal intercourse partner in the past year (PR 1.1 per partner in the past year, 95% CI 1.0-1.2) (22). Further, *M. genitalium* was more prevalent in individuals that ever lived with a sexual partner (PR 11.2, 95% CI 3.2-39.5), and in individuals who reported Black race (PR 7.2, 95% CI 2.9-17.9) and condom use during last sexual intercourse (PR 3.9, 95% CI 1.3-11.5) (22). *M. genitalium* was not associated with age, age at sexual debut, or correct and consistent condom use over the past year (22). In another study conducted in the U.S., Huppert et al tested vaginal swabs from 331 sexually active female adolescents aged 14 to 21 years recruited from inner-city medical center for *M. genitalium* using PCR (68). Sexual intercourse within the last 7 days was associated with a 2-fold increase in the odds of *M. genitalium* infection (OR 2.0, 95% CI 1.1-3.2), after adjusting for *C. trachomatis* (68). *M. genitalium* infection was not independently associated with demographic variables including age, race and sexual behaviors such as inconsistent condom use, new sexual partner, or multiple sexual partners (68). Tosh et al used PCR to test vaginal samples from 383 female adolescents aged 14-17 years enrolled in urban primary health care clinics in the U.S. (14). With the exception of one individual, *M. genitalium* was identified exclusively among individuals reporting history of vaginal intercourse (14). Having a recent sexual partner (OR 1.4, 95% CI 1.2-1.7) was the only behavioral characteristic independently associated with *M. genitalium* (14). There was no difference in age, race, age at first intercourse, condom use, intercourse frequency, or oral sex in the past three months

between those with and without *M. genitalium* (14). The young age of the study participants may have limited these studies. Further, none of the studies compared the risk profile of women with *M. genitalium* to women with other STDs and no attempt was made to assess the potential relationship between *M. genitalium* and non-sexual related behaviors, including alcohol and drug use, smoking and vaginal douching.

Table 5. Select studies of the risk factors associated with *M. genitalium* female lower genital tract infections in the general population (Part A: Study, study population, sample, risk factors examined)

Study	Study Population	Sample	Risk factors examined
Huppert et al, 2008	331 women aged 14 to 21 years recruited from an urban medical center	Endocervical	# of sexual partners Age Condom use History of STI Hormonal contraception use New sexual partner Race
Manhart et al, 2007	1218 men and 1714 women aged 18-27 in the general population in the U.S.	Urine	# of sexual partners Age Alcohol use Co-infections Contraception use Drug use Education level Employment Ever lived with sexual partner Gender History of STI Marital status Race Sexual activity
Tosh et al, 2007	383 adolescent women aged 14-17 from urban primary health care clinics in the U.S.	Vaginal and urine	# of sexual partners Age Age at first intercourse Co-infections Condom use Frequency of sex Oral sex Race

Table 6. Select studies of the risk factors associated with *M. genitalium* female genital tract infections in the general population (Part B. Risk factors significantly associated with *M. genitalium*, results)

Study	Risk factors significantly associated with *M. genitalium*	Results
Huppert et al, 2008	Sexual intercourse	OR[1] 2.0 (95% CI 1.1-3.2)
Manhart et al, 2007	Race Ever lived with a partner Sexually active Condom use during last intercourse	PR[2] 7.2 (95% CI 2.9-17.9) PR 11.2 (95% CI 3.2-39.5) PR 22.5 (95% CI 4.3-116.6) PR 3.9 (95% CI 1.3-11.5)
Tosh et al, 2007	Recent sexual partner	OR 1.4 (95% CI 1.2-1.7)

[1] OR=odds ratio
[2] PR=prevalence ratio

Only one published study has looked at the risk factors among women with *M. genitalium* upper genital tract infection (41). Cohen et al used PCR to analyze cervical, endometrial, and fallopian tube samples from 123 women presenting at an STD clinic with laparoscopically confirmed salpingitis (41). Age, marital status and median number of sexual partners were not associated with *M. genitalium* infection at any site, while being HIV positive was independently associated with infection (Adjusted Hazard Ratio (AHR) 2.2, 95% CI 1.2-3.7) (41). However, the analyses included women with gonorrhea or chlamydial infections, which may have biased the results. More studies of the risk factors of *M. genitalium* upper genital tract infection are needed.

1.2.1.9 Clinical features *M. genitalium* infections

Previous studies have examined the characteristics and clinical manifestations of lower genital tract *M. genitalium* infections, yet the association of *M. genitalium* with lower tract disease in women has not been consistently reported, possibly reflecting differences in the population studied and criteria used to assess signs and symptoms at this site. While some studies have showed an association between *M. genitalium* and cervicitis (33, 34, 37, 69), several PCR studies have failed to find a strong association between symptoms and *M. genitalium* lower genital tract infection (14, 22, 32). Tosh et al studied 383 adolescent females attending a primary care clinic and found that women with *M. genitalium* identified in the lower

genital tract were no more symptomatic than uninfected women (14). In a group of women negative for both *C. trachomatis* and *N. gonorrhoeae*, those who tested positive for *M. genitalium* were not more likely to have signs (presence of vaginal erythema, vulvar erythema or vaginal discharge) (p=0.33) or symptoms (vaginal itching, vaginal burning, dyspareunia) (p=0.35) compared to women negative for *M. genitalium* (14). Manhart et al also found that lower genital tract *M. genitalium* infection was not associated with symptoms. PCR was used to test the urine from 1,714 women enrolled in a population-based study and *M. genitalium* infections were not associated with symptoms, as none of the participants who tested positive for *M. genitalium* reported symptoms of vaginal discharge (22). Conversely, vaginal discharge was more common in women with lower genital tract *M. genitalium* infections compared to women without *M. genitalium* among 390 minority women with an active sexually transmitted infection (*N. gonorrhoeae*, *C. trachomatis Trichomonas pallidum*, or *T. vaginalis*) attending a public health clinic (70). The results were similar after controlling for co-infection with other STDs. However, vaginal discharge was the only genitourinary sign or symptom that was significantly different between women testing positive and women not infected (70). Casin et al found no association between *M. genitalium* identified in lower genital tract and urinary symptoms (OR 1.3, 95% CI 0.7 to 2.5) or pelvic pain (OR 0.9, 95% CI 0.5 to 1.7) among women attending a STD clinic (32). These PCR studies indicate that *M. genitalium* does not produce strong symptoms in women with lower genital tract infections compared to women without *M. genitalium*.

The clinical features of women presenting with upper genital tract *M. genitalium* have not been extensively examined. Two published studies have reported some clinical characteristics of women with *M. genitalium* upper genital tract infection (40, 41). In a study of 115 Kenyan women presenting at a STD clinic, 100% of *M. genitalium* positive women with histologically diagnosed endometritis reported mild abdominal pain, compared to 68% of those with endometritis who were not *M. genitalium* positive (p=0.06) (40). Further, 89% (8/9) of women infected with *M. genitalium*, compared to 53% (59/102) of uninfected women had easily induced cervical bleeding (p=0.08) (40), however, the difference was not

significant. In another study by the same author, *M. genitalium* was not associated with severity of salpingitis (mild, moderate, or severe) by either clinical criteria or laparoscopic scoring system (41).

Clinical characteristics of M. genitalium compared to other STDs

Previous PCR studies indicate that *M. genitalium* does not produce strong symptoms in women with lower genital tract infections compared to women without *M. genitalium* and that the symptomatology induced by *M. genitalium* is similar to that seen for *C. trachomatis* (71). In general, female genital tract *C. trachomatis* infections tend to be less abrupt in onset, and tend to have few or mild symptoms (71). In fact, there are no genital symptoms that are specifically correlated with chlamydial cervical infection, (71) and it has been reported that more than 90% of women testing positive for *C. trachomatis* report no symptoms (72). One study compared the symptoms of *C. trachomatis* and *M. genitalium* in the lower genital tract among 465 women either attending a STD clinic or enrolled in a cervical cancer screening program in Sweden. In this study, no significant differences were reported in symptoms (32% v 23%, RR 1.4, 95% CI 0.6 to 3.4) or signs (71% v 50%, RR 1.4, 95% CI 0.9 to 2.3) between women testing for *C. trachomatis* and *M. genitalium* in the lower genital tract (33). No studies have compared the characteristics of *M. genitalium*-PID to PID caused by other pathogens.

1.2.1.10 Treatment of *M. genitalium*

There is no current standard treatment for *M. genitalium* infection in women. Numerous antibiotics have been used to treat infections, but with varying degrees of success. As mycoplasmal bacteria lack a cell wall, they are therefore resistant to cell-wall-inhibiting antibiotics, including penicillin and cephalosporin (73). Also, because *M. genitalium* grows very slowly, a longer period of therapy may be required (74). Further, as *M. genitalium* can invade epithelial cells, antibiotics may fail to fully eradicate infections, demonstrated by the fact that *M. genitalium* is associated with persistent non-gonococcal urethritis among men treated with tetracyclines (75). An *M. genitalium* strain with increased tetracycline resistance has also been isolated (76), and it has been shown that other genital mycoplasmas, *M. hominis* and *U.*

ureaplasma, which belong to the same class as *M. genitalium*, have genes coding for tetracycline resistance (77). *M. genitalium* has demonstrated variable resistance to fluroquinolones, and susceptibility to macrolides, although azithromycin resistant strains have recently been identified (76, 78). Randomized clinical trials assessing alternative regimens for the treatment of *M. genitalium* are clearly needed.

The efficacy of commonly used PID antimicrobials in treating *M. genitalium* upper genital tract infection is largely unknown, but there is evidence to suggest that cefoxitin and doxycycline, a CDC recommended PID treatment regimen, are not effective (42). In the PEACH study, approximately 41% (23/56) of women who tested positive for *M. genitalium* at baseline again tested positive for *M. genitalium* at 30 days post-treatment with cefoxitin and doxycycline (42). In contrast, only 2% to 4% of women in the PEACH study had persistent or recurrent gonococcal or chlamydial cervicitis when retested at 30 days (79). Women with *M. genitalium* identified in the endometrium were over 4.5 times more likely to experience short-term treatment failure, defined by histological identification of endometritis and persistent pelvic pain at the 30 day follow-up clinic visit (adjusted RR 4.6, 95% CI 1.1 – 20.1) (42).

1.2.1.11 Sequelae associated with *M. genitalium* infection

The pathogenic role of *M. genitalium* has been studied more extensively in men than in women. In fact, *M. genitalium* was first discovered in the 1980s in men with non-gonococcal urethritis (80), and several subsequent clinical studies have shown a strong association between *M. genitalium* and urethritis among men (24, 81-87). Men attending STD clinics, and therefore higher-risk, have been most thoroughly studied. The prevalence of *M. genitalium* in men with urethritis has ranged from 9.4% to 29.2%, while *M. genitalium* was less prevalent in asymptomatic men in the same populations (0.8–8.5%) (23, 82, 87-90). Due to the development of PCR detection methods, recent studies have investigated the pathogenic role of *M. genitalium* in women. The evidence for pathogenicity among women is suggestive but inconclusive. Still, studies have implicated *M. genitalium* with several reproductive morbidities, including cervicitis, PID, infertility, and adverse pregnancy outcomes. *M. genitalium* does not seem to

be associated with BV and few studies have examined the association between *M. genitalium* and urethritis in women.

M. genitalium and cervicitis

M. genitalium has been implicated as an etiological agent of cervicitis, the inflammation of the cervix. Four PCR studies have shown *M. genitalium* to be associated with cervicitis, independent of *C. trachomatis* or *N. gonorrhoeae* (33, 34, 37, 69) (Table 7). However, other studies have not found a strong association between *M. genitalium* and cervicitis. Schlicht et al did not find a difference in the detection of *M. genitalium* in cervical swabs between college-aged sexually active women with clinical signs of cervicitis and asymptomatic sexually active control women (91). Thirteen percent (5/39) of symptomatic women and 8% (4/50) of asymptomatic women tested positive for *M. genitalium* by PCR ($p>0.05$) (92). Casin et al did not find an association between *M. genitalium* detection in the lower genital tract by PCR and criteria for cervicitis (erythematous cervix, mucoplurulent cervical discharge, and presence of >10 PMNs/HPR taken separately) in a population of 170 symptomatic women presenting at a STD clinic in France (32). Further, women with cervicitis were not more likely to be positive for *M. genitalium* than women without cervicitis (42% vs. 32%, $p=0.19$) (32). The samples used for *M. genitalium* detection, the use of less sensitive measures of cervicitis, the use of asymptomatic women and the failure to control for other STDs may have accounted for null findings of these studies.

Table 7. PCR studies of *M. genitalium* (Mg) and cervicitis

Study	Location	Study Population	N	% of Women with cervicitis testing positive for Mg	% of Women without cervicitis testing positive for Mg	p-value
Anagrius, 2005	U.S.	STD clinic attendees	445	13.3	2.6	0.02
Falk, 2005	Sweden	STD clinic attendees and cervical cancer screening program participants	524	10.2	4.0	0.02[1]
Pepin, 2005	Africa	Female sex workers	826	16.5	7.2	0.05[1]
Schlicht, 2004	U.S.	Sexually active symptomatic and asymptomatic college students	89	13	8	> 0.05
Manhart, 2003	U.S.	STD clinic attendees	719	11	5	0.004[1]
Casin, 2002	France	Women attending a STD clinic	170	42	32	0.19
Uno, 1997	Japan	Women with cervicitis and/or adnexititis and asymptomatic pregnant women	144	7.8	0	< 0.05[1]

[1] independent of *C. trachomatis* or *N. gonorrhoeae*

M. genitalium and bacterial vaginosis

BV is a syndrome in which the normal vaginal lactobacilli are partially or completely replaced by a mixed flora with high concentrations of anaerobic and other bacteria, including *Gardneralla vaginalis, Mobiluncus* spp, *Bacteroides* spp, and genital mycoplasmas (93-95). Though BV is a polymicrobial condition, and several anaerobes and mycoplasmal bacteria have been associated with BV, the microbiologic profile of women with BV has not been completely identified. Studies regarding the association between *M. genitalium* and BV suggest that there is not a strong relationship between *M. genitalium* and BV, and this relationship similar to that of *C. trachomatis* and BV.

Keane et al found no evidence of *M. genitalium* lower genital tract infection in any of the 15 women with BV, but in 12% (2/17) of women with normal vaginal flora among women attending an STD clinic (96). Lu et al found no association between BV and *M. genitalium* among women who had experienced a spontaneous preterm delivery; *M. genitalium* was diagnosed in 20% (1/5) of women with

BV versus 18.5% (22/119) of women without BV (97). In a study of 45 PID cases and 37 control women without PID, BV was not diagnosed in any of the women with *M. genitalium* (98). Due to the small sample size of these studies, power may have been limited.

Several larger studies have failed to find an association between *M. genitalium* and BV. In the PEACH study, women with *M. genitalium* identified in the cervix and/or endometrium were not significantly more likely to have BV than women without *M. genitalium* (63.8% vs. 56.1%, p=0.20) (42). Manhart et al conducted a study among 719 STD clinic attendees in which BV was found in only 20% of women with *M. genitalium* detected with PCR, versus 33% of women without *M. genitalium* (p= 0.05) (34). Further, women with BV were less likely to have *M. genitalium* than women without BV; only 4.3% of women with BV tested positive for *M. genitalium* while 9.2% of women without BV tested positive for *M. genitalium* (p=0.05) (34). Falk et al conducted a study comparing the signs and symptoms of 465 female STD clinic attendees with a *C. trachomatis* or *M. genitalium* infection to 59 women in a cancer screening program who acted as controls (33). There was no statistically significant difference in the prevalence of BV between *M. genitalium* negative women and women who tested positive for *M. genitalium* (p=0.22) (33).

In a handful of studies, *M. genitalium* has been more prevalent in women with BV compared to women without BV. Palmer et al studied 57 women attending an STD clinic; *M. genitalium* was found in 30% (3/10) of women with BV but in only 16% (4/16) of women without BV (36). Oakeshott et al found that *M. genitalium* was associated with BV in a group of pregnant women presenting at prenatal visits at less than 10 weeks gestation (99). Women with BV by Gram stain were more likely to test positive for *M. genitalium* in their urine than women with normal vaginal flora (p for trend=0.01) (99). Korte et al found a mild non-significant association between BV and *M. genitalium* (70). Women who were culture positive for *M. genitalium* and women who were PCR positive for *M. genitalium* were more likely to have BV compared to women who did not test positive for *M. genitalium* by either method (p=0.19, p=0.11, respectively) (70).

M. genitalium and urethritis

Although in men it has been established as a cause of nongonococcal urethritis, the inflammation of the urethra, few studies have examined the relationship between *M. genitalium* and urethritis in women. Anagrius et al found a strong association between *M. genitalium* and urethritis among 445 women attending a STD clinic in Sweden (23). Women with microscopic signs of urethritis and without cervicitis were more likely to test positive for *M. genitalium* than women without urethritis (8.5% vs. 2.6%, p=0.02) and *M. genitalium* was still significantly associated with urethritis regardless of concomitant cervicitis (p=0.005) (23). Moi et al also found an association between *M. genitalium* and urethritis among 7646 women attending a STD clinic in Norway (100). The authors found a strong association between detecting *M. genitalium* in FVU by PCR and signs of urethral inflammation (100). Further, women with *M. genitalium* detected in the FVU had a higher probability of having moderate to severe (>10 polymorphic mononuclear leucocytes per high-powered field) inflammation of the urethra compared with women who were *M. genitalium* negative (34% vs. 22%; p<0.001) (100). As few studies have examined this, the relationship between *M. genitalium* and urethritis in women remains unclear.

M. genitalium and Pelvic Inflammatory Disease

In addition to causing infections in the lower genital tract of women, evidence from animal and human studies support a pathogenic role of *M. genitalium* in female upper genital tract infection. Non-human female primates inoculated with *M. genitalium* exhibited mild genital tract infections and developed endometritis and salpingitis (13, 101). *M. genitalium* has been shown to be able to adhere to human fallopian tube epithelial cells in organ culture, resulting in damage to the ciliated cells (102). It has also been shown that *M. genitalium* can adhere to human spermatozoa, allowing it to be carried to the female upper genital tract on motile sperm (103). Further, as previously mentioned, *M. genitalium* has been associated with cervicitis (33, 34, 37, 69), which often precedes upper genital tract infection. Therefore, it is plausible that *M. genitalium* can cause PID. In fact, *M. genitalium* has been identified as a possible etiologic agent of non-gonococcal/non-chlamydial PID (40, 42, 43, 104). It has also been

detected by PCR in cervical and salpingeal samples from women with laparoscopically confirmed salpingitis (41) and in cervical and endometrial specimens from women with endometritis (40, 42). However, serological studies of *M. genitalium* in PID have been less conclusive (46, 105, 106). While data from clinical studies indicate that 10-40% of women with gonococcal infections and 20-40% of women infected with *C. trachomatis* will develop PID if these infections remain untreated (107), the proportion of *M. genitalium* infections that cause upper tract inflammation and tissue damage remains unknown. Table 8 summarizes the studies of *M. genitalium* and PID.

Table 8. Studies of *M. genitalium* (Mg) and female upper genital tract infections

Study	Location	Population	Sample	Results
PCR Studies				
Haggerty, 2008	U.S.	586 women with clinically suspected PID	Cervical Endometrial	Mg associated with baseline endometritis independent of *C. trachomatis* and *N. gonorrhoeae* (OR 2.0, 95% CI 1.0 – 4.2)
Cohen, 2005	Kenya	126 women with laparoscopically confirmed acute salpingitis	Cervical Endometrial Fallopian tube	Mg detected in cervical, endometrial, and fallopian tube samples
Simms, 2003	England	45 women with and 37 women without PID	Endocervical	Cases significantly more likely to test positive for Mg than controls (13% vs. 0% controls, p<0.01)
Cohen, 2002	Kenya	58 women with endometritis and 57 women without endometritis attending a STD clinic	Cervical Endometrial	Mg infection in the endometrium alone was associated with endometritis, even after exclusion of *C. trachomatis* and *N. gonorrhoeae* co-infections (p=0.03) Women with endometritis more likely to test positive for Mg in the cervix and/or endometrium than women without endometritis (16% vs. 2%, p=0.02)
Uno, 1997	Japan	53 women with adnexititis and 80 asymptomatic pregnant women	Cervical	Women with adnexitis more likely to test positive for Mg than control women (4.1% vs. 0%, p=not given)
Serological Studies				
Jurstrand, 2007	Sweden	194 women with and 396 women without PID	Serum	Cases not more likely to be seropositive for Mg compared to controls (17% vs. 15%, p=xxx) No association between PID and Mg (OR 1.0; 95% CI 0.6-1.7)
Lind, 1987	Denmark	21 women with laparoscopically confirmed salpingitis	Serum	No association between salpingitis and Mg 4.8% had a $\geq$ 4-fold change in titer of Mg antibody
Moller, 1984	Denmark	31 women with acute PID	Serum	39% had $\geq$ 4-fold change in titer of Mg antibody

In the PEACH study, *M. genitalium* was detected in 15% of women at baseline, and *M. genitalium* was significantly associated with baseline endometritis, independent of *C. trachomatis* and *N. gonorrhoeae* (OR 2.0, 95% CI 1.0-4.2) (42). Further, having *M. genitalium* identified in the endometrium

at baseline was associated with both an increased risk of incident endometritis at 30 days (adjusted RR 5.0, 95% CI 1.2-20.6) and persistent endometritis, identified histologically at both baseline and 30 days following treatment with cefoxitin and doxycycline (adjusted RR 4.1, 95% CI 1.4-11.9) (42). After adjusting for *N. gonorrhoeae* and *C. trachomatis*, endometrial *M. genitalium* was associated with incident endometritis (adjusted RR 6.0, 95% CI 1.4 -27.1). In a subgroup of women without *N. gonorrhoeae* or *C. trachomatis*, women with endometrial *M. genitalium* at baseline were almost 9 times as likely to have endometritis at 30 days (adjusted RR 8.8, 95% CI 2.0-39.6) and over 13 times as likely to have incident endometritis at 30 days (adjusted RR 13.4, 2.4-75.2) (42).

In a case-control study, *M. genitalium* was more common among women with PID than control women without PID. Simms et al analyzed endocervical samples using PCR in a case-control study among 82 women (45 PID cases and 37 control women undergoing tubal ligation) aged 16-46 years in England and found evidence of *M. genitalium* infection in 13% of the PID cases and none of the controls (43). Cases were significantly more likely to present with *M. genitalium* and/or *C. trachomatis* than the control women ($p<0.001$) (43). However, the control women may have been less likely to be have a STD, due to their older age (43). (The median age of the cases was 25 (range 16–43), whereas that of the controls was 34 (range 21–45), and cases were significantly younger than the controls ($p<0.001$) (43).

Two recent PCR studies have also suggested that *M. genitalium* is associated with both histologically confirmed acute endometritis and laparoscopically confirmed acute salpingitis. Cohen et al conducted a study among 115 Kenyan women aged 18-40 years presenting at a STD clinic with abdominal pain that had lasted 14 or less days (108). *M. genitalium* was detected in the cervix, endometrium, or both in 16% (9/52) of women with histologically confirmed endometritis and in 2% (1/57) of women without endometritis ($p=0.02$) (40). Endometrial *M. genitalium* infection was associated with endometritis, even after exclusion of *N. gonorrhoeae* and/or *C. trachomatis* co-infections ($p=0.03$) (40) In a second study conducted in Kenya among 126 women with laparoscopically confirmed acute salpingitis, *M. genitalium* was identified by PCR in 7% of all samples, 3% of cervical samples and 1% of endometrium samples (41). It was also found in a fallopian tube sample from one women (41).

Serological studies of *M. genitalium* in PID have been less conclusive (46, 105, 106). Moller et al used micro-immunofluorescence to test sera from 31 women diagnosed with acute PID in Denmark, in whom antibodies to *C. trachomatis* and *M. hominis* could not be detected (106). Approximately 48% of the samples tested had antibodies to *M. genitalium,* while 38.7% of patients had a 4-fold or greater change in titre during the one month after the onset of PID (106). However, the authors failed to provide information regarding the changes in *M. genitalium* titre among women without PID. Using indirect haemagglutination and indirect immunofluorescence tests, Lind and Kristensen examined sera from 21 women with laparoscopically confirmed salpingitis without evidence of *C. trachomatis* or *N. gonorrhoeae* (105). Only 1 women (4.8%) had a 4-fold or greater change in titer (105). Jurstrand et al did not find an association between serological evidence of *M. genitalium* and PID (46). They used lipid-associated membrane protein-enzyme immunoassay (LAMP-EIA) to analyze sera from 194 women hospitalized with clinically diagnosed PID, 246 healthy pregnant woman, and 150 healthy female blood donors in Sweden aged 15 to 50 years (46). Among women with PID, 17% (33/193) were seropositive for *M. genitalium*, while 15% (36/246) of pregnant controls and 3% (5/150) of the blood donor controls were seropositive (46). However, *M. genitalium* antibodies were not associated with PID in univariate analysis (OR 1.3, 95% CI 0.7 -2.2), or after adjustment of *C. trachomatis* infection (OR 1.0, 95% CI 0.6-1.7) (46). As serology measures past infection, women could have had a current infection which would have remain undetected with use of serology, resulting in the null findings.

Common sequelae of PID include infertility, recurrent PID, ectopic pregnancy and chronic pelvic pain (109). Therefore, *M. genitalium* PID may lead to subsequent reproductive morbidity. In the PEACH study, rates of infertility (22%), recurrent PID (31%), and chronic pelvic pain (42%) at follow-up were high among women testing positive for endometrial *M. genitalium* at baseline (42). Further, women who tested positive for *M. genitalium* in the endometrium were less likely to become pregnant (ARR 0.7, 95% CI 0.3-1.4) or have a live birth (ARR 0.6, 95% CI 0.3-1.3) and more likely to report infertility (ARR 1.1 95% CI 0.7-4.0), chronic pelvic pain (ARR 1.3, 95% CI 0.6-2.7) and recurrent PID (ARR1.8, 95% CI 0.8-3.8) than women without *M. genitalium* (42). These results were found after adjusting for age, race, *N.*

gonorrhoeae, and *C. trachomatis* infection, suggesting that infection of *M. genitalium* alone is sufficient to cause these morbidities. Although the association between *M. genitalium* and these sequelae did not reach significance, they were in the hypothesized direction and the findings were similar with another reported PEACH analyses, showing that chlamydial and gonococcal upper genital tract infection was not associated with subsequent morbidity (110). This could be explained by the fact that women in the comparison groups who did not test positive for *M. genitalium*, *C. trachomatis* or *N. gonorrhoeae* had signs and symptoms of PID, thus all women in the PEACH study were at high risk of sequelae because they had clinically suspected PID.

M. genitalium and Tubal Factor Infertility

Tubal factor infertility can follow an episode of PID if the fallopian tubes undergo cellular and sub-cellular damage and ciliary motion of damaged epithelial cells is reduced (18). In the large Scandinavian prospective cohort studies, almost 20% of women who had at least one episode of PID experienced TFI (109). Other studies have found that tubal occlusion with infertility occurs in approximately 11-50% of PID patients (111). The risk of TFI increases with each episode of PID. After a single episode of PID, the relative risk for TFI is approximately 10%, and each subsequent episode of PID doubles the risk of TFI; after two episodes of PID the risk of TFI is nearly 20%, and it is 40% after three or more episodes (112). The severity of inflammation has also been associated with post-PID infertility (mild/moderate/severe; RRs 1/1.8/5.6) (112).

The association between *M. genitalium* and tubal factor infertility (TFI) has been assessed by two serological studies and results from these studies indicate that there is a possible association between *M. genitalium* and TFI (113, 114). Clausen et al conducted a study among 308 infertile women and found that antibodies against the MgPa gene were identified more frequently among women with TFI compared to women with normal fallopian tubes (22% vs. 6.3%, p=0.005) (113). Women with TFI had nearly a 4-fold greater risk of being seropositive compared to women with normal tubes (OR 3.8, 95% CI 1.7-9.4) (113). Additionally, over 27% of *M. genitalium* positive women were seronegative to *C. trachomatis,*

suggesting an association between *M. genitalium* and TFI, independent of *C. trachomatis* (113). In another study by the same group among 194 women attending fertility clinics, 17% (5/30) of women with TFI assessed by culdoscopy and/or laparoscopy had antibodies to *M. genitalium*, compared with only 4% (7/164) of women with normal tubes ($p<0.01$) (114). The association between TFI and *M. genitalium* was significant (OR 4.5; 95% CI 1.3-15.2), even after adjusting for *C. trachomatis* and age (OR 4.5; 95% CI 1.2-15.6) (114). In the same study, none of the cervical swabs analyzed for *M. genitalium* were positive, indicating that a previous infection was most likely the cause of damage to the fallopian tubes.

1.2.1.12 *M. genitalium* in pregnancy

Because *M. genitalium* may be associated with PID and TFI, it is plausible that *M. genitalium* can infect the upper genital tract during pregnancy, resulting in adverse pregnancy outcomes. The prevalence of *M. genitalium* in pregnant women has ranged from less than 1% to over 20% (44, 97, 99, 115, 116) However, the consequences of prenatal *M. genitalium* are unknown.

Definition and causes of spontaneous abortion

Spontaneous abortion (SAB) is the most common adverse outcome of pregnancy, occurring in an estimated 15% of clinically recognized pregnancies (117), and up to 50% of all pregnancies (118). Nearly half of SABs are due to chromosomal abnormalities (119). However, the cause of the remaining 50% is poorly understood. As the percentage of SAB caused by chromosomal aberrations peaks at 11 weeks gestation, (118) other factors are more likely to have a strong impact after 11 weeks gestation. In fact, after 11 weeks gestation there are 2.9 to 3.5 times more chromosomally normal SABs (118). Therefore, the gestational age at the time of the SAB can provide clues about the cause. Evidence suggests that lower genital tract and intrauterine infections play a role in SAB (93, 120, 121). However, due to the design of many studies, it is difficult to conclude whether infectious agents cause pregnancy loss or if they arise secondary after loss which is caused by a non-infectious etiology. Still, several

different theories have been postulated to explain how infectious agents may cause SAB, including: 1) toxic metabolic byproducts, endotoxin, exotoxin, or cytokines may have a direct effect on the uterus, fetus or placenta; 2) fetal infection may cause fetal death or severe malformation incompatible with fetal viability; 3) placental infection may result in placental insufficiency, with subsequent fetal death; 4) chronic infection of the endometrium caused by the ascension of organisms from the lower genital tract may interfere with implantation (122).

M. genitalium, SAB, and other adverse pregnancy outcomes

Few published studies have looked at *M. genitalium* and adverse pregnancy outcomes (Tables 9 and 10). In a study that aimed to identify causes of and risk factors for mucopurulent cervicitis among STD clinic attendees, Manhart et al reported a borderline significant association between *M. genitalium* lower genital tract infection and history of SAB (OR 2.4, 95% CI 1.0-5.8) (34). Two PCR-based studies have failed to find a positive association between *M. genitalium* and SAB. Oakeshott et al used PCR to test urine samples from 1,014 pregnant women at less than 10 weeks gestation (99). Only 0.66% (95% CI 0.1-1.2) of the samples tested positive for *M. genitalium* and 1% of the women who experienced a SAB at less than 16 weeks gestation tested positive for *M. genitalium* compared to 0.6% of women who did not experience a SAB (p=non-significant) (99). Selection bias may have been introduced since participants were women presenting at prenatal care at less than 10 weeks gestation in general practice and family planning clinics and were generally at low risk for STDs. Though Labbe et al found a higher prevalence among 1,014 women in Guinea-Bissau (6.2%), *M. genitalium* at time of pregnancy loss was not associated with SAB (OR 0.44, 95% CI 0.01-2.75) (116). As the number of women with *M. genitalium* infection who miscarried was very low in these two studies, insufficient power may have limited the ability to detect a significant association. Further studies are needed to assess the relationship between *M. genitalium* and SAB.

Table 9. Studies of *M. genitalium* (Mg) and adverse pregnancy outcomes (Part A: Study, location, population, sample size, sample, gestational week, pregnancy outcome)

Study	Location	Population	N	Sample	Gestational week	Pregnancy Outcome
Edwards, 2006	U.S.	Pregnant women with signs and symptoms of preterm labor and intact membranes	137	Vaginal	23-32	PTD[1]
Kataoka, 2006	Japan	Pregnant women with singleton pregnancies	877	Vaginal	<11	PTD SAB[2]
Oakeshott, 2004	England	Pregnant women	1014	Urine	<10	SAB
Labbe, 2002	Africa	Pregnant women	1014	Cervical	After delivery or SAB	PTD SAB
Lu, 2001	U.S.	Women delivering preterm	124	Vaginal	21-25	PTD

[1] PTD= preterm delivery
[2] SAB= spontaneous abortion

Table 10. Studies of *M. genitalium* (Mg) and adverse pregnancy outcomes (Part B: Overall prevalence of Mg, results)

Study	Overall Prevalence of Mg	Results
Edwards, 2006	20.2%	Mg associated with PTD[1] (OR 3.5, 95% CI 1.4-8.6)
Kataoka, 2006	0.8%	Mg not associated with SAB[2] or PTD
Oakeshott, 2004	0.6%	Cases not more likely to test positive for Mg than controls (0.6% vs. 1.1%, p=NS[3])
Labbe, 2002	6.2%	Mg not associated with SAB (OR 0.4, 95% CI 0.01-2.7) Mg not associated with PTD (OR 1.4, 95% CI 0.7-2.6)
Lu, 2001	3.9%	Mg positive did not delivery at an earlier gestational age than Mg negative (p=0.62)

[1] PTD= preterm delivery
[2] SAB= spontaneous abortion
[3] NS=non-significant

The role of *M. genitalium* in other adverse pregnancy outcomes is also largely unknown, however, it has been associated with an increased risk of preterm birth in one published study (115). Edwards et al tested the vaginal fluid of 137 women at 23-32 weeks of gestation with signs and symptoms

of preterm labor and intact membranes (115). *M. genitalium* was found in over 20% of samples tested with PCR and was found to be independently associated with a 3-fold increase risk of spontaneous preterm delivery (OR 3.5, 95% CI 1.4-8.6) (115). Other studies have not found an association between *M. genitalium* and preterm birth (44, 97, 116). In a study of 124 women with spontaneous preterm birth, the occurrence of *M. genitalium* in the vagina at mid-trimester was infrequent; only 3.9% (5/124) of the samples tested positive for *M. genitalium* by PCR (97). Further, women with *M. genitalium* did not deliver at an earlier gestational age than women without *M. genitalium*; the mean delivery gestational age was similar for women with a positive PCR (34.6 +/- 2.2 weeks) and a negative PCR (34.0 +/- 2.7 weeks) (p=0.62) (97). Labbe et al found that *M. genitalium* was not significantly associated with premature delivery (OR 1.4, 95% CI 0.7-2.6) in a population of 1014 pregnant women in Guinea-Bissau (116). Kataoka et al conducted a prospective cohort study of 877 women presenting at hospitals in Japan for prenatal care with singleton pregnancies at less than 11 weeks gestation to test for numerous mycoplasmas, including, *M. genitalium, M. hominis, U. parvum*, and *U. urealyticum* using PCR (44). The authors found that vaginal colonization with *M. genitalium* was not associated with preterm birth, as none of the women who experienced a preterm birth tested positive for *M. genitalium* (44), while only 0.8% of all women enrolled in the study tested positive for *M. genitalium*. However, these were women seeking prenatal care at less than 11 weeks gestation and were at low risk for STDs in general.

Other mycoplasmas and adverse pregnancy outcomes

More data is available on other genital tract mycoplasmas, including *U. urealyticum* and *M. hominis*. In fact, in some studies, *M. hominis* and *U. urealyticum* has been associated with preterm labor and delivery and chorioamnionitis, the inflammation of the fetal membranes (placenta tissue) and amniotic fluid (44, 66, 123-125). Other studies have suggested that there is an increased risk of SAB in women testing positive for mycoplasmas (126-128). Donders et al found that *M. hominis* and *U. urealyticum* infection at less than 14 weeks gestation were independently associated with an increased risk of pregnancy loss before 20 weeks (RR 12.5, 95% CI 3.0-52 and RR 5.8, 95% CI 2.1-16,

respectively) (128). Berg et al found that treatment of an amniotic mycoplasmal colonization with erythromycin was associated with fewer mid-trimester losses after genetic amniocentesis (126); pregnancy loss was 11.4% (4/35) and 44.4% (4/9) ($p = 0.04$) in the treated and untreated groups, respectively. However, preterm delivery was similar in the two groups, 19.4% and 20% (p = NS). (126). Naessens et al found that women with a history of SAB were more likely to test positive for ureaplasmas in endocervical samples than control women ($p<0.05$) (129). In a study of 40 women who experienced a SAB and 20 healthy pregnant women, *U. urealyticum* was isolated more often in aborted tissue and cervical mucus from women who experienced a SAB than control women (55.0% vs. 10.0%) (127).

Recently, previously unclassified ureaplasmas (formally called *U. urealyticum*) were separated into two species: *U. parvum* (biovar 1) and *U. urealyticum* (biovar 2). The role of the two newly specified ureaplasmas in adverse pregnancy outcomes have been studied, but with conflicting results. Kataoka et al found a significant association between *U. parvum* and preterm birth (OR 3.0, 95% CI 1.1-8.5), but not with *M. hominis* or *U. urealyticum* and preterm birth (44). The authors did not assess BV. Conversely, Edwards et al found that *U. urealyticum* but not *U. parvum* was associated with preterm birth (OR 2.3, 95% CI 1.0-5.1) in a study of 137 women with signs and symptoms of preterm labor and intact membranes between 23 and 32 weeks gestation (115).

However, whether these mycoplasmas are independently associated with these pregnancy disorders and outcomes remains controversial due to their carriage rate in the general population. It has been estimated that 5-49% of all women and 43-81% of pregnant women are colonized with *M. hominis* and *U. urealyticum*, respectively (30). Thus, these mycoplasmas may simply be frequent colonizers of the reproductive tract, and not pathogenic. Further, as women with BV have *M. hominis* or *U. urealyticum* in the vagina more often as well as in larger numbers than women without BV (130), failure to adjust for BV may confound study results. It is also possible that these pathogens invade the upper genital tract after a SAB, and therefore fetal death is not a measureable outcome.

C. trachomatis and SAB

The prevalence of *C. trachomatis* in pregnancy has varied between 2%-30% (131). Results from studies assessing the relationship between *C. trachomatis* infections and SAB are inconclusive. Many of these studies have been conducted among women who had recurrent abortions or who had a history of unexplained infertility, which limit the generalizability of the findings. Further, most of the studies relied on measures of past infections and did not examine current infections. Still, few studies using DNA based methods of detection have been conducted to examine the relationship between *C. trachomatis* and SAB and results have been inconsistent. Among women undergoing in vitro fertilization (IVF), Witkin et al found a strong correlation between endocervical *C. trachomatis* and SAB after embryo transfer (p=0.004) (132). Further, *C. trachomatis* was identified in more women who had SABs than women who had term deliveries (27.3% vs. 1.8%) (132). As all women were asymptomatic, the authors concluded that an undetected *C. trachomatis* infection may be responsible for SAB after IVF and embryo transfer (132). Contrary to this, Sozio and Ness did not find a significant relationship between acute lower genital tract *C. trachomatis* infection and SAB in their nested case-control pilot study of 52 women who experienced a SAB and 59 control women who maintained their pregnancies (133). 3.8% (2/52) of cases and 8.5% (5/59) of control women tested positive for *C. trachomatis* in their urine by ligase chain reaction, and women who experienced a SAB were not significantly more likely to have *C. trachomatis* infection than controls (OR 1.8, 95% CI 0.3-10.7) (133). Thus, the role of *C. trachomatis* in SAB remains unexplained.

Serology studies have found the presence of antibodies to *C. trachomatis* in the sera of women who have experienced repeated pregnancy loss. Licciardi et al assessed the sera from 145 women undergoing in-vitro fertilization and found that anti-chlamydial antibodies were found in 69% (20.29) of women who experienced a SAB compared to 24% (9/38) of women who did not (p<0.001) (134). Witkin and Ledger tested sera from the female partners of 258 couples with unexplained infertility, no history of chlamydial infection, and negative cervical cultures for *C. trachomatis,* and found that high-titer antibody to *C. trachomatis* was associated with recurrent spontaneous abortions (135). Forty one percent (7/17) of

women with three abortions and 60% (6/10) of women with four abortions had chlamydial antibodies as opposed to 13.5% (20/148) women with no abortions, 12.8% (6/47) of women with one abortion, and 12.1% (4/33) of women with two abortions ($p<0.01$) (135). The incidence of 3 or more SABs was 31.8% among women with high-titer chlamydial antibodies and 7.5% among women who had seronegative results ($p<0.001$) (135). Results from this study suggest that a chronic, "silent" infection may increase the risk of SAB, for none of the women had a positive *C. trachomatis* culture test.

Other studies have failed to find a significant association between *C. trachomatis* antibodies and SAB. Rae did not find a significant association between antibodies to *C. trachomatis* and recurrent SAB in a population of 106 recurrent aborters and 3890 sera from a general antenatal population (136). Nearly 25% (26/106) of recurrent aborters had antichlamydial antibodies compared 20.3% (788/3890) of the general antenatal population ($p < 0.05$) (136). Sugiura-Ogasawara et al found that *C. trachomatis* antibodies were not related to pregnancy outcome in women with a history of two or more miscarriages (137). Over 33% of women (10/30) positive for IgA and/or IgG antibodies to *C. trachomatis* and 23.9% (48/201) of women negative for IgA and IgG antibodies to *C. trachomatis* experienced a SAB ($p>0.05$) (137). Osser and Perrson also failed to find an association between previous chlamydial infection and SAB, as SAB cases were not significantly more likely to have chlamydial antibodies than controls (39.3% vs. 33.2%) (138). Paukku et al found no statistically significant difference in the frequencies of chlamydial IgG or IgA antibodies between 70 women with recurrent pregnancy loss (≥ 3 consecutive losses) and 134 control women (139). Small sample sizes may have limited the power of these studies. Further, the generalizability of these studies is limited to recurrent aborters.

Bacterial vaginosis, SAB and other adverse pregnancy outcomes

BV is a common condition among pregnant women. In fact, up to 50% of pregnant women are have BV (131). BV and BV-related organisms have emerged as important infections possibly associated with several serious obstetrical complications. Although results from cross-sectional studies are varied, (120, 140-143), several prospective cohort studies have reported that BV in early pregnancy is strongly

and significantly associated with a 2 to 5-fold increased risk of subsequent SAB (99, 128, 144-148). BV has also been shown to be associated with an increased risk of preterm birth (143, 145, 149-152). Two case-control studies have found over a 2-fold increased risk of preterm delivery among women with BV (143, 150), while numerous cohort studies have reported similar findings. In fact, BV in pregnancy is strongly and significantly associated with up to a 7-fold increased risk of subsequent preterm delivery (145, 149, 151, 152).

Additional evidence of BV and preterm delivery comes from studies among populations of women with a high risk of preterm delivery in which oral treatment of BV has been shown to decrease the rate of recurrent preterm delivery (153-155). However, a large multi-centered randomized control trial failed to find a significant association between treatment of asymptomatic BV during pregnancy and preterm delivery (156). The timing of therapy may contribute to the effectiveness in preventing subsequent pregnancy complications. Therefore, more studies are needed to assess the importance of BV treatment during pregnancy.

1.2.1.13 Summary

M. genitalium is a sexually transmitted bacterium that infects the genital tract of male and females. In some populations, *M. genitalium* is as prevalent as *C. trachomatis* and *N. gonorrhoeae*. *M. genitalium* is emerging as a possible etiological agent of numerous female reproductive tract morbidities, including cervicitis, PID, and TFI. However, the risk factors for and clinical characteristics of *M. genitalium* infections have not been well described, and no studies have compared the clinical, demographic, and behavioral characteristic of women with *M. genitalium* to women with other STDs. Further, the prevalence and consequences of prenatal *M. genitalium* have not been thoroughly examined. With the recent development of more sensitive and quicker PCR-based methods of detecting *M. genitalium*, more research is warranted.

2.0 MANUSCRIPT 1: CLINICAL PRESENTATION OF *MYCOPLASMA GENITALIUM* INFECTION VERSUS *NEISSERIA GONORRHOEAE* INFECTION AMONG WOMEN WITH PELVIC INFLAMMATORY DISEASE

Clinical Infectious Diseases 2009 January 48(1):41-7

Vanessa L. Short, MPH[1], Patricia A. Totten, PhD[2], Roberta B. Ness, MD, MPH[3], Sabina G. Astete, PhD[2], Sheryl F. Kelsey, PhD[1], Catherine L. Haggerty, PhD, MPH[1]

[1] University of Pittsburgh, Pittsburgh, PA, [2] University of Washington, Seattle, WA, [3]The University of Texas School of Public Health, Houston, TX

Funding Support: HS08358-05 from the Agency for Healthcare Research and Quality Development and 1 R01 AI067661-01A2 from the National Institute of Allergy and Infectious Diseases

2.1 ABSTRACT

Background: Women with pelvic inflammatory disease (PID) frequently present with a spectrum of symptoms. The characteristics of non-gonococcal/non-chlamydial PID are not well described. Our objective was to examine the characteristics of *Mycoplasma genitalium* infection among women with clinically suspected PID.

Methods: We evaluated 722 women enrolled in the PID Evaluation and Clinical Health study. Women with *M. genitalium* only were compared to women with only *Neisseria gonorrhoeae* or *Chlamydia trachomatis*.

Results: Compared to women with gonococcal PID, women with *M. genitalium* were less likely to have elevated systemic inflammatory markers, including erythrocyte sedimentation rate >15mm/hr (22.7% vs. 60.8%, p=0.002), white blood cell count >10,000 mm^3 (28.6% vs. 64.6%, p=0.018), and oral temperature $\geq$ 38.3° C (0.0% vs. 13.9%, p=0.085). Further, they were less likely to present with mucopurulent cervicitis (47.4% vs. 83.3%, p=.001), elevated vaginal pH (p=0.018), and high pelvic pain score (p=0.014). In contrast, women with chlamydial PID had similar signs and symptoms as women with infected with *M. genitalium*.

Conclusions: As symptoms may be mild, women with *M. genitalium* may not seek PID treatment. Further studies are needed to assess the potential reproductive tract sequelae of *M. genitalium* upper genital tract infection.

2.2 INTRODUCTION

Pelvic inflammatory disease (PID), an inflammation of the female upper genital tract caused by ascension of organisms from the lower genital tract, affects approximately 8% of reproductive-aged women in the United States at some time in their lives (157). Serious sequelae, including recurrent PID, tubal factor infertility, ectopic pregnancy and chronic pelvic pain frequently follow PID (158). Approximately 30% to 50% of PID cases are caused by the sexually transmitted pathogens *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (79, 159, 160). Although the etiology of PID is unknown in the majority of cases, it has been epidemiologically linked to bacterial vaginosis (161).

M. genitalium has been identified as a possible etiologic agent of non-gonococcal/non-chlamydial PID (40, 43, 69). It has also been detected in cervical and salpingeal samples from women with laparoscopically confirmed salpingitis (41) and in cervical and endometrial specimens from women with endometritis (40). Because *M. genitalium is* extremely difficult to culture, epidemiologic studies assessing the role of this organism in reproductive diseases among women was dependent upon the development and application of PCR-based assays. Although little is known about the clinical characteristics of *M. genitalium* PID, evidence suggests that, like women with chlamydia, lower genital tract infections tend to be asymptomatic (14, 162).

Presenting characteristics of women with PID vary and may include pelvic pain, abnormal vaginal discharge, bleeding, itching and/or odor. Although the most common symptom of PID is pelvic pain, many women with PID may have mild or absent pain, despite evidence of infection and inflammation (163, 164). The presence and severity of PID symptoms varies by microbiologic etiology, with chlamydial patients more likely to experience asymptomatic PID than women with gonococcal PID (165-168).

The purpose of our study was to describe the clinical features of women with lower and/or upper genital tract *M. genitalium* infection in a population of women presenting with clinically suspected PID. We hypothesize that presenting clinical characteristics, symptoms, and pelvic pain will be less frequent

and severe among women with *M. genitalium* or *C. trachomatis* compared to women with gonococcal PID.

2.3 METHODS

2.3.1 Study Population

We used data from the baseline interview of the PID Evaluation and Clinical Health Study, described in detail elsewhere (169). Briefly, the PEACH Study evaluated the effectiveness of inpatient versus outpatient treatment for PID in preventing infertility. Between March 1996 and February 1999, non-pregnant women between the ages of 14 and 36 years were recruited from emergency departments and outpatient facilities (OB/GYN clinics, sexually transmitted disease clinics, and private practices) from seven primary (Atlanta, GA; Birmingham, AL; Charleston, SC; Detroit, MI; Philadelphia, PA; Pittsburgh, PA; and Providence, RI) and six secondary sites in the U.S.. Women were eligible to participate if they had clinically suspected PID as defined by: 1) complaints of acute pain (<30 days); 2) a clinical finding of pelvic tenderness; and 3) evidence of lower genital tract inflammation. Women were excluded from the study if they had severe disease requiring inpatient management, could not tolerate an outpatient regimen due to vomiting, had an allergy to antibiotics, experienced a delivery, abortion or gynecologic surgery within the past 45 days, had a prior hysterectomy, bilateral salpingectomy, or bilateral tubal ligation, had a tubo-ovarian abscess documented by ultrasound or laparoscope and/or had appendicitis, hemorrhagic ovarian cyst or other condition requiring surgery by ultrasound or laparoscopy. Eligible women were informed and consented, and 831 participants were enrolled into the PEACH Study. IRB approval was obtained for the parent PEACH study as well as for subsequent *M. genitalium* PCR testing of stored specimens. For this analysis, stored cervical and endometrial specimens and *M. genitalium* PCR assays were available on a subset of 722 women. The demographic, behavioral and clinical characteristics of the

111 women who did not have *M. genitalium* PCR assays did not differ significantly from the women included in our analyses.

2.3.2 Data Collection

Baseline data were collected by trained research staff at each study center using standardized interview, examination, and specimen collection techniques. Information was collected on demographic characteristics, medical, gynecologic and sexual histories, presenting complaints, substance use, current medications and contraception. Cervical and vaginal swabs, endometrial biopsies, and serum and urine samples were obtained from participants.

2.3.3 Detection of *M. genitalium*

Previously collected cervical and endometrial samples stored at -70°C were tested for *M. genitalium* using the MgPa-IMW PCR assay targeting the MgPa gene (170). This assay has an analytical sensitivity of 15 genomes (170) and a high clinical sensitivity and specificity relative to TMA, another *M. genitalium* NAAT assay (28). For all samples testing positive, a repeat MgPa PCR assay (170) was performed using another aliquot of the sample to rule out PCR product contamination or cross-contamination; all samples initially positive were verified as positive in this confirmatory test.

2.3.4 Detection of *N. gonorrhoeae* and *C. trachomatis*

Baseline cervical and endometrial samples were assessed for *N. gonorrhoeae* by culture and *C. trachomatis* by PCR at a central laboratory, as previously described (79).

2.3.5 Clinical Characteristics

The following are the baseline signs and symptoms that we evaluated as potential characteristics of *M. genitalium* infection: elevated oral temperature ($\geq$38.3^{o} C), elevated white blood cell count (>10,000 mm^{3}), elevated erythrocyte sedimentation rate (ESR) (>15 mm/hr), C-Reactive Protein (>5mg/dL), bilateral adnexal tenderness, mucopurulent cervicitis, and bacterial vaginosis (BV), defined using Gram stain (171) and Amsel's criteria (172). An oral temperature of 38.3°C was considered as clinically elevated. Mucopurulent cervicitis was defined as the presence of a grossly yellow or green exudates observed on a swab with a specimen taken from the cervix. Presenting complaints that we evaluated as potential characteristics of *M. genitalium* infection included: nausea or vomiting, non-menstrual vaginal bleeding or spotting, more prolonged or heavier menstrual bleeding than usual, vaginal bleeding during or after sex, abnormal vaginal discharge, more frequent urination than usual, and overall self-rated pelvic pain. A pelvic pain score was calculated as the mean scores for pain at worst, on average, and in the past 24 hours, measured on a Likert scale and multiplied by 10 (range 0-100).

2.3.6 Statistical Methods

The Chi-square test, Fischer's exact test, and analysis of variance were used to evaluate the baseline characteristics and presenting complaints. Women with *M. genitalium* identified in the cervix and/or endometrium and who tested negative for both *N. gonorrhoeae* and *C. trachomatis* were compared to: 1) women who tested positive only for *N. gonorrhoeae* in the cervix and/or endometrium; and 2) women who tested positive only for *C. trachomatis* in the cervix and/or endometrium. Women who tested positive for only *N. gonorrhoeae* were also compared to women co-infected with *M. genitalium* and *N. gonorrhoeae*. Similarly, women who tested positive only for *C. trachomatis* were compared to women co-infected with *M. genitalium* and *C. trachomatis*. We also examined the differences between women with gonococcal PID and women with chlamydial PID. All data were analyzed using SAS version 9.1.

2.4 RESULTS

Compared to women with gonococcal PID, women with *M. genitalium* were generally less likely to have elevated systemic inflammatory markers, including erythrocyte sedimentation rates greater than 15mm/hr (22.7% vs. 60.8%, p=0.002), white blood cell counts greater than 10,000 mm^3 (28.6% vs. 64.6%, p=0.018), and oral temperatures greater than or equal to 38.3° C (0.0% vs. 13.9%, p=0.085) (Table 11). They were also significantly less likely to present with mucopurulent cervicitis (47.4% vs. 83.3%, p=0.001) and an elevated vaginal pH (68.4% vs. 92.3%, p=0.018). Further, they had significantly lower mean composite pain scores at baseline (p=0.014). Compared to women testing positive for *N. gonorrhoeae*, women with *M. genitalium* were marginally more likely to have discharge consistent with BV identified upon clinical examination (57.1% vs. 34.7%, p=0.065). The clinical characteristics of women with only *N. gonorrhoeae* were not different compared to women who tested positive for both *N. gonorrhoeae* and *M. genitalium.* After adjusting for BV status (normal or intermediate vs. BV flora), vaginal pH was no longer significantly different between women with *M. genitalium* and women with *N. gonorrhoeae* (data not shown). All other results remained the same.

In contrast to women with *N. gonorrhoeae*, the clinical features of women who tested positive only for *M. genitalium* were similar to women who tested positive only for *C. trachomatis*. The clinical characteristics of women with only *C. trachomatis* were not different compared to women who tested positive for both *C. trachomatis* and *M. genitalium* (Table 12). The results remained the same after adjusting for BV (data not shown).

Compared to women with gonococcal PID, women with *C. trachomatis* PID were generally less symptomatic and less likely to have elevated systemic inflammatory markers, including elevated oral temperature (0% vs. 13.9%, p=0.013) or elevated white blood cell count (22.5% vs. 64.6%, p<0.001). They were less likely to present with cervicitis (52.4% vs. 83.3%, p<0.001) or bilateral adnexal tenderness (77.8% vs. 82.4%, p=0.049), and had significantly lower mean composite pain scores (p=0.020).

2.5 DISCUSSION

To our knowledge, this is the first study to compare the clinical characteristics of women with clinically suspected PID who had genital tract infections with *M. genitalium*, *N. gonorrhoeae* and/or *C. trachomatis*. Our study suggests that like chlamydial PID, *M. genitalium* upper genital tract infection is less symptomatic than gonococcal PID. However, it should be noted that all women in the PEACH study had clinically suspected PID, and therefore they all presented with some signs or symptoms. Since the inclusion criteria minimized the selection of asymptomatic patients, differences in the clinical characteristics between women with and without *M. genitalium* may be muted. It would be important to repeat these analyses in a population of women including symptomatic and sub-clinical or "silent" PID.

Mucopurulent cervicitis, an elevated vaginal pH, and numerous systemic markers of inflammation, including an elevated oral temperature, white blood cell count and ESR, were more prevalent in women with *N. gonorrhoeae* compared to women with only *M. genitalium.* Further, pelvic pain scores were higher among women with *N. gonorrhoeae*. Women with only *N. gonorrhoeae* infections had similar clinical features as those women that tested positive for both *N. gonorrhoeae* and *M. genitalium*. This suggests that in co-infections, the clinical picture of *N. gonorrhoeae* dominates.

The clinical features of women presenting with upper genital tract *M. genitalium* infection have not been extensively examined. In a study of 115 women with histologically confirmed endometritis, 100% of women with *M. genitalium* reported mild abdominal pain, compared to 68% of women without *M. genitalium* (p=0.06) (108). The association of *M. genitalium* with lower tract disease in women has not been consistently reported, possibly reflecting differences in the population studied and criteria used to assess signs and symptoms at this site. While some studies have showed an association between *M. genitalium* and cervicitis (33, 34, 37, 69) several PCR studies have failed to find a strong association between symptoms and *M. genitalium* lower genital tract infection (14, 22, 32). Tosh et al studied 383 adolescent females attending a primary care clinic and found that women with *M. genitalium* identified in the lower genital tract were no more symptomatic than uninfected women (14). In a group of women

negative for both *C. trachomatis* and *N. gonorrhoeae*, those who tested positive for *M. genitalium* were not more likely to have signs (presence of vaginal erythema, vulvar erythema or vaginal discharge) (p=0.33) or symptoms (vaginal itching, vaginal burning, dyspareunia) (p=0.35) compared to women negative for *M. genitalium* (14). Manhart et al also found that lower genital tract *M. genitalium* infection was not associated with symptoms. PCR was used to test the urine from 1714 women enrolled in a population-based study and *M. genitalium* infections were not associated with symptoms, as none of the participants who tested positive for *M. genitalium* reported symptoms of vaginal discharge (22). Conversely, vaginal discharge was more common in women with lower genital tract *M. genitalium* infections compared to women without *M. genitalium* among 390 minority women with an active sexually transmitted infection attending a public health clinic (70). The results were similar after controlling for co-infection with other STDs. However, vaginal discharge was the only genitourinary sign or symptom that was significantly different between women testing positive and women not infected. Casin et al found no association between *M. genitalium* identified in lower genital and urinary symptoms (OR 1.34, 95% CI 0.72 to 2.50) or pelvic pain (OR 0.93, 95% CI 0.50 to 1.73) among women attending a STD clinic (32). These PCR studies indicate that *M. genitalium* does not produce strong symptoms in women with lower genital tract infections compared to women without *M. genitalium*. The lower symptomatology induced by *M. genitalium* is similar to that seen for *C. trachomatis* (71).

Our study is unique in that we compared the clinical characteristics of lower and/or upper genital tract *M. genitalium* infection to infections caused by other known bacterial STDs. In our study, while women with *M. genitalium* tended to be less symptomatic than women with gonococcal PID, they were similar to women with chlamydial PID. No characteristics differed significantly between women with *M. genitalium* and women with *C. trachomatis*. While, to our knowledge, no other study has compared the clinical characteristics of women with clinically suspected PID, our results are similar to a study comparing the symptoms of *C. trachomatis* and *M. genitalium* in the lower genital tract conducted among 465 women either attending a STD clinic or enrolled in a cervical cancer screening program in Sweden. In this study, no significant differences were reported in symptoms (32% v 23%, RR 1.4, 95% CI 0.6 to

3.4) or signs (71% v 50%, RR 1.4, 95% CI 0.9 to 2.3) between women testing for *C. trachomatis* and *M. genitalium* in the lower genital tract (33).

Although women with *M. genitalium* infections present with fewer clinical signs and symptoms, there is evidence from animal and human studies supporting a pathogenic role of *M. genitalium* in female upper genital tract infection. *M. genitalium* has been found to induce salpingitis experimentally in monkeys (173), and adheres to human fallopian tube epithelial cells in organ culture, resulting in damage to the ciliated cells (102). This bacterium can adhere to human spermatozoa, potentially allowing it to be carried to the female upper genital tract on motile sperm (103).

M. genitalium PID may lead to subsequent reproductive morbidity, including infertility, recurrent PID and pelvic pain. In a previous analysis of the PEACH data, Haggerty et al found that rates of short term treatment failure (persistent endometritis and pelvic pain post treatment with cefoxitin and doxycycline) (41%); infertility (22%); recurrent PID (31%); and chronic pelvic pain (42%) were high among women testing positive for endometrial *M. genitalium* at baseline (42). These results were similar in a subset of women testing negative for *N. gonorrhoeae* and *C. trachomatis.* Although the association between *M. genitalium* and these sequelae did not reach significance, the findings were similar with previously reported PEACH analyses, showing that chlamydial and gonococcal upper genital tract infection was not associated with subsequent morbidity (110). This could be explained by the fact that women in the comparison groups who did not test positive for *M. genitalium*, *C. trachomatis* or *N. gonorrhoeae* did have signs and symptoms of PID; thus all women in the PEACH study were at high risk of sequelae because they had clinically suspected PID.

Infertility after infection with *M. genitalium* could result from inflammation and scaring of the fallopian tubes because of frequent PID treatment failure, as 44% of women who tested positive for *M. genitalium* at baseline tested positive again at 30 days-post treatment (174). A relationship between *M. genitalium* and tubal factor infertility (TFI) has also been identified in serological studies (113). Specifically, *M. genitalium* antibodies were identified more frequently among women with TFI compared to women with non-tubal factor infertility (22% vs. 6%) (113). In another serological study, 17% of

women with TFI had antibodies to *M. genitalium*, compared with only 4% of women with normal tubes (114).

The ability to test for concomitant infections of *C. trachomatis*, *N. gonorrhoeae*, and BV was a strength of our study. However, the unavailability of data on other pathogens may limit the interpretation of our findings. It may be possible that specific BV-associated bacteria, anaerobes and other mycoplasmal bacteria confound our analysis. However, adjustment for these bacteria was not possible in our current analyses, as only a subset of PEACH women were tested for these bacteria.

In this study, we compared clinical characteristics and presenting complaints by microbial etiology among a population of women with clinically suspected PID. As our study suggests, women with *M. genitalium* may have less symptomatic PID, which, left untreated, can lead to serious reproductive morbidity, including tubal factor infertility, ectopic pregnancy, chronic pelvic pain and recurrent PID (109). Since the etiology of up to 70% of PID cases is unknown, and *M. genitalium* has frequently been found in women with PID, detection of the pathogen may help reduce the burden of untreated PID. However, as clinical symptoms may be mild, and since PID is typically diagnosed through clinical suspicion, women with *M. genitalium* may not seek PID treatment and cases of *M. genitalium* PID may go undiagnosed. Additional studies are needed to determine a diagnostic approach for *M. genitalium* PID and to assess the potential reproductive tract sequelae of *M. genitalium* upper genital tract infection.

2.6 TABLES

Table 11. Clinical characteristics of women with *M. genitalium* (Mg) only, women with *N. gonorrhoeae* (Gc) only and women with *M. genitalium* and *N. gonorrhoeae*

	Mg+/Gc-/Ct-[a] (N=22) n (%)	Gc+/Mg-/Ct- (N=74) n (%)	p-value[b]	Gc+/Mg+/Ct- (N=16) n (%)	p-value[c]
Presenting Complaints					
Nausea/vomiting	10 (45.5)	30 (40.5)	0.682	8 (50.0)	0.487
Non-menstrual vaginal bleeding	9 (40.9)	28 (37.8)	0.795	3 (18.8)	0.245
Heavier menstrual bleeding	10 (45.5)	26 (35.1)	0.380	5 (31.3)	0.767
Bleeding during or after sex	4 (18.2)	6 (8.1)	0.230	2 (12.5)	0.629
Abnormal vaginal discharge	14 (63.6)	50 (67.6)	0.731	8 (50.0)	0.183
More frequent urination	11 (50.0)	31 (41.9)	0.501	10 (62.5)	0.170
Markers of Inflammation					
Elevated temperature ($\geq$38.3^{o} C)	0 (0.0)	10 (13.9)	0.085	1 (6.67)	0.681
Elevated white blood cell count (>10,000 mm^{3})	4 (28.6)	42 (64.6)	0.018	5 (41.7)	0.134
Erythrocyte Sedimentation Rate (>15 mm/hr)	5 (22.7)	45 (60.8)	0.002	8 (50.0)	0.426
C-Reactive Protein (>5mg/dL)	1 (4.5)	9 (12.2)	0.305	2 (12.5)	0.970
Bilateral adnexal tenderness	17 (77.3)	61 (82.4)	0.586	15 (93.8)	0.257
Mucopurulent cervicitis	9 (47.4)	60 (83.3)	0.001	9 (56.3)	0.017
Bacterial vaginosis					
by Gram Stain[d]	13 (59.1)	50 (76.9)	0.106	11 (84.6)	0.540
by Amsel's criteria	7 (38.9)	16 (42.1)	0.819	4 (44.4)	1.00
Pelvic Pain					
Mean composite pain score[e] (standard deviation)	58.0 (21.9)	72.3 (23.9)	0.014	75.2 (20.7)	0.658

[a] *C. trachomatis*
[b] p-value in comparison to the Mg+/Gc-/Ct- group
[c] p-value in comparison to the Gc+/Mg-/Ct-
[d] Normal or intermediate vs. BV flora
[e] Mean composite pain score=(mean of current pelvic pain score, average pelvic pain score and worst pelvic pain score) X 10

Table 12. Clinical characteristics of women with *M. genitalium* (Mg) only, women with *C. trachomatis* (Ct) only, and women with *M. genitalium* and *C. trachomatis*

	Mg+/Ct-/Gc-[a] (N=22) n (%)	Ct+/Mg-/Gc- (N=45) n (%)	p-value[b]	Ct+/Mg+/Gc- (N=9) n (%)	p-value[c]
Presenting Complaints					
Nausea/vomiting	10 (45.5)	21 (46.7)	0.926	4 (44.4)	1.00
Non-menstrual vaginal bleeding	9 (40.9)	26 (57.8)	0.194	4 (44.4)	0.489
Heavier menstrual bleeding	10 (45.5)	17 (37.8)	0.547	1 (11.1)	0.244
Bleeding during or after sex	4 (18.2)	12 (26.7)	0.444	1 (11.1)	0.428
Abnormal vaginal discharge	14 (63.6)	32 (71.1)	0.536	7 (77.8)	0.684
More frequent urination	11 (50.0)	21 (46.7)	0.798	5 (55.6)	0.626
Markers of Inflammation					
Elevated temperature ($\geq$38.3^{o} C)	0 (0.0)	0 (0.0)	---	1 (14.3)	0.137
Elevated white blood cell count (>10,000 mm^{3})	4 (28.6)	9 (22.5)	0.722	2 (22.2)	0.986
Erythrocyte Sedimentation Rate (>15 mm/hr)	5 (22.7)	19 (42.2)	0.118	6 (66.7)	0.179
C-Reactive Protein (>5mg/dL)	1 (4.5)	6 (13.3)	0.269	3 (33.3)	0.161
Bilateral adnexal tenderness	17 (77.3)	35 (77.8)	0.963	7 (77.8)	1.00
Mucopurulent cervicitis	9 (47.4)	22 (52.4)	0.717	6 (66.7)	0.434
Bacterial vaginosis					
by Gram Stain[d]	13 (59.1)	28 (65.1)	0.634	4 (50.0)	0.450
by Amsel's criteria	7 (38.9)	15 (46.9)	0.585	2 (33.3)	0.672
Pelvic Pain					
Mean composite pain score[e] (standard deviation)	58.0 (21.9)	61.8 (22.9)	0.517	64.4 (26.8)	0.764

[a] *N. gonorrhoeae*
[b] p-value in comparison to the Mg+ /Ct-/Gc- group
[c] p-value in comparison to the Ct+/Mg-/Gc-
[d] Normal or intermediate vs. BV flora
[e] Mean composite pain score=(mean of current pelvic pain score, average pelvic pain score and worst pelvic pain score) X 10

3.0 MANUSCRIPT 2: THE DEMOGRAPHIC, SEXUAL HEALTH AND BEHAVIORAL CORRELATES OF *MYCOPLASMA GENITALIUM* INFECTION AMONG WOMEN WITH CLINICALLY SUSPECTED PELVIC INFLAMMATORY DISEASE

Manuscript in preparation

Vanessa L. Short, MPH[1], Patricia A. Totten, PhD[2], Roberta B. Ness, MD, MPH[3], Sabina G. Astete, PhD[2], Sheryl F. Kelsey, PhD[1], Pamela Murray, MD, MPH[4], Catherine L. Haggerty, PhD, MPH[1]

[1] Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA, [2] Department of Medicine, Division of Infectious Diseases, University of Washington, Seattle, WA, [3] The University of Texas School of Public Health, Houston, TX [4] Children's Hospital of Pittsburgh of University of Pittsburgh Medical Center, Pittsburgh, PA

Funding Support: HS08358-05 from the Agency for Healthcare Research and Quality Development and 1 R01 AI067661-01A2 from the National Institute of Allergy and Infectious Diseases

3.1 ABSTRACT

Objective: *Mycoplasma genitalium* has been identified as a cause of pelvic inflammatory disease (PID), the inflammation of the female upper genital tract which may result in serious reproductive sequelae. As the demographic, behavioral and sexual risk profile of women with *M. genitalium* is not well understood, we sought to describe the risk factors of *M. genitalium* among women presenting with clinically suspected PID.

Methods: Data from 586 participants in the PID Evaluation and Clinical Health Study were analyzed. Demographic, sexual history, and behavioral characteristics, including age, race, marital status, education level, sexually activity, number of sexual partners, history of STDs, bacterial vaginosis and PID, contraception use, oral and anal sex, age at sexual debut, douching practices, and drug, alcohol and tobacco use, were compared between women testing positive and negative for *M. genitalium* as determined by polymerase chain reaction (PCR), and between *M. genitalium* positive women and *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* positive women. As the profiles of women with cervical *M. genitalium* were similar to women with endometrial *M. genitalium*, comparisons using combined cervical and/or endometrial *M. genitalium* results are presented.

Results: Being younger than 25 years of age (OR 2.3, 95% CI 1.3-4.1), douching 2 or more times per month (OR 1.9, 95% CI 1.2-3.3), and smoking cigarettes (OR 1.9, 95% CI 1.2-3.1) were significantly associated with *M. genitalium*. After adjusting for *C. trachomatis* and/or *N. gonorrhoeae* infection, age (OR 3.5, 95% CI 1.6-8.1) and douching (OR 2.1, 95% CI 1.1-4.2) were significantly associated with *M. genitalium* infection. Women with *M. genitalium* only were significantly less likely to be African-American (59.1% vs. 86.0%, p=0.001) than women with *N. gonorrhoeae* and/or *C. trachomatis*.

Conclusion: PID patients who tested positive for *M. genitalium* had some characteristics that are commonly associated with other STDs and PID. The demographic, sexual and behavioral characteristics of *M. genitalium* positive women were generally similar to women with chlamydial and/or gonococcal-PID.

3.2 INTRODUCTION

Pelvic inflammatory disease (PID) is the inflammation of the female upper genital tract caused by ascension of organisms from the lower genital tract. PID is a polymicrobial condition and in many studies only 30% to 50% of PID cases have been associated with the sexually transmitted pathogens *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (79, 159, 160). Bacterial vaginosis-associated anaerobes (161) and *Mycoplasma genitalium* (42) have also been identified as an etiologic agents of PID. Since serious sequelae, including recurrent PID, tubal factor infertility (TFI), ectopic pregnancy and chronic pelvic pain frequently follow an episode of PID (158), it is important to identify risk factors associated with the pathogens that can cause PID. An understanding of modifiable risk factors may aid in efforts to reduce the risk of infection and subsequent serious sequelae.

Evidence suggests that *M. genitalium* may be sexually transmitted. First, it has been associated with a history of sexual intercourse (21, 22). Second, the concordance rates among female sexual partners of *M. genitalium* infected males have been high, ranging from 45% to 71% (23, 33, 96, 162). Third, in a study of 19 couples conducted by Hjorth et al, sequence-based typing of *M. genitalium* revealed sexual transmission, for the sequence type found in specimens from the female partner was identical to that found in the male partner in all the couples studied (26).

Given that data suggests *M. genitalium* may be sexually transmitted, the demographic, behavioral and sexual risk profile of women with *M. genitalium* should be similar to women with other bacterial sexually transmitted diseases (STDs). However, few studies have examined the risk factors associated with *M. genitalium* infection. Therefore, we sought to describe the demographic, sexual health and behavioral risk factors of genital tract *M. genitalium* infection identified by polymerase chain reaction (PCR) in a population of women presenting with clinically suspected PID. We hypothesize that women with *M. genitalium* will be more likely to exhibit characteristics associated with other bacterial STDs, such as younger age (59), black race (60-62), history of STDs or PID, young age at sexual debut (47, 48), rare or inconsistent condom use (67), hormonal contraceptive use (66), douching (51-54), and drug,

tobacco or alcohol use (55, 65) than women without *M. genitalium*. We also hypothesize that the risk factors for *M. genitalium* will be similar to risk factors of *C. trachomatis* and *N. gonorrhoeae*.

3.3 METHODS

3.3.1 Study Population

Data from the baseline interview of the PID Evaluation and Clinical Health (PEACH) Study, described in detail elsewhere (169), were analyzed. The PEACH Study evaluated the effectiveness of inpatient versus outpatient treatment for PID in preventing infertility. Between March 1996 and February 1999, non-pregnant women between the ages of 14 and 36 years were recruited from emergency departments and outpatient facilities (OB/GYN clinics, sexually transmitted disease clinics, and private practices) from seven primary (Atlanta, GA; Birmingham, AL; Charleston, SC; Detroit, MI; Philadelphia, PA; Pittsburgh, PA; and Providence, RI) and six secondary sites in the U.S.. Institutional Review Board approval was obtained from each site. Women were eligible to participate if they had clinically suspected PID as defined by: 1) complaints of acute pelvic pain (<30 days); 2) a clinical finding of pelvic tenderness; and 3) evidence of lower genital tract inflammation. Women were excluded from the study if they had severe disease requiring inpatient management, could not tolerate an outpatient regimen due to vomiting, had an allergy to antibiotics, experienced a delivery, abortion or gynecologic surgery within the past 45 days, had a prior hysterectomy, bilateral salpingectomy, or bilateral tubal ligation, had a tubo-ovarian abscess documented by ultrasound or laparoscope and/or had appendicitis, hemorrhagic ovarian cyst or other condition requiring surgery by ultrasound or laparoscopy. Eight hundred thirty one women were enrolled into the PEACH Study. For this analysis, stored cervical and endometrial specimens were tested for *M.*

genitalium as part of a subsequent ancillary study to PEACH, which has been described in detail elsewhere (42). *M. genitalium* PCR assays were available on a subset of 586 women.

3.3.2 Data Collection

Baseline data were collected by trained research staff at each study center using standardized interview, examination, and specimen collection techniques. Information was collected on demographic characteristics, medical, gynecologic and sexual histories, presenting complaints, substance use, current medications and contraception. Cervical and vaginal swabs, endometrial biopsies, and serum and urine samples were obtained from participants.

3.3.3 Detection of *M. genitalium*

Previously collected cervical and endometrial samples stored at -70°C were tested for *M. genitalium* using the MgPa-IMW PCR assay targeting the MgPa gene (170). This assay has an analytical sensitivity of 15 genomes (170) and a high clinical sensitivity and specificity relative to TMA, another *M. genitalium* NAAT assay (28). For all samples testing positive, a repeat MgPa PCR assay was performed using another aliquot of the sample to rule out PCR product contamination or cross-contamination. All samples initially positive were verified as positive in this confirmatory test.

3.3.4 Detection of *N. gonorrhoeae* and *C. trachomatis*

Previously collected and stored baseline cervical and endometrial samples were assessed for *N. gonorrhoeae* by culture and *C. trachomatis* by PCR at a central laboratory, as previously described (79).

3.3.5 Risk Factors

The following are the baseline variables that we evaluated as potential risk factors for *M. genitalium* infection: age, race, marital status, level of education, sexual activity, number of lifetime sexual partners, new sexual partner in the past 4 weeks, history of STDs, history of BV, history of PID, hormonal contraceptive use, condom use, oral sex, anal sex, age at sexual debut, vaginal douching practices, and drug, alcohol use, and tobacco use.

3.3.6 Statistical Methods

The Chi-square and Fischer's exact tests were used to evaluate the risk profile of *M. genitalium* infection. Women with *M. genitalium* only were compared to: 1) women without *M. genitalium*; and 2) women with *N. gonorrhoeae* and/or *C. trachomatis*. Cervical and endometrial results were considered in separate and combined analyses.

The associations between *M. genitalium* genital tract infection and potential risk factors were assessed with univariate and multivariate logistic regression models. Variables for which the p-value was less than 0.20 in univariate analysis were considered for multivariable analysis. We used multivariate logistic models to quantify the risk of having *M. genitalium* for the various risk factors while controlling for other risk factors simultaneously. If multi-collinearity existed among some covariates being considered for model inclusion, one of the collinear variables was removed. The result of such removal is typically to reduce the variance in the model without introducing bias into the model estimates, since the deleted variable contains redundant information. The decision regarding which variable to remove was made on the basis of biological plausibility and strength of association. The model was rerun adjusting for *C. trachomatis* and/or *N. gonorrhoeae* infection. All data were analyzed using SAS version 9.2 for windows.

3.4 RESULTS

Table 13 provides the baseline demographic, sexual health and behavioral characteristics of study participants according to *M. genitalium* PCR result. As risk factors for lower and upper genital tract *M. genitalium* infection were generally similar, only risk factors for combined cervical and/or endometrial *M. genitalium* are presented. Approximately 15% (88/586) of the study participants tested positive for *M. genitalium* in the cervix and/or endometrium. Being younger than 25 years of age (OR 2.3, 95% CI 1.3-4.1), douching 2 or more times per month (OR 1.9, 95% CI 1.2-3.3), and smoking cigarettes (OR 1.9, 95% CI 1.2-3.1) were significantly associated with *M. genitalium* infection. There were non-significant trends towards associations between *M. genitalium* and African-American race (OR 1.4, 95% CI 0.8-2.5), sexual activity (OR 1.6, 95% CI 0.8-3.1), history of BV (OR 0.6, 95% CI 0.3-1.0), rare/occasional condom use (OR 1.4, 95% CI 0.8-2.6), and illicit drug use (OR 1.7, 95% CI 1.0-2.7).

In the multivariate model, being less than 25 years old (AOR 2.8, 95% CI 1.5-5.2) and douching 2 or more times in the previous month (AOR 2.3, 95% CI 1.3-4.0) and smoking (AOR 2.0, 95% CI 1.2-3.3). were independently associated with *M. genitalium* infection (Table 14). After adjusting for *C. trachomatis* and/or *N. gonorrhoeae* infection, age (AOR 3.6, 95% CI 1.6-8.1) and douching (AOR 2.1, 95% CI 1.1-4.0) were significantly associated with *M. genitalium* infection, while the trend towards association between smoking and *M. genitalium* remained (AOR 1.9, 95% CI 1.0-3.4), but was no longer significant.

Among women who tested positive for *M. genitalium,* 22 women did not test positive for *N. gonorrhoeae* or *C. trachomatis.* The demographic, sexual health and behavioral characteristics between women with and without *M. genitalium* were generally no different when analyzed in this subset of women without *N. gonorrhoeae* or *C. trachomatis* (Table 15). Power may have been limited due to the small number of participants in this subgroup analysis.

The baseline characteristics of women testing positive for *M. genitalium* only were generally similar to women with *N. gonorrhoeae* and/or *C. trachomatis* identified in cervical and/or endometrial

specimens. Women with *M. genitalium* only were significantly less likely to be African-American (59.1% vs. 86.0%, p=0.001) than women with *N. gonorrhoeae* and/or *C. trachomatis* (Table 16). However, this was the only characteristic that differed between these two groups.

3.5 DISCUSSION

In our study population of women with mild to moderate clinically suspected PID, compared to women who tested negative for *M. genitalium*, women who tested positive for *M. genitalium* in the cervix and/or endometrium were more likely to have some characteristics and behaviors that are commonly associated with other STDs and PID, including young age, smoking, and vaginal douching. Further, after adjusting for *N. gonorrhoeae* and/or *C trachomatis* co-infection, women with *M. genitalium* were significantly more likely to be less than 25 years of age and douche than women without *M. genitalium*, while the trend towards association between smoking and *M. genitalium* remained but did not reach significance.

Biologically, younger females are more susceptible to bacterial infections than older females due to cervical ectopy. During adolescence, endocervical columnar epithelial cells extend to the vaginal surface, increasing the surface area and increasing the number of receptive cells which may favor the growth of some mucosal pathogens (59), including *M. genitalium*. Younger women may also be at a greater risk for *M. genitalium* because they may be more likely to engage in risky sexual behaviors, such as unprotected intercourse and multiple sexual partners, or have less immunity. Douching can create an environment favorable to facultative aerobes and anaerobes over the usually predominant hydrogen-peroxide-producing lactobacilli (63). Douching can alter the vaginal microflora, remove protective components from the vagina or cervix, and/or promote the ascension of microorganisms from the lower to the upper genital tract, all increasing a woman's susceptibility to infection (64). Smoking is thought to exert a biologic effect on the genital tract via compromised immunity or altered estrogen status (55, 65),

and has been positively associated with PID (55). Further, smoking may mark poor health-seeking behaviors.

To our knowledge, this is the first study to compare the risk factors associated with *M. genitalium* lower and/or upper female genital tract infection to women with other bacterial STDs. As we hypothesized, the characteristics of *M. genitalium* were similar to those of other sexually transmitted bacteria, *N. gonorrhoeae* and *C. trachomatis*. The only characteristic that differed between the two groups of women was race. Only one published study has looked at the risk factors among women with *M. genitalium* upper genital tract infection (41). Cohen et al used PCR to analyze cervical, endometrial, and fallopian tube samples from 123 women presenting at an STD clinic with laparoscopically confirmed salpingitis (41). Age, marital status and median number of sexual partners were not associated with *M. genitalium* infection at any site, while being HIV positive was independently associated with infection (Adjusted Hazard Ratio (AHR) 2.2, 95% CI 1.2-3.7) (41). However, the analyses included women with gonorrhea or chlamydial infections, which may have biased the results.

In our study, *M. genitalium* infection was not associated with all traditional markers of STDs, including sexual activity, number of sexual partners, new sexual partner, history of STDs or PID, hormonal contraception and condom use, oral and anal sex, and age at sexual debut. Our findings are not consistent with other studies that have examined the risk factors for *M. genitalium* infection (14, 22, 68). Manhart et al used data from a subsample of participants aged 18 to 27 years in Wave III of the National Longitudinal Study of Adolescent Health (Add Health) to examine the potential risk factors of *M. genitalium* (22). PCR was used to test the urine of 1714 women and 1218 men for *M. genitalium* (22). *M. genitalium* infection was strongly associated with ever having engaged in vaginal intercourse (Prevalence Ratio (PR) 22.5, 95% CI 4.3-116.6), and in multivariate analyses the prevalence of *M. genitalium* increased by 10% with each additional vaginal intercourse partner in the past year (PR 1.1 per partner in the past year, 95% CI 1.0-1.2) (22). Further, *M. genitalium* was more prevalent in individuals that ever lived with a sexual partner (PR 11.2, 95% CI 3.2-39.5) and in individuals who reported condom use during last sexual intercourse (PR 3.9, 95% CI 1.3-11.5) (22). Huppert et al tested vaginal swabs

from 331 sexually active female adolescents aged 14 to 21 years recruited from inner-city medical center for *M. genitalium* using PCR (68). Sexual intercourse within the last 7 days was associated with a 2-fold increase in the odds of *M. genitalium* infection (OR 2.0, 95% CI 1.1-3.2), after adjusting for *C. trachomatis* (68). Tosh et al used PCR to test vaginal samples from 383 female adolescents aged 14-17 years enrolled in urban primary health care clinics in the U.S. (14). With the exception of one individual, *M. genitalium* was identified exclusively among individuals reporting history of vaginal intercourse and having a recent sexual partner (OR 1.4, 95% CI 1.2-1.7) was independently associated with *M. genitalium* (14). Our study is unique in that we examined the risk factors of lower and/or upper genital tract *M. genitalium* infection. However, all women enrolled in the PEACH study had clinically suspected PID and they may have had homogeneity of risk behaviors, resulting in our null findings. Further, in the PEACH study, *M. genitalium* infection was associated with *C. trachomatis* and/or *N. gonorrhoeae* co-infection (42), which may have biased our results. In fact, approximately two-thirds (43/63, 66.2%) of women who tested positive for *M. genitalium* in the cervix and/or endometrium were also infected with *N. gonorrhoeae* and/or *C trachomatis* in the cervix and/or endometrium (42). Still, after excluding women with co-infections, *M. genitalium* was not significantly associated with these known STD risk factors. However, due to the small number of participants in this subgroup analysis, power may have been limited.

We previously reported that among PEACH participants, women testing positive for *M. genitalium* tended to be less symptomatic than women testing positive for *N. gonorrhoeae* (175). Compared to women with gonococcal PID, women with *M. genitalium* were less likely to present with elevated systemic inflammatory markers, an elevated vaginal pH, and mucopurulent cervicitis and had lower pelvic pain scores. Several other PCR studies have failed to find a strong association between symptoms and *M. genitalium* lower genital tract infection (14, 22, 32). If *M. genitalium* is indeed sexually transmitted, a high number of unrecognized infected individuals may provide the reservoir for spreading *M. genitalium* to others via sexual activity. Further, asymptomatic individuals may not seek treatment for lower genital tract infections, increasing the risk of developing PID and PID-associated

sequelae. Future studies should explore factors associated with asymptomatic *M. genitalium* infections in the general population. The identification of correlates associated with *M. genitalium* will help to identify women at risk for disease acquisition. This may help direct screening programs and ultimately prevent serious reproductive and gynecologic morbidities, including PID and its associated sequelae.

3.6 TABLES

Table 13. Characteristics of study participants and association with *M. genitalium* cervical and/or endometrial infection

Characteristic	*M. genitalium* positive N=88 n (%)[1]	*M. genitalium* negative N=498 n (%)[2]	OR (95% CI)
Demographic			
Age			
< 25 years	71 (18.2)	319 (81.8)	2.3 (1.3-4.1)
≥ 25 years	17 (8.7)	179 (91.3)	
Race/Ethnicity			
African-American	69 (16.2)	358 (83.8)	1.4 (0.8-2.4)
White/Hispanic/Other	19 (11.9)	140 (99.1)	
Marital Status			
Unmarried	71 (14.7)	411 (85.3)	1.4 (0.6-3.1)
Married	7 (11.3)	55 (88.7)	
Education			
< High school	38 (16.7)	189 (83.3)	1.2 (0.8-2.0)
≥ High school	50 (13.9)	308 (86.0)	
Sexual Health			
Sexually active			
Yes	78 (15.8)	415 (84.2)	1.6 (0.8-3.1)
No	10 (10.7)	83 (89.2)	
≥ 2 sexual partners			
Yes	10 (17.9)	46 (82.1)	1.3 (0.6-2.6)
No	78 (14.7)	452 (85.3)	
New sexual partner in last month			
Yes	10 (17.5)	47 (82.5)	1.2 (0.6-2.5)
No	78 (14.7)	451 (85.3)	
History of STD[3]			
Yes	52 (15.0)	295 (85.0)	1.0 (0.6-1.6)
No	35 (15.0)	198 (85.0)	
History of BV			
Yes	13 (10.0)	117 (90.0)	0.6 (0.3-1.0)
No	73 (16.7)	365 (83.3)	

Table 13 continued

History of PID			
Yes	23 (12.9)	155 (87.1)	0.8 (0.5-1.3)
No	64 (15.9)	338(84.1)	
Rare/occasional condom use[4]			
Yes	60 (17.2)	289 (82.8)	1.4 (0.8-2.6)
No	18 (12.5)	126 (87.5)	
Consistent condom use[5]			
Yes	7 (10.9)	57 (89.1)	1.2 (0.5-2.7)
No	71 (16.5)	358 (83.5)	
Oral sex			
Yes	23 (17.6)	108 (82.4)	1.2 (0.7-2.0)
No	63 (15.0)	356 (85.0)	
Anal sex			
Yes	4 (21.1)	15 (78.9)	1.5 (0.5-4.7)
No	84 (14.8)	483 (85.2)	
Age at sexual debut			
≤ 15 years	48 (15.8)	255 (84.2)	1.1 (0.7-1.8)
> 15 years	40 (14.1)	243 (85.9)	
Behavioral			
Vaginal douche ≥ 2 times in past month			
Yes	26 (22.8)	88 (77.8)	1.9 (1.2-3.3)
No	62 (13.1)	410 (86.9)	
Illicit drug use			
Yes	32 (20.4)	125 (79.6)	1.7 (1.0-2.7)
No	56 (13.1)	370 (86.9)	
Current smoker			
Yes	49 (20.2)	194 (79.8)	1.9 (1.2-3.1)
No	39 (11.5)	301 (88.5)	
Alcohol use			
Yes	48 (14.8)	277 (85.2)	0.9 (0.6-1.5)
No	40 (15.5)	218 (84.5)	
Alcohol drinks per week			
> 7 drinks	13 (19.1)	55 (80.9)	1.4 (0.7-2.7)
≤ 7 drinks	75 (14.6)	440 (85.4)	

***** Missing observations: marital status, n=42; education, n=1; history of STD, n=6; history of BV, n=18; history of PID, n=6; hormonal contraception use, n=93; condom use, n=93; oral sex, n=36; drug use, n=3; smoking, n=3, alcohol use, n=3.

[1] % of total study population with characteristic that tested positive for *M. genitalium*
[2] % of total study population with characteristic that tested negative for *M. genitalium*
[3] History of *N. gonorrhoeae*, *C. trachomatis*, or *Trichonomas vaginalis*
[4] Condoms used 0 to 5 out of 10 sexual encounters
[5] Condoms used 10 out of 10 sexual encounters

Table 14. Multivariate model of cervical and/or endometrial *M. genitalium* infection

Characteristic	AOR (95% CI)	AOR[1] (95% CI)
Demographic		
Age < 25 years	2.8 (1.5-5.2)	3.6 (1.6-8.1)
Black race	1.4 (0.8-2.6)	1.2 (0.6-2.4)
Sexual Health		
History of BV	0.5 (0.2-1.0)	0.5 (0.2-1.2)
Rare/occasional condom use[2]	1.4 (0.8-2.6)	1.1 (0.6-2.2)
Behavioral		
Vaginal douche $\geq$ 2 times in past month	2.3 (1.3-4.1)	2.1 (1.1-4.0)
Smoking	2.0 (1.2-3.3)	1.9 (1.0-3.4)

[1] Additionally adjusted for *C. trachomatis* and/or *N. gonorrhoeae* co-infection
[2] Condoms used 0 to 5 out of 10 sexual encounters

Table 15. Characteristics of study participants and association with *M. genitalium* cervical and/or endometrial infection among women without *C. trachomatis* or *N. gonorrhoeae* cervical and/or endometrial infection

Characteristic	*M. genitalium* positive N=22 n (%)[1]	*M. genitalium* negative N=263 n (%)[2]	OR (95% CI)
Demographic			
Age			
< 25 years	17 (10.7)	142 (89.3)	2.9 (1.0-8.1)
≥ 25 years	5 (3.9)	121 (96.0)	
Race/Ethnicity			
African-American	13 (7.5)	160 (92.5)	0.9 (0.4-2.2)
White/Hispanic/Other	9 (8.0)	103 (92.0)	
Marital Status			
Unmarried	20 (8.6)	213 (91.4)	3.9 (0.5-30.2)
Married	1 (2.3)	42 (97.7)	
Education			
< High school	7 (7.6)	85 (92.4)	1.0 (0.4-2.5)
≥ High school	15 (7.8)	178 (92.2)	
Sexual Health			
Sexually active			
Yes	19 (8.1)	216 (91.9)	1.4 (0.4-4.8)
No	3 (6.0)	47 (94.0)	
≥ 2 sexual partners			
Yes	3 (14.3)	18 (85.7)	2.1 (0.6-7.9)
No	19 (7.2)	245 (92.8)	
New sexual partner in past month			
Yes	4 (14.8)	23 (85.2)	2.3 (0.7-7.4)
No	18 (7.0)	240 (93.0)	
History of STD[3]			
Yes	12 (7.5)	147 (92.5)	0.9 (0.4-2.2)
No	10 (8.3)	111 (91.7)	
History of BV			
Yes	5 (6.6)	71 (93.4)	0.8 (0.3-2.1)
No	17 (8.3)	187 (91.7)	
History of PID			
Yes	7 (8.4)	76 (91.6)	1.1 (0.4-2.9)
No	15 (7.5)	185 (92.5)	
Hormonal contraception use			
Yes	4 (7.5)	49 (92.5)	0.9 (0.3-2.9)
No	15 (8.2)	167 (91.8)	

Table 15 continued

Rare/occasional condom use[4]			
Yes	15 (8.7)	158 (91.3)	1.4 (0.4-4.3)
No	4 (6.4)	58 (93.6)	
Consistent condom use[5]			
Yes	1 (3.2)	30 (96.8)	1.1 (0.2-5.1)
No	18 (8.8)	186 (91.2)	
Oral sex			
Yes	5 (8.2)	56 (91.8)	1.0 (0.4-2.9)
No	16 (8.0)	185 (92.0)	
Anal sex			
Yes	1 (16.7)	5 (83.3)	2.5 (0.3-2.6)
No	21 (7.5)	258 (92.5)	
Age at sexual debut			
$\leq$ 15 years	11 (8.0)	126 (92.0)	1.1 (0.5-2.6)
> 15 years	11 (7.4)	137 (92.6)	
Behavioral			
Vaginal douche $\geq$ 2 times in past month			
Yes	6 (13.0)	40 (87.0)	2.1 (0.8-5.7)
No	16 (6.7)	223 (93.3)	
Illicit drug use			
Yes	7 (11.1)	56 (88.9)	1.7 (0.7-4.4)
No	15 (6.8)	205 (93.2)	
Current smoker			
Yes	9 (8.1)	102 (91.9)	1.1 (0.4-2.6)
No	13 (7.5)	160 (92.5)	
Alcohol use			
Yes	14 (8.5)	151 (91.5)	1.3 (0.5-3.2)
No	8 (6.7)	111 (93.3)	
Alcohol drinks per week			
> 7 drinks	4 (12.9)	27 (87.1)	1.9 (0.6-6.1)
$\leq$ 7 drinks	18 (7.1)	235 (92.9)	

***** Missing observations: marital status, n=9; history of STD, n=5; history of BV, n=5; history of PID, n=2; hormonal contraception use, n=50; condom use, n=50; oral sex, n=23; drug use, n=2; alcohol use, n=1.

[1] % of total study population with characteristic that tested positive for *M. genitalium*
[2] % of total study population with characteristic that tested negative for *M. genitalium*
[3] History of *N. gonorrhoeae*, *C. trachomatis*, or *Trichonomas vaginalis*
[4] Condoms used 0 to 5 out of 10 sexual encounters
[5] Condoms used 10 out of 10 sexual encounters

Table 16. Characteristics of women with *M. genitalium* only identified in the cervix and/or endometrium compared to women with *N. gonorrhoeae* and/or *C. trachomatis* in the cervix and/or endometrium

Characteristic	*M. genitalium* positive n=22 n (%)	*N. gonorrhoeae* and/or *C. trachomatis* positive n=172 n (%)	p-value
Demographic			
Age < 25 years	17 (77.3)	132 (76.7)	0.96
African-American race/ethnicity	13 (59.1)	148 (86.0)	0.001
Unmarried	20 (95.2)	151 (95.0)	1.00
< High school education	7 (31.8)	82 (47.7)	0.16
Sexual History			
Sexually active	19 (86.4)	146 (84.9)	0.85
≥ 2 sexual partners	3 (13.6)	24 (13.9)	1.00
New sexual partner in past month	4 (18.2)	17 (9.9)	0.27
History of STD[1]	12 (54.4)	108 (62.8)	0.45
History of *N. gonorrhoeae* or *C. trachomatis*	11 (50.0)	92 (54.4)	0.69
History of BV	5 (22.7)	32 (19.0)	0.68
History of PID	7 (31.8)	50 (29.2)	0.80
Hormonal contraception use	4 (21.0)	30 (20.5)	1.00
Rare/occasional condom use[2]	15 (78.9)	101 (69.2)	0.38
Consistent condom use[3]	1 (5.3)	19 (13.0)	0.47
Oral sex	5 (23.8)	32 (19.5)	0.58
Anal sex	1 (4.5)	7 (4.07)	1.00
Age at sexual debut ≤15 years	11 (50.0)	94 (54.6)	0.68
Behavioral			
Vaginal douche ≥2 times in past month	6 (27.3)	4 (24.4)	0.77
Illicit drug use	7 (31.8)	50 (29.1)	0.79
Current smoker	9 (40.9)	74 (43.2)	0.85
Alcohol use	14 (63.6)	98 (57.0)	0.55
Alcohol use, > 7 drinks/week	4 (18.2)	24 (13.9)	0.53

[1] History of *N. gonorrhoeae*, *C. trachomatis*, or *Trichonomas vaginalis*
[2] Condoms used 0 to 5 out of 10 sexual encounters
[3] Condoms used 10 out of 10 sexual encounters

4.0 MANUSCRIPT 3: NEITHER *MYCOPLASMA GENITALIUM* NOR *CHLAMYDIA TRACHOMATIS* IN EARLY PREGNANCY ARE ASSOCIATED WITH SPONTANEOUS ABORTION AMONG YOUNG WOMEN RECRUITED FROM AN URBAN EMERGENCY DEPARTMENT

Manuscript in preparation

Vanessa L. Short, MPH[1], Jørgen S. Jensen, MD, PhD, DMedSci[2], Deborah B. Nelson, PhD[3], Pamela Murray, M.D., M.P.H.[4], Roberta B. Ness, MD, MPH[5], Catherine L. Haggerty, PhD, MPH[1]

[1]Deparment of Epidemiology, University of Pittsburgh, Pittsburgh, PA, [2]Statens Serum Institut, Copenhagen, Denmark, [3]Temple University, Philadelphia, PA, [4]Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA, [5]The University of Texas School of Public Health, Houston, TX

Funding Support: R01 HD36918-04 from the National Institute of Child Health and Human Development

4.1 ABSTRACT

Objectives: Spontaneous abortion (SAB), the loss of a conceptus prior to 20 weeks, is the most common adverse outcome of pregnancy. As genital tract infections may cause SABs and the prevalence and consequences of the sexually transmitted pathogen *Mycoplasma genitalium* in pregnant women is unknown, we sought to examine the relationship between prenatal *M. genitalium* infection and SAB.

Methods: We conducted a nested case-control study among predominately young, African-American pregnant women recruited from an urban emergency department. *M. genitalium* was measured by polymerase chain reaction (PCR) in urine collected at enrollment from 82 women who subsequently experienced a SAB and 134 control women who maintained their pregnancies past 22 weeks gestation. Clinical, demographic and behavioral characteristics of cases and controls were compared. The relationship between *M. genitalium* and subsequent SAB was evaluated, adjusting for age, smoking, *Chlamydia trachomatis* infection, and previous SAB.

Results: The prevalence of *M. genitalium* at enrollment was only slightly less than that of *C. trachomatis* (5.6% vs. 6.9%). Compared to women testing negative for *M. genitalium*, *M. genitalium* positive women were more likely to report a history of other STDs (100% vs. 59.6%, p=0.008). After adjusting for *C. trachomatis* PCR, *M. genitalium* was associated with nulliparity (AOR 3.4, 95% CI, 1.0-11.6), history of pelvic inflammatory disease (PID) (AOR 3.9, 95% CI 0.9-16.1), prior *C. trachomatis* infection (AOR 3.0, 95% CI 0.8-10.5) and problems getting pregnant (AOR 4.8, 95% CI 0.9-25.7), although not all relationships were statistically significant. Neither *M. genitalium* (AOR 0.6, 95% CI 0.1-2.3) nor *C. trachomatis* (AOR 0.4 95% CI 0.1-1.6) were independently associated with SAB. Results did not differ after excluding women with vaginal bleeding at enrollment (*M. genitalium* OR 0.4, 95% CI 0.1-3.8; *C. trachomatis* OR 1.2, 95% CI 0.3-5.1).

Conclusions: Out study suggests that pregnant women with positive urine PCR for *M. genitalium* do not have an increased risk of SAB, but report a history of gynecologic and reproductive morbidities, including other STDs and PID.

4.2 INTRODUCTION

Spontaneous abortion (SAB), the loss of a conceptus prior to 20 weeks, is the most common adverse outcome of pregnancy, occurring in an estimated 15% of clinically recognized pregnancies (117) and up to 50% of all pregnancies (118). Many SABs occurring in the first trimester are due to phenotypic and/or chromosomal abnormalities, while environmental factors may have a greater impact on SABs occurring in later pregnancy (118). Evidence suggests that sexually transmitted bacterial and viral infections play a role in adverse pregnancy outcomes. Syphilis, an infection caused by the spirochaete *Treponema pallidum*, is a recognized cause of SAB (176, 177). Bacterial vaginosis (BV) in early pregnancy has been associated with a 2 to 5 fold increased risk of subsequent SAB (99, 128, 144-148), but others studies have not supported this association (120, 140-143). The association between *Chlamydia trachomatis* and SAB has not been consistently reported.

Mycoplasma genitalium, a sexually transmitted bacterium, has been linked to various adverse gynecologic and reproductive events. It has been implicated as an etiological agent of cervicitis (33, 34, 37, 69) and pelvic inflammatory disease (PID) (40, 42, 43, 104), independent of *C. trachomatis* and *Neisseria gonorrhoeae*. Further, *M. genitalium* has been detected in cervical and salpingeal samples from women with laparoscopically confirmed salpingitis (41) and in cervical and endometrial specimens from women with histologically confirmed endometritis (108, 178). A serologic relationship between *M. genitalium* and tubal factor infertility has also been identified (113, 114).

Because *M. genitalium* has been associated with these morbidities, it is plausible that *M. genitalium* can infect the upper genital tract during pregnancy, resulting in adverse pregnancy outcomes. Data on *M. genitalium* in pregnancy are sparse, although a handful of studies among low-risk women have reported no relationship between *M. genitalium* and SAB (99, 116). We conducted a nested case-

control study to examine the role of *M. genitalium* in early pregnancy and subsequent SAB among predominantly young, single, African-American women with a history of STDs.

4.3 METHODS

4.3.1 Study Population

For our analyses, data from participants enrolled in the prospective Early Pregnancy Study (EPS), designed to examine the influence of violence on SAB risk, were used. During the period of January 1999 through August 2001, adolescent girls and women aged 14 to 40 years who presented to the emergency department (ED) of the Hospital of the University of Pennsylvania and who resided in selected Philadelphia zip codes were screened for eligibility. Nurse interviewers administered a brief screening questionnaire to identify all pregnant women less than 22 weeks gestation seen in the ED regardless of the reason for the visit. Among the 1,249 pregnant adolescent and adult women who were eligible for the study, 96% agreed to participate (n =1,199). The mean gestational age at enrollment was 10.3 weeks. All participants provided written informed consent, and the protocol and consent forms were approved by the institutional review board of the University of Pennsylvania.

4.3.2 Data Collection

At enrollment, nurse interviewers administered an in-person questionnaire. The interview contained information regarding medical and reproductive history, sociodemographic factors, current level of social support, living arrangements, and complications of the pregnancy. Hair samples were collected to measure cocaine use, and urine samples were collected to assess recent tobacco and alcohol use. Follow-

up telephone interviews were conducted at 16 and 22 weeks of gestation to determine pregnancy status and to collect additional information. Medical record reviews were conducted to confirm pregnancy outcome, which was obtained for 93% of participants. The 807 women who remained pregnant through 22 weeks of gestation were classified as controls (67%), and the 392 women who experienced a non-induced pregnancy loss before 22 weeks of gestation were classified as cases (33%). Of the cases, 212 experienced a SAB at enrollment and 180 experienced a SAB during the follow-up period. For our subsequent EPS sub-analyses, women who were experiencing a SAB at enrollment and women who did not have a urine sample available for analysis were excluded. The total sample for this sub-analysis was 82 cases and 132 controls.

4.3.3 Detection of *M. genitalium* and *C. trachomatis*

Previously collected urine samples stored at -70°C were tested for *M. genitalium* and *C. trachomatis*. *M. genitalium* was tested for using a PCR with primers deduced from the 16 S rRNA gene sequence of *M. genitalium* (179). All positive results were confirmed by a second PCR amplifying a part of the *M. genitalium* MgPa adhesion gene with primers MgPa-1 and MgPa-3 as previously described (179, 180). The methods for the PCR detection of *M. genitalium* in urine have been previously validated (181). *C. trachomatis* DNA was detected in the urine samples by amplification of a sequence of the cryptic plasmid using primers CP24 (5′GGG ATT CCT GTA ACA ACA AGT CAG G) and CP27 (5′CCT CTT CCC CAG AAC AAT AAG AAC AC) (182). Positive results were confirmed with an inhibitor-controlled PCR detecting the 16S rRNA gene of chlamydia species modified from Pollard et al (183, 184).

4.3.4 Microbiologic, demographic, current pregnancy and reproductive health history variables

The following is a list of microbiologic, demographic, current pregnancy, and reproductive health history variables that were compared between SAB cases and pregnant controls: *M. genitalium* and *C.*

trachomatis infection detected by PCR, age, race, level of education, marital status, recipient of government assistance, gestational age at enrollment, vaginal bleeding during pregnancy, *N. gonorrhoeae* or BV infection during pregnancy, parity, history of STDs, history of PID, history of problems getting pregnant, history of prior SAB, prior ectopic pregnancies and alcohol, cigarette, marijuana and crack/cocaine use during current pregnancy. All variables were self-reported during the baseline interview. Cocaine use was additionally measured in hair specimens as previously described (185). Gestational age was calculated on the basis of self-reported date of the last menstrual period.

4.3.5 Statistical Methods

The microbiologic, demographic, current pregnancy and reproductive health history characteristics among cases and controls were compared using t, chi-square, and Fisher's exact tests for continuous and dichotomous variables as appropriate. The same characteristics were compared among women who tested positive for *M. genitalium* and women who did not test positive for *M. genitalium.*

Logistic regression models were used to investigate the relationship between *M. genitalium* urine PCR and SAB. Analyses were repeated controlling for age, history of SAB, smoking, and *C. trachomatis* PCR. Regression models were repeated including only women without a history of SAB, as including the history of SAB variable in the model may bias the relationship toward the null if there is an association between *M. genitalium* and habitual SABs. We also repeated our analyses categorizing SAB by gestational age (<11 weeks, ≥11 weeks), since gestational age of 11 weeks or greater at time of SAB can be used as a marker for pregnancies which are more likely to be chromosomally normal and other factors such as infectious agents are more likely to cause pregnancy loss (118). Finally, we repeated our analyses excluding women who were experienced vaginal bleeding at enrollment, as these women may have already been experiencing a SAB and this may have compromised temporality.

4.4 RESULTS

Among all study participants, 5.6% (12/216) tested positive for *M. genitalium* and 6.9% (15/216) tested positive for *C. trachomatis* at enrollment. Only one woman (0.05%) was co-infected with both bacteria. Participants were predominantly less than 30 years old (82.2%), African-American (89.5%), not married (81.3%) and receiving government assistance at time of enrollment (59.4%). Nearly one-quarter (23.4%) of the participants had cocaine detected in their hair sample and 35.3% smoked cigarettes while pregnant. Most women (81.3%) reported at least one live birth and a history of a prior STD (61.7%), while nearly a third of women had a history of at least one SAB (31.5%).

Compared to control women, cases were significantly more likely to report vaginal bleeding both within 24 hours of the enrollment visit (57.3% vs. 21.8%, $p<0.0001$) and during any time during pregnancy (65.4% vs. 37.3%, $p<0.0001$) (Table 17). Cases were also significantly more likely to report a previous SAB (40.2% vs. 26.1%, $p=0.030$) than controls. These were the only characteristics that differed between cases and controls. Neither *M. genitalium* (AOR 0.6, 95% CI 0.1-2.8) nor *C. trachomatis* (AOR 0.41 95% CI 0.1-1.6) were independently associated with SAB, after adjusting for age, history of SAB, and smoking during pregnancy (Table 18). Results were similar after excluding women who experienced vaginal bleeding at enrollment (*M. genitalium* OR 0.4, 95% CI 0.1-3.8; *C. trachomatis* OR 1.2, 95% CI 0.3-5.1) (Table 19). When history of SAB was removed from the multivariate model, neither *M. genitalium* nor *C. trachomatis* were associated with SAB.

Nearly 28% (22/79) of the cases experienced a SAB at less than 11 weeks gestation. Among these earlier cases, none tested positive for *M. genitalium* and 3% (1/33) tested positive for *C. trachomatis*. Among the cases who experienced a SAB at 11 or greater weeks of gestation, 4.3% (2/46) tested positive for *M. genitalium* and 4.3% (2/46) tested positive for *C. trachomatis*. When stratifying by gestational age of pregnancy loss, neither *M. genitalium* nor *C. trachomatis* were associated with SAB (<11 weeks, *C. trachomatis* OR 0.5, 95% CI 0.1-4.0; $\geq$11 weeks *M. genitalium* OR 0.6, 95% CI 0.1-3.0, *C. trachomatis* OR 0.5, 95% CI 0.7-2.9) (Table 20).

Compared to women without *M. genitalium*, women with *M. genitalium* were significantly more likely to report nulliparity (OR 3.4, 95% CI 1.0-11.3), history of PID (OR 3.9, 95% CI 0.9-16.0), prior *C. trachomatis* infection (OR 3.0, 95% CI 0.9-10.6) and problems getting pregnant (OR 4.9, 95% CI 0.9-25.9) (Table 21). Although results were not statistically significant, trends remained after adjusting for *C. trachomatis* PCR (nulliparity AOR 3.4, 95% CI, 1.0-11.6; history of PID AOR 3.9, 95% CI 0.9-16.1; prior *C. trachomatis* infection AOR 3.0, 95% CI 0.8-10.5; problems getting pregnant AOR 4.8, 95% CI 0.9-25.7) (Table 22). After adjusting for age, nulliparous women were still more likely to test positive for *M. genitalium* than women who did not report nulliparity, but the results did not reach significance (AOR 2.8, 95% CI 0.8-9.8) (results not shown).

4.5 DISCUSSION

The prevalence of *M. genitalium* in our study population (5.6%) was higher than reported in other studies of pregnant women (44, 97, 99). The higher prevalence of *M. genitalium* we report here is not all that surprising, as women in our study population were probably at high risk for STDs in general, as they were predominantly young, single, African-American, of lower socioeconomic status (SES) and reported a history of STDs. Still, we did not find a statistically significant relationship between *M. genitalium* in pregnancy and subsequent SAB. Our null findings are consistent with those of two other studies of *M. genitalium* and SAB. Oakeshott et al used PCR to test urine samples from 915 pregnant women presenting at prenatal care at less than 10 weeks gestation in general practice and family planning clinics in London (99). Only 0.66% of the samples tested positive for *M. genitalium* (99). Women who experienced a subsequent SAB were not more likely to test positive for *M. genitalium* compared to the women who did not experience a SAB (1% vs. 0.6%, p=NS) (99). In a case-control study of 1,014 recently pregnant women in Guinea-Bissau, *M. genitalium* detected by PCR in cervical samples collected

within 24 hours of a SAB or delivery of a term neonate was not associated with SAB (OR 0.61, 95% CI 0.07-2.51) (116).

Similar to *M. genitalium, C. trachomatis* was not associated with SAB in our study population. Results from previous studies assessing the relationship between *C. trachomatis* infections and SAB are inconclusive. Several serology studies found the presence of antibodies to *C. trachomatis* in the sera of women who have experienced repeated pregnancy loss (134, 135), while other studies have failed to find a significant association between *C. trachomatis* antibodies and SAB (136-138). However, given the method of diagnosis used in these studies, women could have a current infection which might be undetected by serology. Nevertheless, few studies using DNA based methods of detection have been conducted to examine the relationship between *C. trachomatis* and SAB and results have been inconsistent. Among women undergoing in vitro fertilization (IVF), Witkin et al found a strong correlation between endocervical *C. trachomatis* and SAB after embryo transfer (p=0.004) (132). Further, *C. trachomatis* was identified in more women who had SABs than women who had term deliveries (27.3% vs. 1.8%) (132). As all women were asymptomatic, the authors concluded that an undetected *C. trachomatis* infection may be responsible for SAB after IVF and embryo transfer (132). Contrary to this, Sozio and Ness did not find a significant relationship between acute lower genital tract *C. trachomatis* infection and SAB in their nested case-control pilot study of 52 women who experienced a SAB and 59 control women who maintained their pregnancies (133). 3.8% (2/52) of cases and 8.5% (5/59) of control women tested positive for *C. trachomatis* in their urine by ligase chain reaction, and women who experienced a SAB were not significantly more likely to have *C. trachomatis* infection than controls (OR 1.8, 95% CI 0.3-10.7) (133). Thus, the role of *C. trachomatis* in SAB remains unexplained.

In our study, the clinical, demographic and behavioral characteristics of pregnant women who tested positive for *M. genitalium* in early pregnancy and women who did not were similar. Our results are similar to those reported by Labbe et al, in which *M. genitalium* was not associated with any demographic, behavioral, clinical or laboratory characteristic, including age, age at sexual debut, number of sexual partners, previous pregnancies, syphilis, HIV serology, *N. gonorrhoeae* and *T. vaginalis*

(p>0.05 for all) (116). Conversely, Oakshott et al found that *M. genitalium* was more common in pregnant women who were less than 20 years of age (p=0.002), black (p=0.034), single (p=0.017) and had lower SES (p=0.007). Our findings were likely due to homogeneity of women, which may have biased our results toward the null.

Although *M. genitalium* was not associated with SAB or demographic and behavioral characteristics in this study, *M. genitalium* was associated with a history of other gynecologic and reproductive morbidities, including history of PID, prior chlamydial infection and problems getting pregnant. Women who tested positive for *M. genitalium* were also more likely to report nulliparity, which may indicate problems getting pregnant. Further, our results suggest an independent relationship between *M. genitalium* and these conditions, as the results remained the same even after adjusting for *C. trachomatis* PCR. Our results are consistent with other studies that have identified *M. genitalium* as a possible etiologic agent of other adverse reproductive conditions, such as non-gonococcal/non-chlamydial PID (42, 43, 69, 108, 178) and PID-associated sequelae (42), including tubal factor infertility (113, 114). It may be that *M. genitalium* was not associated with SAB in our study population of pregnant women because women with *M. genitalium* are more likely to develop PID and sub-fertility and would therefore not be enrolled in a pregnancy related study. Future prospectively designed studies should examine the long-term sequelae of *M. genitalium* infections among pregnant and non-pregnant women.

There are a few limitations to recognize as part of this study. First, the small sample sizes of some of our subgroups limited statistical power. Second, the mean gestational age at enrollment was approximately 10 weeks, so earlier SABs would have been missed. Third, only archived urine samples were available for *M. genitalium* testing in this sub-study, and this may have limited our analyses. The optimal specimen type used for *M. genitalium* PCR is unknown and has not been thoroughly assessed. Jensen et al compared the efficacy of first void urine (FVU) with cervical and urethral swab specimens for detection of *M. genitalium* using the in-house inhibitor-controlled PCR assays used in our study and found that urine samples were the most sensitive (31). More infections were detected using urine samples (88%) than cervical (71%) and urethral swab specimens (57%), and the urine specimen was significantly

more efficient than both the cervical (p=0.049) and the urethral swab specimen (p=0.0009) (31). However, for optimal sensitivity, urine should be supplemented with a cervical specimen (31). In our study, cervical PCR may have increased the sensitivity and detected more *M. genitalium* positive women. However, cervical samples were not available for testing. Therefore, the reported prevalence of *M. genitalium* in our study population may be an underestimate of the true prevalence in our study population. The null relationship between *M. genitalium* urine PCR and SAB in our study may further be explained by the fact that some women did not have upper genital tract infection, but instead had only urinary or lower genital tract infection, insufficient to cause SAB.

In conclusion, this study is one of only a few to examine the role of *M. genitalium* in early pregnancy and subsequent SAB. Among this population of mostly young, single, African-American pregnant women, a positive urine PCR for *M. genitalium* or *C. trachomatis* did not predict SAB. However, trends between *M. genitalium* and a history of gynecologic and reproductive morbidities, including other STDs, PID, and problems getting pregnant were evident, even after adjusting for concurrent *C. trachomatis* infection.

4.6 TABLES

Table 17. Characteristics of women who experienced a SAB and women who maintained their pregnancies past 22 weeks gestation

Characteristic	Women who experienced a SAB n=82 n (%)	Women who maintained their pregnancies n=134 n (%)	p-value
Infection			
M. genitalium (n=12)	3 (3.7)	9 (6.7)	0.54
C. trachomatis (n=15)	3 (3.7)	12 (9.0)	0.17
M. genitalium and *C. trachomatis* (n=1)	0	1 (0.7)	1.00
Demographic			
Mean age (standard deviation)	24.8 (6.2)	23.9 (5.7)	0.33
Age			
14-19 years	17 (20.7)	28 (20.9)	0.98
20-29 years	50 (61.0)	83 (61.9)	
30-40 years	15 (18.3)	23 (17.2)	
Race/Ethnicity			
African-American	72 (88.9)	120 (89.6)	0.89
Other	9 (11.1)	14 (10.4)	
Marital Status			
Single	68 (82.9)	107 (79.8)	0.57
Married	14 (17.1)	27 (20.2)	
Education			
< High school	25 (30.5)	50 (37.3)	0.31
≥ High school	57 (69.5)	84 (62.7)	
Receiving government assistance	31 (37.8)	58 (43.3)	0.43
Current Pregnancy History			
Mean gestational age[1] (standard deviation)	8.7 (3.2)	9.5 (3.4)	0.08
Vaginal bleeding at enrollment	47 (57.3)	29 (21.8)	<0.0001
Vaginal bleeding since LMP	53 (65.4)	50 (37.3)	<0.0001
Alcohol use	30 (36.6)	44 (32.8)	0.57
Cigarette smoking	31 (38.3)	44 (32.8)	0.42
Marijuana use	17 (21.0)	25 (18.7)	0.68

Table 17 continued

Crack/cocaine use[2]	16 (25.4)	21 (22.8)	0.71
N. gonorrhoeae	1 (1.2)	0	0.38
Bacterial vaginosis	3 (3.7)	6 (4.5)	1.00
Reproductive health history			
Nulliparous	14 (17.1)	27 (20.1)	0.58
Prior SAB	34 (40.2)	35 (26.1)	0.03
Prior ectopic pregnancy	5 (7.3)	6 (5.6)	0.75
History of STD[3]	48 (60.0)	82 (62.1)	0.76
History of PID	6 (7.4)	15 (11.3)	0.36
History of problems getting pregnant	5 (6.3)	6 (4.5)	0.75

* Missing observations: race, n=1; vaginal bleeding, n=1; cigarette smoking, n=1; marijuana use, n=1; crack/cocaine use, n=61; *N. gonorrhoeae,* n=2; bacterial vaginosis, n=3; prior incompetent cervix, n=2; prior ectopic pregnancy, n=41; history of STD, n=5; history of PID, n=3; history of problems getting pregnant, n=2

[1] Gestational age at enrollment in weeks
[2] Detected in hair sample collected at enrollment and/or self-report use
[3] *C. trachomatis*, *N. gonorrhoeae*, syphilis, genital warts, *Trichomonas vaginalis*, and/or BV

Table 18. Odds of spontaneous abortion according to infection at enrollment

Infection	Women who experienced a SAB n=82 n (%)	Women who maintained their pregnancies n=134 n (%)	OR (95% CI)	AOR (95% CI)[1]
M. genitalium				
Yes	3 (3.7)	9 (6.7)	0.5 (0.1-2.0)	0.6 (0.1-2.5)
No	79 (6.3)	125 (93.3)		
C. trachomatis				
Yes	3 (3.7)	12 (9.0)	0.4 (0.1-1.4)	0.4 (0.1-1.6)
No	79 (6.3)	122 (91.0)		

[1] Adjusted for age, history of SAB, smoking, and *C. trachomatis* or *M. genitalium*

Table 19. Odds of spontaneous abortion according to infection at enrollment, among women who did not report vaginal bleeding at enrollment

	Women who did not report vaginal bleeding at enrollment N=139		
Infection	**Cases N=35 n(%)**	**Controls N=104 n(%)**	**AOR (95% CI)[1]**
M. genitalium			
Yes	1 (2.9)	7 (6.7)	0.4 (0.1-3.8)
No	34 (97.1)	97 (93.3)	
C. trachomatis			
Yes	3 (8.6)	8 (7.7)	1.2 (0.3-5.1)
No	32 (91.4)	96 (92.3)	

[1] Adjusted for age, history of SAB, smoking, and *C. trachomatis* or *M. genitalium*

Table 20. Odds of spontaneous abortion according to infection at enrollment, stratified by gestational age at spontaneous abortion

Infection	Cases who experienced a SAB ≥11 weeks N=46 n(%)	Controls N=134 n(%)	AOR (95% CI)[1]	Cases who experienced a SAB <11 weeks N=33 n(%)	Controls N=134 n(%)	AOR (95% CI)[1]
M. genitalium						
Yes	2 (4.3)	9 (6.7)	0.6 (0.1-3.0)	0 (0)	9 (6.7)	---
No	44 (95.7)	125 (93.3)		33 (100)	125 (93.3)	
C. trachomatis						
Yes	2 (4.3)	12 (9.0)	0.5 (0.7-2.9)	1 (3.0)	12 (9.0)	0.5 (0.1-4.0)
No	44 (95.7)	122 (91.0)		32 (97.0)	122 (91.0)	

[1] Adjusted for age, history of SAB, smoking, and *C. trachomatis* or *M. genitalium*

Table 21. Characteristics of all study participants according to *M. genitalium* PCR result

Characteristic	*M. genitalium* positive n=12 n (%)	*M. genitalium* negative n=207 n (%)	OR (95% CI)
Co-infection			
C. trachomatis			
Yes	1 (8.3)	14 (6.8)	1.3 (0.2-10.4)
No	11 (91.7)	193 (93.2)	
Demographic			
Age			
< 25 years	10 (83.3)	133 (64.2)	2.8 (0.6-13.0)
≥ 25 years	2 (16.7)	74 (35.8)	
Race/Ethnicity			
African American	11 (91.7)	184 (89.3)	1.3 (0.2-10.7)
White/Other	1 (8.3)	22 (10.7)	
Marital Status			
Unmarried	12 (100)	166 (80.2)	---
Married	0	41 (19.8)	
Education			
< High school	5 (41.7)	72 (34.8)	0.7 (0.2-2.4)
≥ High school	7 (58.3)	135 (65.2)	
Current Pregnancy History		9.2 (3.3)	
Mean gestational age[1] (standard deviation)	9.5 (2.9)		---[4]
Vaginal bleeding at enrollment		74 (35.9)	
Yes	4 (33.3)	132 (64.1)	0.9 (0.3-3.1)
No	8 (66.7)		
Vaginal bleeding since LMP			
Yes	5 (41.7)	101 (49.0)	0.7 (0.2-2.4)
No	7 (58.3)	105 (51.0)	
Alcohol use			
Yes	5 (41.7)	70 (33.8)	1.4 (0.4-4.6)
No	7 (58.3)	137 (66.2)	
Cigarette use			
Yes	6 (50.0)	71/206 (34.5)	1.9 (0.6-6.1)
No	6 (50.)	135/206 (65.5)	
Marijuana use			
Yes	4 (33.3)	40/206 (19.4)	2.1 (0.6-7.2)
No	8 (66.7)	166/206 (80.6)	
Crack/cocaine use[2]			
Yes	2 (20.0)	36 (24.3)	0.8 (0.2-3.8)
No	8 (80.0)	112 (75.1)	

Table 21 continued

N. gonorrhoeae			
Yes	0 (0)	1 (0.5)	---
No	11 (100)	205 (99.5)	
Bacterial vaginosis			---
Yes	0 (0)	9 (4.4)	
No	11 (100)	197 (95.6)	
Reproductive Health History			
Nulliparous			
Yes	5 (41.7)	36 (17.4)	3.4 (1.0-11.3)
No	7 (58.3)	171 (82.6)	
History of SAB			
Yes	1 (8.3)	68 (32.8)	0.2 (0.02-1.5)
No	11 (91.7)	139 (67.2)	
Prior ectopic pregnancy			
Yes	0 (0)	11 (6.4)	---
No	7 (100)	160 (93.6)	
History of STD[3]			
Yes	11 (100)	121 (59.6)	---
No	0 (0)	82 (40.4)	
History of *C. trachomatis*			
Yes	7 (63.6)	76 (36.9)	3.0 (0.9-10.6)
No	4 (36.4)	130 (63.1)	
History of PID			
Yes	3 (27.3)	18 (8.8)	3.9 (0.9-16.0)
No	7 (72.7)	187 (91.2)	
History of problems getting pregnant			
Yes	2 (18.2)	9 (4.4)	4.9 (0.9-25.9)
No	9 (81.8)	197 (95.6)	

* Missing observations: race, n=1; vaginal bleeding, n=1; cigarette smoking, n=1; marijuana use, n=1; crack/cocaine use, n=61; *N. gonorrhoeae,* n=2; BV, n=3; prior incompetent cervix, n=2; prior ectopic pregnancy, n=41; history of STD, n=5; history of PID, n=3; history of problems getting pregnant, n=2

[1] Gestational age in weeks
[2] Detected in hair sample collected at enrollment and/or self-reported use
[3] *C. trachomatis*, *N. gonorrhoeae*, syphilis, genital warts, *Trichomonas vaginalis*, and/or BV
[4] p-value=0.78

Table 22. Odds of *M. genitalium* infection adjusted for *C. trachomatis* PCR

Characteristic	**AOR (95% CI)**
Nulliparous	3.4 (1.0-11.6)
History of *C. trachomatis*	3.0 (0.8-10.5)
History of PID	3.9 (0.9-16.1)
History of problems getting pregnant	4.8 (0.9-25.7)

5.0 CONCLUSION

In this study we used data from 2 cohorts of women at high risk of STDs and were able to examine three important features of *M. genitalium* infections in women, including: 1) the clinical characteristics; 2) the sociodemographic, sexual and behavioral correlates; and 3) the association with spontaneous abortion. The results from our study add to the growing literature on *M. genitalium* and suggest that women with *M. genitalium* present with few symptoms, have risk factors commonly associated with other STDs, are not at an increased risk of SAB, but report a history of reproductive morbidities.

Our findings suggest that, among women with PID, women with *M. genitalium* do not present with strong clinical signs or symptoms and this symptomology is similar to that of *C. trachomatis*. On the other hand, women with *M. genitalium* presented with fewer symptoms than women with gonococcal-PID and had lower pelvic pain scores on average. As symptoms may be mild, women with *M. genitalium* may not seek PID treatment. Delayed care of PID increases the risk of developing serious sequelae that often follows an episode of PID (186). Therefore, identifying the risk factors associated with *M. genitalium* can help identify women at risk for infection or women that may have a less symptomatic infection. In the PEACH cohort, *M. genitalium* cervical and/or endometrial infection was associated with young age, smoking and douching; variables that have been associated with other STDs and PID in previous studies. Further, the demographic, sexual health and behavioral characteristics of women with *M. genitalium* were similar to women with *C. trachomatis* and/or *N. gonorrhoeae*.

While the relationship between reproductive morbidities and *M. genitalium* is becoming better understood, the effects of *M. genitalium* in pregnancy have not been extensively studied. In the EPS cohort of predominately young, unmarried, African-American pregnant women, *M. genitalium* did not

increase the risk of SAB, but was associated with several serious reproductive morbidities, including previous STDs, PID and problems getting pregnant. Interestingly, the trends towards association remained after adjusting for *C. trachomatis*. However, the cross-sectional design of our study and the small sample sizes of some of our sub-groups may have limited our findings. Still, our findings support the need for further research on *M. genitalium.*

5.1 FUTURE RESEARCH

In our two study populations, *M. genitalium* was as prevalent as other STDs. The relatively high prevalence of *M. genitalium* infection in these populations, along with its association with reproductive morbidities, raises important concerns about the importance of *M. genitalium* as a significant sexually transmitted infection. Further, as *M. genitalium* was strongly associated with having a concurrent *C. trachomatis* and/or *N. gonorrhoeae* infection, questions arise regarding the transmission of *M. genitalium*. That is, is *M. genitalium* an opportunistic organism, or does it pave the way for other STDs? If it is indeed a pathogenic organism, then understanding the consequences of infection is important. The long-term sequelae of *M. genitalium* infection have been alluded to in previous studies that showed an association between *M. genitalium,* PID and tubal factor infertility, however, as these findings come from cross-sectional analyses of high-risk populations, future prospectively designed studies with larger sample sizes and appropriately chosen control groups are needed. Such studies should further explore: 1) *M. genitalium* infection in asymptomatic individuals and the general population; 2) the relationship between *M. genitalium* and other STDs; 3) factors related to the persistence of *M. genitalium* infection; and 4) the long-term reproductive sequelae associated with persistent *M. genitalium* genital tract infections. Such investigations should help explain factors associated with transmission of *M. genitalium* and the pathogenecity of this organism. Still, even if *M. genitalium* is pathogenic, effective treatment options are

not yet available for women with *M. genitalium* PID. Therefore, future randomized, controlled clinical trials should be designed to assess effective treatment options. The development of commercially available assay for *M. genitalium* detection is also needed in order to be able to screen those at risk for *M. genitalium* and to study *M. genitalium* outside of structured research settings.

5.2 APPLICATION TO PUBLIC HEALTH

Sexually transmitted diseases are prevalent, serious public health issues. In fact, according to the CDC, the two most commonly-reported notifiable diseases (chlamydia and gonorrhea) in the U.S. are sexually transmitted (60). STDs are a huge burden to the national health care system, costing an estimated $15.3 billion annually (60).

Women are more commonly infected with STDs and disproportionately suffer from the consequences of STDs compared to men (60). In women, STDs can cause PID, a significant public health issue in the U.S. and worldwide. PID is a common, costly condition, affecting nearly 8% of American women at some time in their lives. Each year in the U.S. an estimated 1 million women are treated for PID. Adverse sequelae, including tubal factor infertility, chronic pelvic pain, recurrent PID and ectopic pregnancy can occur if PID treatment is delayed or avoided. Although PID is a polymicrobial disorder, non-gonococcal/non-chlamydial PID has not been studied extensively. Therefore, studies of other possible organisms involved in PID are essential in order to decrease the incidence of this condition.

M. genitalium has been identified as a possible etiologic agent of non-gonococcal/non-chlamydial PID (40, 42, 43, 104), and our study increased the understanding of *M. genitalium* PID. We showed that *M. genitalium* infections tend not to produce strong symptoms in women with clinically suspected PID. We were also able to identify correlates associated with *M. genitalium* infections in women with PID. Due to the asymptomatic nature, *M. genitalium* lower and upper genital tract infections may go

undetected, which, left untreated, can lead to serious reproductive morbidity. Therefore, identifying the factors associated with *M. genitalium* could help predict women at high risk of disease. Further, screening for *M. genitalium* among high-risk women may help identify infected women, which may ultimately reduce PID and its sequelae.

In addition to our findings related to *M. genitalium* and PID, our study provided an increased understanding of *M. genitalium* in adverse pregnancy outcomes. Specifically, we were able to examine the association between *M. genitalium* and SAB, an area that has not been extensively studied. SAB is the most common adverse outcome of pregnancy, occurring in an estimated 15% of clinically recognized pregnancies (117), and up to 50% of all pregnancies (118). SABs are another important public health issue, for they cause considerable maternal psychological and physical morbidity and impart substantial costs to healthcare systems (147, 187). Our study was the first to prospectively examine the role of *M. genitalium* in SAB among a high-risk pregnant population and adds to the growing literature on the role of *M. genitalium* on reproductive health.

APPENDIX A: SUPPLEMENTARY TABLES FOR MANUSCRIPT 1

Table 23. Unadjusted and adjusted p-values for the clinical characteristics of women with *M. genitalium* (Mg) only, women with *N. gonorrhoeae* (Gc) only and women with *M. genitalium* and *N. gonorrhoeae* enrolled in the PEACH Study

	Unadjusted p- value	Adjusted p-value[a]	Unadjusted p- value	Adjusted p-value[b]
Presenting Complaints				
Nausea/vomiting	0.68	0.56	0.48	0.52
Non-menstrual vaginal bleeding	0.79	0.75	0.24	0.24
Heavier menstrual bleeding	0.38	0.29	0.77	0.42
Bleeding during or after sex	0.23	0.10	0.63	0.93
Abnormal vaginal discharge	0.73	0.67	0.18	0.45
More frequent urination	0.50	0.22	0.17	0.14
Markers of Inflammation				
Elevated temperature ($\geq 101^{o}$ F)	0.08	0.10	0.68	0.28
Elevated white blood cell count (>10,000 mm^3)	0.02	0.01	0.13	0.51
Erythrocyte Sedimentation Rate (>15 mm/hr)	0.002	0.01	0.43	0.49
C-Reactive Protein (>5mg/dL)	0.30	0.48	0.97	0.36
Bilateral adnexal tenderness	0.59	0.51	0.26	0.42
Mucopurulent cervicitis	0.001	0.01	0.02	0.01
BV by Gram Stain[c]	0.11	---	0.54	---
Amsel's Criteria				
BV by Amsel's criteria	0.82	0.42	1.00	0.77
Discharge consistent with BV	0.06	0.09	0.79	0.97
Vaginal pH >4.5	0.02	0.09	0.39	0.96
Positive whiff test	0.92	0.88	0.17	0.11
Clue cells >20%	0.53	0.83	0.75	0.92

[a] p-value adjusted for BV (Mg+/Gc-/Ct- vs. Gc+/Mg-/Ct-)
[b] p-value adjusted for BV (Gc+/Mg-/Ct- vs. Gc+/Mg+/Ct-)
[c] Normal or intermediate vs. BV flora
[d] Mean composite pain score= (mean of current pelvic pain score, average pelvic pain score and worst pelvic pain score) X 10
[e] sd=standard deviation

Table 24. Unadjusted and adjusted p-values for the clinical characteristics of women with *M. genitalium* (Mg) only, women with *C. trachomatis* (Ct) only, and women with *M. genitalium* and *C. trachomatis* enrolled in the PEACH Study

	Unadjsuted p- value	Adjusted p-value[a]	Unadjsuted p- value	Adjusted p-value[b]
Presenting Complaints				
Nausea/vomiting	0.93	0.94	1.00	0.81
Non-menstrual vaginal bleeding	0.19	0.25	0.49	0.38
Heavier menstrual bleeding	0.55	0.55	0.24	0.16
Bleeding during or after sex	0.44	0.41	0.43	0.40
Abnormal vaginal discharge	0.54	0.57	0.68	0.85
More frequent urination	0.80	0.63	0.63	0.73
Markers of Inflammation				
Elevated temperature ($\geq 101^{o}$ F)	---	---	0.14	0.06
Elevated white blood cell count (>10,000 mm^3)	0.72	0.76	0.99	0.58
Erythrocyte Sedimentation Rate (>15 mm/hr)	0.12	0.16	0.18	0.26
C-Reactive Protein (>5mg/dL)	0.27	0.24	0.16	0.16
Bilateral adnexal tenderness	0.96	0.98	1.00	0.88
Mucopurulent cervicitis	0.72	0.74	0.43	0.57
BV by Gram Stain[c]	0.63	---	0.45	---
Amsel's Criteria				
BV by Amsel's criteria	0.58	0.55	0.67	0.75
Discharge consistent with BV	0.53	0.74	0.39	0.451
Vaginal pH >4.5	0.08	0.12	0.21	0.40
Positive whiff test	0.67	0.63	0.13	0.15
Clue cells >20%	0.67	0.78	0.27	0.35

[a] p-value adjusted for BV (Mg+/Gc-/Ct- vs. Ct+/Mg-/Gc-)
[b] p-value adjusted for BV (Ct+/Mg-/Gc- vs. Ct+/Mg+/Gc-)
[c] Normal or intermediate vs. BV flora
[d] Mean composite pain score= (mean of current pelvic pain score, average pelvic pain score and worst pelvic pain score) X 10
[e] sd=standard deviation

APPENDIX B: SUPPLEMENTARY TABLES FOR MANUSCRIPT 2

Table 25. Characteristics of PEACH Study participants and association with *M. genitalium* cervical infection

Characteristic	*M. genitalium* positive n (%)[1]	*M. genitalium* negative n (%)[1]	OR (95% CI)
Demographic			
Age			
< 25 years	63 (14.1)	384 (85.9)	3.3 (1.7-6.5)
≥25 years	11 (4.7)	224 (95.3)	
Race/Ethnicity			
African-American	55 (10.8)	454 (89.2)	0.9 (0.6-1.7)
White/Hispanic/Other	19 (11.0)	154 (89.0)	
Marital Status			
Unmarried	60 (10.7)	499 (89.3)	1.5 (0.6-3.9)
Married	5 (7.2)	64 (92.7)	
Education			
< High school	33 (12.5)	231 (87.5)	1.3 (0.8-2.1)
≥ High school	41 (9.9)	375 (90.1)	
Sexual Health			
Sexually active			
Yes	68 (11.8)	510 (88.2)	2.2 (0.9-5.2)
No	6 (5.8)	98 (94.2)	
≥ 2 life time sexual partners			
Yes	10 (15.6)	54 (84.4)	1.6 (0.8-3.3)
No	64 (10.4)	554 (89.6)	
New sexual partner in past month			
Yes	9 (14.1)	55 (85.9)	1.4 (0.7-2.9)
No	65 (10.5)	553 (89.5)	
History of STD[3]			
Yes	43 (10.5)	366 (89.5)	0.9 (0.5-1.4)
No	31 (11.7)	234 (88.3)	
History of BV			
Yes	12 (7.9)	139 (92.1)	0.6 (0.3-1.2)
No	60 (11.8)	450 (88.2)	

Table 25 continued

History of PID			
Yes	19 (9.1)	190 (90.9)	0.8 (0.4-1.3)
No	54 (11.6)	411 (88.4)	
Hormonal contraception use			
Yes	16 (12.5)	112 (87.5)	1.1 (0.6-2.)
No	52 (11.6)	398 (88.4)	
Rare/occasional condom use[4]			
Yes	51 (12.6)	355 (87.4)	1.3 (0.7-2.3)
No	17 (9.9)	155 (90.1)	
Consistent condom use[5]			
Yes	7 (9.3)	68 (90.7)	1.8 (0.7-4.7)
No	61 (12.1)	442 (87.9)	
Oral sex			
Yes	22 (14.0)	135 (86.0)	1.4 (0.8-2.4)
No	50 (10.3)	434 (89.7)	
Anal sex			
Yes	4 (18.2)	18 (81.8)	1.9 (0.6-5.7)
No	70 (10.6)	590 (89.4)	
Age at sexual debut			
$\leq$ 15 years	41 (11.7)	310 (88.3)	1.2 (0.7-1.9)
> 15 years	33 (10.0)	298 (90.0)	
Behavioral			
Vaginal douche $\geq$ 2 times in past month			
Yes	21 (16.1)	109 (83.8)	1.8 (1.0-3.1)
No	53 (9.6)	499 (90.4)	
Drug use			
Yes	29 (15.3)	161 (84.7)	1.8 (1.1-2.9)
No	45 (9.2)	443 (90.8)	
Current smoker			
Yes	43 (14.7)	250 (85.3)	2.0 (1.2-3.2)
No	31 (8.0)	354 (91.9)	
Alcohol use			
Yes	41 (10.8)	338 (89.2)	1.0 (0.6-1.6)
No	33 (11.0)	266 (89.0)	
Alcohol drinks per week			
> 7 drinks	11 (13.4)	71 (86.6)	1.3 (0.7-2.6)
$\leq$ 7 drinks	63 (10.6)	533 (89.4)	

[1] % of total study population with characteristic that tested positive for *M. genitalium*;
[2] Normal or intermediate vaginal flora vs. BV flora;
[3] History of *N. gonorrhoeae*, *C. trachomatis*, or *Trichonomas vaginalis*;
[4] Condoms used 0 to 5 out of 10 sexual encounters;
[5] Condoms used 10 out of 10 sexual encounters

Table 26. Characteristics of PEACH Study participants and association with *M. genitalium* endometrial infection

Characteristic	*M. genitalium* positive n (%)[1]	*M. genitalium* negative n (%)[1]	OR (95% CI)
Demographic			
Age			
< 25 years	41 (10.0)	362 (90.0)	2.5 (1.2-5.3)
≥25 years	9 (4.3)	199 (35.5)	
Race/Ethnicity			
African-American	42 (9.5)	399 (71.1)	2.1 (0.98-4.6)
White/Hispanic/Other	8 (4.7)	162 (95.3)	
Marital Status			
Unmarried	39 (7.8)	461 (92.2)	1.2 (0.43-3.6)
Married			
Education			
< High school	22 (9.3)	215 (38.4)	1.3 (0.70-2.3)
≥ High school	28 (7.5)	345 (61.6)	
Sexual Health			
Sexually active			
Yes	42 (8.2)	471 (91.8)	1.0 (0.5-2.2)
No	8 (8.2)	90 (91.8)	
≥ 2 life time sexual partners			
Yes	6 (10)	54 (92.0)	1.3 (0.5-3.1)
No	44 (8)	507 (90.4)	
New sexual partner in past month			
Yes	6 (9.4)	58 (10.3)	1.2 (0.5-2.9)
No	44 (8.0)	503 (92.0)	
History of STD[3]			
Yes	29 (8.1)	330 (91.9)	1.2 (0.5-2.9)
No	20 (8.1)	226 (91.9)	
History of BV			
Yes	8 (5.7)	132 (94.3)	0.6 (0.3-1.3)
No	42 (9.2)	412 (90.7)	
History of PID			
Yes	12 (6.3)	177 (93.7)	0.7 (0.3-1.3)
No	38 (9.1)	378 (90.9)	
Hormonal contraception use			
Yes	9 (8.1)	102 (91.9)	1.0 (0.5-2.1)
No			
Rare/occasional condom use[4]			
Yes	33 (9.2)	326 (90.8)	1.6 (0.8-3.5)
No	9 (5.8)	145 (94.2)	

Table 26 continued

Consistent condom use[5]			
Yes	3 (4.5)	64 (95.5)	0.7 (0.3-1.9)
No	39 (8.7)	407 (91.3)	
Oral sex			
Yes	11 (7.9)	128 (92.1)	0.9 (0.4-1.8)
No	39 (8.9)	397 (91.1)	
Anal sex			
Yes	3 (14.3)	18 (85.7)	1.9 (0.6-6.8)
No	47 (14.3)	543 (92.0)	
Age at sexual debut			
≤ 15 years	29 (9.1)	289 (90.9)	1.3 (0.7-2.3)
> 15 years	21 (7.2)	272 (92.8)	
Behavioral			
Vaginal douche ≥ 2 times in past month			
Yes	17 (14.3)	102 (85.7)	2.3 (1.2-4.3)
No	33 (6.7)	459 (93.3)	
Drug use			
Yes	22 (13.0)	147 (87.0)	2.2 (1.2-3.9)
No	28 (6.4)	411 (73.7)	
Current smoker			
Yes	28 (10.7)	234 (89.3)	1.8 (0.9-3.2)
No	22 (6.4)	324 (93.6)	
Alcohol use			
Yes	27 (7.9)	314 (7.9)	0.9 (0.5-1.6)
No	23 (8.6)	244 (43.7)	
Alcohol drinks per week			
> 7 drinks	8 (11.0)	65 (89.0)	1.4 (0.6-3.2)
≤ 7 drinks	42 (7.8)	493 (92.1)	

[1] % of total study population with characteristic
[2] Normal or intermediate vaginal flora vs. BV flora;
[3] History of *N. gonorrhoeae*, *C. trachomatis*, or *Trichonomas vaginalis*;
[4] Condoms used 0 to 5 out of 10 sexual encounters;
[5] Condoms used 10 out of 10 sexual encounters

APPENDIX C: SUPPLEMENTARY TABLES FOR MANUSCRIPT 3

Table 27. Odds of spontaneous abortion according to infection at enrollment among participants in the Early Pregnancy Study

Infection	OR (95% CI)	AOR (95% CI)[1]	AOR (95% CI)[2]	AOR (95% CI)[3]
M. genitalium	0.5 (0.1-2.0)	0.6 (0.1-2.5)	0.5 (0.1-2.0)	06 (0.2-2.3)
C. trachomatis	0.4 (0.1-1.4)	0.4 (0.1-1.6)	0.4 (0.1-1.4)	0.4 (0.1-1.6)

[1] Adjusted for age, history of SAB, smoking, cocaine use and *C. trachomatis* or *M. genitalium* PCR
[2] Adjusted for age, smoking, and *C. trachomatis* or *M. genitalium* PCR
[3] Adjusted for age, history of SAB, smoking, alcohol use and *C. trachomatis* or *M. genitalium* PCR

Table 28. Unadjusted odds of spontaneous abortion among participants in the Early Pregnancy Study

Characteristic	OR (95% CI)
Previous SAB	1.9 (1.1-3.4)
Smoking	1.3 (0.7-2.3)
Cocaine use	1.2 (0.5-2.4)
Alcohol use	1.2 (0.7-21)
Maternal Age	1.1 (0.5-2.2)

BIBLIOGRAPHY

1. Taylor-Robinson D. Mycoplasmas. In: Greenwood D, Slack R, Peutherer J, Barer M, editors. Medical Microbiology A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control. Edinburgh: Churchill Livingstone Elsevier; 2007. p. 395-405.

2. Razin S, Yogev D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. Microbiol Mol Biol Rev 1998;62(4):1094-156.

3. Uuskula A, Kohl PK. Genital mycoplasmas, including Mycoplasma genitalium, as sexually transmitted agents. Int J STD AIDS 2002;13(2):79-85.

4. Blanchard A, Bebear C. Mycoplasmas of Humans. In: Razin, Herrmann, editors. Molecular Biology and Pathogenicity of Mycoplasmas. New York: Kluwar Academic/Plenum Publishers; 2002.

5. Fraser C, Gocayne J, White O, al. E. The minimal gene complement of Mycoplasma genitalium. Science 1995;270:397-403.

6. Taylor-Robinson D, Gilroy CB, Keane FE. Detection of several Mycoplasma species at various anatomical sites of homosexual men. Eur J Clin Microbiol Infect Dis 2003;22(5):291-3.

7. de Barbeyrac B, Bernet-Poggi C, Febrer F, Renaudin H, Dupon M, Bebear C. Detection of Mycoplasma pneumoniae and Mycoplasma genitalium in clinical samples by polymerase chain reaction. Clin Infect Dis 1993;17 Suppl 1:S83-9.

8. Samra Z, Borin M, Bukowsky Y, Lipshitz Y, Sompolinsky D. Non-occurrence of Mycoplasma genitalium in clinical specimens. Eur J Clin Microbiol Infect Dis 1988;7(1):49-51.

9. Hu PC, Schaper U, Collier AM, Clyde WA, Jr., Horikawa M, Huang YS, et al. A Mycoplasma genitalium protein resembling the Mycoplasma pneumoniae attachment protein. Infect Immun 1987;55(5):1126-31.

10. Taylor-Robinson D. Mycoplasma genitalium -- an up-date. Int J STD AIDS 2002;13(3):145-51.

11. Jensen JS, Blom J, Lind K. Intracellular location of Mycoplasma genitalium in cultured Vero cells as demonstrated by electron microscopy. Int J Exp Pathol 1994;75(2):91-8.

12. Taylor-Robinson D, Horner PJ. The role of Mycoplasma genitalium in non-gonococcal urethritis. Sex Transm Infect 2001;77(4):229-31.

13. Taylor-Robinson D, Furr PM, Tully JG, Barile MF, Moller BR. Animal models of Mycoplasma genitalium urogenital infection. Isr J Med Sci 1987;23(6):561-4.

14. Tosh AK, Van Der Pol B, Fortenberry JD, Williams JA, Katz BP, Batteiger BE, et al. Mycoplasma genitalium among adolescent women and their partners. J Adolesc Health 2007;40(5):412-7.

15. Cohen CR, Nosek M, Meier A, Astete SG, Iverson-Cabral S, Mugo NR, et al. Mycoplasma genitalium infection and persistence in a cohort of female sex workers in Nairobi, Kenya. Sex Transm Dis 2007;34(5):274-9.

16. Schachter J. Biology of Chlamydia trachomatis. In: Holmes K, Sparling P, Mardh P-A, Lemon S, Stamm W, Piot P, et al., editors. Sex Transm Dis; 1999. p. 391-405.

17. Ward M. Chlamydia. In: Greenwood D, Slack R, Peutherer J, Barer M, editors. Medical Micorbiology A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratoy Diagnosis and Control. 17 ed. Edinburgh: Churchill Livingstone Elsevier; 2007.

18. Wiesenfeld H, Cates W. Sexually Transmitted Diseases and Infertility. In: Holmes K, editor. Sexually Transmitted Diseases 4th ed New York: McGraw-Hill Medical; 2008.

19. Melly MA, Gregg CR, McGee ZA. Studies of toxicity of Neisseria gonorrhoeae for human fallopian tube mucosa. J Infect Dis 1981;143(3):423-31.

20. McGee ZA, Johnson AP, Taylor-Robinson D. Pathogenic mechanisms of Neisseria gonorrhoeae: observations on damage to human fallopian tubes in organ culture by gonococci of colony type 1 or type 4. J Infect Dis 1981;143(3):413-22.

21. Andersen B, Sokolowski I, Ostergaard L, Kjolseth Moller J, Olesen F, Jensen JS. Mycoplasma genitalium: prevalence and behavioural risk factors in the general population. Sex Transm Infect 2007;83(3):237-41.

22. Manhart LE, Holmes KK, Hughes JP, Houston LS, Totten PA. Mycoplasma genitalium among young adults in the United States: an emerging sexually transmitted infection. Am J Public Health 2007;97(6):1118-25.

23. Anagrius C, Lore B, Jensen JS. Mycoplasma genitalium: prevalence, clinical significance, and transmission. Sex Transm Infect 2005;81(6):458-62.

24. Keane FE, Thomas BJ, Gilroy CB, Renton A, Taylor-Robinson D. The association of Chlamydia trachomatis and Mycoplasma genitalium with non-gonococcal urethritis: observations on heterosexual men and their female partners. Int J STD AIDS 2000;11(7):435-9.

25. Falk L, Fredlund H, Jensen JS. Symptomatic urethritis is more prevalent in men infected with Mycoplasma genitalium than with Chlamydia trachomatis. Sex Transm Infect 2004;80(4):289-93.

26. Hjorth SV, Bjornelius E, Lidbrink P, Falk L, Dohn B, Berthelsen L, et al. Sequence-based typing of Mycoplasma genitalium reveals sexual transmission. J Clin Microbiol 2006;44(6):2078-83.

27. Lind K, Lindhardt BO, Schutten HJ, Blom J, Christiansen C. Serological cross-reactions between Mycoplasma genitalium and Mycoplasma pneumoniae. J Clin Microbiol 1984;20(6):1036-43.

28. Wroblewski J, Manhart L, Dickey K, Hudspeth M, Totten P. Comparison of transcription-mediated amplification and PCR assay results for various genital specimen types for detection of Mycoplasma genitalium. Journal of Clinical Microbiology 2006;44:3306-3312.

29. Totten P, Taylor-Robinson D, Jensen J. Genital Mycoplasmas. In: Holmes K, editor. Sexually Transmitted Diseases 4th ed New York: McGraw-Hill Medical; 2008.

30. Luki N, Lebel P, Boucher M, Doray B, Turgeon J, Brousseau R. Comparison of polymerase chain reaction assay with culture for detection of genital mycoplasmas in perinatal infections. Eur J Clin Microbiol Infect Dis 1998;17(4):255-63.

31. Jensen JS, Bjornelius E, Dohn B, Lidbrink P. Comparison of first void urine and urogenital swab specimens for detection of Mycoplasma genitalium and Chlamydia trachomatis by polymerase chain reaction in patients attending a sexually transmitted disease clinic. Sex Transm Dis 2004;31(8):499-507.

32. Casin I, Vexiau-Robert D, De La Salmoniere P, Eche A, Grandry B, Janier M. High prevalence of Mycoplasma genitalium in the lower genitourinary tract of women attending a sexually transmitted disease clinic in Paris, France. Sex Transm Dis 2002;29(6):353-9.

33. Falk L, Fredlund H, Jensen JS. Signs and symptoms of urethritis and cervicitis among women with or without Mycoplasma genitalium or Chlamydia trachomatis infection. Sex Transm Infect 2005;81(1):73-8.

34. Manhart LE, Critchlow CW, Holmes KK, Dutro SM, Eschenbach DA, Stevens CE, et al. Mucopurulent cervicitis and Mycoplasma genitalium. J Infect Dis 2003;187(4):650-7.

35. Moi H, Reinton N, Moghaddam A. Mycoplasma genitalium in women with lower genital tract inflammation. Sex Transm Infect 2009;85(1):10-4.

36. Palmer HM, Gilroy CB, Claydon EJ, Taylor-Robinson D. Detection of Mycoplasma genitalium in the genitourinary tract of women by the polymerase chain reaction. Int J STD AIDS 1991;2(4):261-3.

37. Pepin J, Labbe AC, Khonde N, Deslandes S, Alary M, Dzokoto A, et al. Mycoplasma genitalium: an organism commonly associated with cervicitis among west African sex workers. Sex Transm Infect 2005;81(1):67-72.

38. Tsunoe H, Tanaka M, Nakayama H, Sano M, Nakamura G, Shin T, et al. High prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium in female commercial sex workers in Japan. Int J STD AIDS 2000;11(12):790-4.

39. Hamasuna R, Imai H, Tsukino H, Jensen JS, Osada Y. Prevalence of Mycoplasma genitalium among female students in vocational schools in Japan. Sex Transm Infect 2008;84(4):303-5.

40. Cohen CR, Manhart LE, Bukusi EA, Astete S, Brunham RC, Holmes KK, et al. Association between Mycoplasma genitalium and acute endometritis. Lancet 2002;359(9308):765-6.

41. Cohen CR, Mugo NR, Astete SG, Odondo R, Manhart LE, Kiehlbauch JA, et al. Detection of Mycoplasma genitalium in women with laparoscopically diagnosed acute salpingitis. Sex Transm Infect 2005;81(6):463-6.

42. Haggerty CL, Totten PA, Astete SG, Lee S, Hoferka SL, Kelsey SF, et al. Failure of cefoxitin and doxycycline to eradicate endometrial Mycoplasma genitalium and the consequence for clinical cure of pelvic inflammatory disease. Sex Transm Infect 2008;84(5):338-42.

43. Simms I, Eastick K, Mallinson H, Thomas K, Gokhale R, Hay P, et al. Associations between Mycoplasma genitalium, Chlamydia trachomatis and pelvic inflammatory disease. J Clin Pathol 2003;56(8):616-8.

44. Kataoka S, Yamada T, Chou K, Nishida R, Morikawa M, Minami M, et al. Association between preterm birth and vaginal colonization by mycoplasmas in early pregnancy. J Clin Microbiol 2006;44(1):51-5.

45. Lyss SB, Kamb ML, Peterman TA, Moran JS, Newman DR, Bolan G, et al. Chlamydia trachomatis among patients infected with and treated for Neisseria gonorrhoeae in sexually transmitted disease clinics in the United States. Ann Intern Med 2003;139(3):178-85.

46. Jurstrand M, Jensen JS, Magnuson A, Kamwendo F, Fredlund H. A serological study of the role of Mycoplasma genitalium in pelvic inflammatory disease and ectopic pregnancy. Sex Transm Infect 2007;83(4):319-23.

47. Marks C, Tideman RL, Estcourt CS, Berry G, Mindel A. Assessment of risk for pelvic inflammatory disease in an urban sexual health population. Sex Transm Infect 2000;76(6):470-3.

48. Miller HG, Cain VS, Rogers SM, Gribble JN, Turner CF. Correlates of sexually transmitted bacterial infections among U.S. women in 1995. Fam Plann Perspect 1999;31(1):4-9, 23.

49. Sweet R, Blankfort-Doyle M, MO MR, Schacter J. The occurrence of chlamydial and gonococcal salpingitis during the menstrual cycle. JAMA 1986;2555(15):2062-4.

50. Jacobson L, Westrom L. Objectivized diagnosis of acute pelvic inflammatory disease. Diagnostic and prognostic value of routine laparoscopy. Am J Obstet Gynecol 1969;105(7):1088-98.

51. Aral SO, Mosher WD, Cates W, Jr. Self-reported pelvic inflammatory disease in the United States, 1988. Jama 1991;266(18):2570-3.

52. Scholes D, Daling JR, Stergachis A, Weiss NS, Wang SP, Grayston JT. Vaginal douching as a risk factor for acute pelvic inflammatory disease. Obstet Gynecol 1993;81(4):601-6.

53. Wolner-Hanssen P, Eschenbach DA, Paavonen J, Stevens CE, Kiviat NB, Critchlow C, et al. Association between vaginal douching and acute pelvic inflammatory disease. Jama 1990;263(14):1936-41.

54. Ness RB, Soper DE, Holley RL, Peipert J, Randall H, Sweet RL, et al. Douching and endometritis: results from the PID evaluation and clinical health (PEACH) study. Sex Transm Dis 2001;28(4):240-5.

55. Marchbanks P, Lee N, Peterson H. Cigarette smoking as a risk factor for pelvic inflammatory disease. Am J Obstet Gynecol 1990;162(639-44).

56. Scholes D, Daling JR, Stergachis AS. Current cigarette smoking and risk of acute pelvic inflammatory disease. Am J Public Health 1992;82(10):1352-5.

57. Wølner-Hanssen P ED, Paavonen J, Kiviat N, Stevens CE, Critchlow C, DeRouen T, Holmes KK. Decreased risk of symptomatic chlamydial pelvic inflammatory disease associated with oral contraceptive use. JAMA 1990;263(1):54-9

58. Ness R, Keder L, Soper D, Amortegui A, Gluck J, Wiesenfeld H. Oral contraception and the recognition of endometritis. Am J Obstet Gynecol 1997;176:580-85.

59. Steele CB, Meléndez-Morales L, Campoluci R, DeLuca N, Dean HD. Health Disparities in HIV/AIDS, Viral Hepatitis, Sexually Transmitted Diseases, and Tuberculosis: Issues, Burden, and Response, A Retrospective Review, 2000–2004. Atlanta, GA: Department of Health and Human Services, Centers for Disease Control and Prevention; November 2007.

60. CDC. Sexually Transmitted Disease Surveillance, 2007. In. Atlanta, GA: U.S. Department of Health and Human Sevices; 2008.

61. Goldenberg RL KM, Nugent R, Krohn MA, Hillier S, Andrews WW. Bacterial colonization of the vagina during pregnancy in four ethnic groups. Vaginal Infections and Prematurity Study Group. Am J Obstet Gynecol 1996;174(5):1618-21.

62. Ness RB, Hillier S, Richter HE, Soper DE, Stamm C, Bass DC, et al. Can known risk factors explain racial differences in the occurrence of bacterial vaginosis? J Natl Med Assoc 2003;95(3):201-12.

63. Ness RB, Hillier SL, Richter HE, Soper DE, Stamm C, McGregor J, et al. Douching in relation to bacterial vaginosis, lactobacilli, and facultative bacteria in the vagina. Obstet Gynecol 2002;100(4):765.

64. Hillier S. Normal Genital Flora. In: Holmes K, editor. Sexually Transmitted Diseases 4th ed New York: McGraw-Hill Medical; 2008. p. 289-308.

65. Barton S, Jenkins D, Cuzick J, Maddox PH, Edwards R, Singer A. Effect of cigarette smoking on cervical epithelial immunity: a mechanism for neoplastic change? The Lancet 1988;332(8612):652-654.

66. Harrison HR, Costin M, Meder JB, Bownds LM, Sim DA, Lewis M, et al. Cervical Chlamydia trachomatis infection in university women: relationship to history, contraception, ectopy, and cervicitis. Am J Obstet Gynecol 1985;153(3):244-51.

67. Shlay JC, McClung MW, Patnaik JL, Douglas JM, Jr. Comparison of sexually transmitted disease prevalence by reported level of condom use among patients attending an urban sexually transmitted disease clinic. Sex Transm Dis 2004;31(3):154-60.

68. Huppert JS, Mortensen JE, Reed JL, Kahn JA, Rich KD, Hobbs MM. Mycoplasma genitalium detected by transcription-mediated amplification is associated with Chlamydia trachomatis in adolescent women. Sex Transm Dis 2008;35(3):250-4.

69. Uno M, Deguchi T, Komeda H, Hayasaki M, Iida M, Nagatani M. Mycoplasma genitalium in Cervices of Japanese Women. Sex Transm Dis 1997;24:284-86.

70. Korte J, Baseman J, Cagle M, Herrera C, Piper J, Holden A, et al. Cervicitis and Genitourinary Symptoms in Women Culture Positive for *Mycoplasma genitalium*. American Journal of Reproductive Immunology 2006;55:265-275.

71. Stamm W. Chlamydia trachomatis infections of the adult. In: Holmes K, Sparling P, Mardh P-A, Lemon S, Stamm W, Piot P, et al., editors. Sex Transm Dis; 1999. p. 407-422.

72. Jones R, Wasserheit J. Introduction to the biology and natural history of sexually transmitted diseases. In: Wasserheit J, editor. Research Issues in Human Behavior and Sexually Transmitted Diseases in the AIDS Era. Washington, DC: American Society for Microbiology; 1991. p. 11-37.

73. Haggerty CL. Evidence for a role of Mycoplasma genitalium in pelvic inflammatory disease. Curr Opin Infect Dis 2008;21(1):65-9.

74. Ross JD, Jensen JS. Mycoplasma genitalium as a sexually transmitted infection: implications for screening, testing, and treatment. Sex Transm Infect 2006;82(4):269-71.

75. Horner P, Thomas B, Gilroy CB, Egger M, Taylor-Robinson D. Role of Mycoplasma genitalium and Ureaplasma urealyticum in acute and chronic nongonococcal urethritis. Clin Infect Dis 2001;32(7):995-1003.

76. Hamasuna R, Osada Y, Jensen JS. Antibiotic susceptibility testing of Mycoplasma genitalium by TaqMan 5' nuclease real-time PCR. Antimicrob Agents Chemother 2005;49(12):4993-8.

77. Samra Z, Rosenberg S, Soffer Y. In vitro susceptibility of Mycoplasma hominis clinical isolates to tetracyclines, quinolones and macrolides. Diagn Microbiol Infect Dis 2002;44(4):359-61.

78. Horner PJ, Thomas B, Gilroy CB, Egger M, Taylor-Robinson D. Do all men attending departments of genitourinary medicine need to be screened for non-gonococcal urethritis? Int J STD AIDS 2002;13(10):667-73.

79. Ness RB, Soper DE, Holley RL, Peipert J, Randall H, Sweet RL, et al. Effectiveness of inpatient and outpatient treatment strategies for women with pelvic inflammatory disease: results from the Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) Randomized Trial. Am J Obstet Gynecol 2002;186(5):929-37.

80. Tully JG, Taylor-Robinson D, Cole RM, Rose DL. A newly discovered mycoplasma in the human urogenital tract. Lancet 1981;1(8233):1288-91.

81. Bjornelius E, Lidbrink P, Jensen JS. Mycoplasma genitalium in non-gonococcal urethritis--a study in Swedish male STD patients. Int J STD AIDS 2000;11(5):292-6.

82. Jensen JS. Mycoplasma genitalium: a cause of non-gonococcal urethritis? Genitourin Med 1994;70(5):363.

83. Mena L, Wang X, Mroczkowski TF, Martin DH. Mycoplasma genitalium infections in asymptomatic men and men with urethritis attending a sexually transmitted diseases clinic in New Orleans. Clin Infect Dis 2002;35(10):1167-73.

84. Janier M, Lassau F, Casin I, Grillot P, Scieux C, Zavaro A, et al. Male urethritis with and without discharge: a clinical and microbiological study. Sex Transm Dis 1995;22(4):244-52.

85. Maeda S, Tamaki M, Nakano M, Uno M, Deguchi T, Kawada Y. Detection of Mycoplasma genitalium in patients with urethritis. J Urol 1998;159(2):405-7.

86. Pepin J, Sobela F, Deslandes S, Alary M, Wegner K, Khonde N, et al. Etiology of urethral discharge in West Africa: the role of Mycoplasma genitalium and Trichomonas vaginalis. Bull World Health Organ 2001;79(2):118-26.

87. Totten PA, Schwartz MA, Sjostrom KE, Kenny GE, Handsfield HH, Weiss JB, et al. Association of Mycoplasma genitalium with nongonococcal urethritis in heterosexual men. J Infect Dis 2001;183(2):269-276.

88. Luo D, Zhu W, Zhang X, Chen X, Wang S, Wang Z, et al. Molecular epidemiologic study of Mycoplasma genitalium infection in high risk populations of sexually transmitted diseases in China. Chin Med J (Engl) 2000;113(11):1015-8.

89. Horner PJ, Gilroy CB, Thomas BJ, Naidoo RO, Taylor-Robinson D. Association of Mycoplasma genitalium with acute non-gonococcal urethritis. Lancet 1993;342(8871):582-5.

90. Johannisson G, Enstrom Y, Lowhagen GB, Nagy V, Ryberg K, Seeberg S, et al. Occurrence and treatment of Mycoplasma genitalium in patients visiting STD clinics in Sweden. Int J STD AIDS 2000;11(5):324-6.

91. Paavonen J, Aine R, Teisala K, Heinonen PK, Punnonen R. Comparison of endometrial biopsy and peritoneal fluid cytologic testing with laparoscopy in the diagnosis of acute pelvic inflammatory disease. Am J Obstet Gynecol 1985;151(5):645-50.

92. Schlicht M, Lovrich S, Sartin J, Kaprinsky P, Callister S, Agger W. High Prevalence of Genital mycoplasmas among sexually active young adults with urethritis or cervicitis symptoms in La Crosse, Wisconsin. Journal of Clinical Microbiology 2004;42(10):4636-4640.

93. Leitich H, Bodner-Adler B, Brunbauer M, Kaider A, Egarter C, Husslein P. Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis. Am J Obstet Gynecol 2003;189(1):139-47.

94. Klebanoff MA, Regan JA, Rao AV, Nugent RP, Blackwelder WC, Eschenbach DA, et al. Outcome of the Vaginal Infections and Prematurity Study: results of a clinical trial of erythromycin among pregnant women colonized with group B streptococci. Am J Obstet Gynecol 1995;172(5):1540-5.

95. Beigi RH, Wiesenfeld HC, Hillier SL, Straw T, Krohn MA. Factors associated with absence of H2O2-producing Lactobacillus among women with bacterial vaginosis. J Infect Dis 2005;191(6):924-9.

96. Keane F, Thomas B, Gilroy C, Renton A, Taylor-Robinson D. The association of Mycoplasma hominis, Ureaplasma urealyticum and Mycoplasma genitalium with bacterial vaginosis: observations on heterosexual women and their male partners. Int J STD AIDS 2002;11:356-60.

97. Lu GC, Schwebke JR, Duffy LB, Cassell GH, Hauth JC, Andrews WW, et al. Midtrimester vaginal Mycoplasma genitalium in women with subsequent spontaneous preterm birth. Am J Obstet Gynecol 2001;185(1):163-5.

98. Simms I, Eastick K, Mallison H, Thomas K, Gokhale R, Hay P, et al. Associations between Mycoplasma genitalium, Chlamydia trachomatis, and pelvic inflammatory disease. Sex Transm Infect 2003;79:154-156.

99. Oakeshott P, Hay P, Taylor-Robinson D, Hay S, Dohn B, Kerry S, et al. Prevalence of Mycoplasma genitalium in early pregnancy and relationship between its presence and pregnancy outcome. Bjog 2004;111(12):1464-7.

100. Moi H, Reinton N, Moghaddam A. *Mycoplasma genitalium* in women with lower genital tract inflammation. Sex Transm Infect 2009;85:10-14.

101. Moller B, Taylor-Robinson D, Furr P, Freundt E. Acute upper genital-tract disease in female monkeys provoked experimentally by *Mycoplasma genitalium*. Br J Exp Pathol 1985;66:417-426.

102. Baczynska A, Funch P, Fedder J, Knudsen HJ, Birkelund S, Christiansen G. Morphology of human Fallopian tubes after infection with Mycoplasma genitalium and Mycoplasma hominis--in vitro organ culture study. Hum Reprod 2007;22(4):968-79.

103. Svenstrup HF, Fedder J, Abraham-Peskir J, Birkelund S, Christiansen G. Mycoplasma genitalium attaches to human spermatozoa. Hum Reprod 2003;18(10):2103-9.

104. Uno M, Deguchi T, Komeda H, Hayasaki M, Iida M, Nagatani M, et al. Mycoplasma genitalium in the cervices of Japanese women. Sex Transm Dis 1997;24(5):284-6.

105. Lind K, Kristensen GB. Significance of antibodies to Mycoplasma genitalium in salpingitis. Eur J Clin Microbiol 1987;6(2):205-7.

106. Moller BR, Taylor-Robinson D, Furr PM. Serological evidence implicating Mycoplasma genitalium in pelvic inflammatory disease. Lancet 1984;1(8386):1102-3.

107. CDC. Sexually Transmitted Disease Surveillance 1994. Atlanta: U.S. Department of Health and Human Services; 1995.

108. Cohen C, Manhart L, Bukusi E, Astete S, Brunham R, Holmes K. Association between Mycoplasma genitalium and acute endometritis. Lancet 2002;359:765-66.

109. Westrom L, Joesoef R, Reynolds G, Hagdu A, Thompson SE. Pelvic inflammatory disease and fertility. A cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic results. Sex Transm Dis 1992;19(4):185-92.

110. Haggerty C, Ness R, Amortegui A, Hendrix S, Hillier S, Holley R, et al. Endometritis does not predict reproductive morbidity after pelvic inflammatory disease. Am J Obstet Gynecol 2003;188:141-8.

111. Westrom L. Effect of pelvic inflammatory disease on fertility. Venereology 1995;8(4):219-22.

112. Westrom L. Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. Am J Obstet Gynecol 1980;138(7 Pt 2):880-92.

113. Clausen H, Fedder J, Drasbek M, Nielsen P, Toft B, Ingerslev H. Serological investigation of Mycoplasma genitalium in infertile women. Hum Reproduct 2001;16:1866-74.

114. Svenstrup HF, Fedder J, Kristoffersen SE, Trolle B, Birkelund S, Christiansen G. Mycoplasma genitalium, Chlamydia trachomatis, and tubal factor infertility-a prospective study. Fertil Steril 2007;90(3):513-20.

115. Edwards RK, Ferguson RJ, Reyes L, Brown M, Theriaque DW, Duff P. Assessing the relationship between preterm delivery and various microorganisms recovered from the lower genital tract. J Matern Fetal Neonatal Med 2006;19(6):357-63.

116. Labbe AC, Frost E, Deslandes S, Mendonca AP, Alves AC, Pepin J. Mycoplasma genitalium is not associated with adverse outcomes of pregnancy in Guinea-Bissau. Sex Transm Infect 2002;78(4):289-91.

117. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of early loss of pregnancy. N Engl J Med 1988;319(4):189-94.

118. Kline K, Stein Z, Susser M. Conception to Birth Epidemiology of Prenatal Development. New York: Oxford University Press; 1989.

119. Goddijn M, Leschot N. Genetic aspects of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:855-65.

120. Carey J, Klebanoff M. Is a change in the vaginal flora associated with an increased risk of preterm birth? American Journal of Obstetrics and Gynecology 2005;192:1341-1347.

121. Hack M, Merkatz IR. Preterm delivery and low birth weight--a dire legacy. N Engl J Med 1995;333(26):1772-4.

122. Taylor-Robinson D. The role of mycoplasmas in pregnancy outcome. Best Practice & Research Clinical Obstetrics and Gynaecology 2007;21(3):425-438.

123. Horowitz S, Mazor M, Romero R, Horowitz J, Glezerman M. Infection of the amniotic cavity with Ureaplasma urealyticum in the midtrimester of pregnancy. J Reprod Med 1995;40(5):375-9.

124. Abele-Horn M, Peters J, Genzel-Boroviczeny O, Wolff C, Zimmermann A, Gottschling W. Vaginal Ureaplasma urealyticum colonization: influence on pregnancy outcome and neonatal morbidity. Infection 1997;25(5):286-91.

125. Yoon BH, Chang JW, Romero R. Isolation of Ureaplasma urealyticum from the amniotic cavity and adverse outcome in preterm labor. Obstet Gynecol 1998;92(1):77-82.

126. Berg TG, Philpot KL, Welsh MS, Sanger WG, Smith CV. Ureaplasma/Mycoplasma-infected amniotic fluid: pregnancy outcome in treated and nontreated patients. J Perinatol 1999;19(4):275-7.

127. Cheng SY, Ling TS, Fu QH. [Ureaplasma urealyticum infection in spontaneous abortion]. Zhonghua Fu Chan Ke Za Zhi 1994;29(4):230-1, 254.

128. Donders GG, Van Bulck B, Caudron J, Londers L, Vereecken A, Spitz B. Relationship of bacterial vaginosis and mycoplasmas to the risk of spontaneous abortion. Am J Obstet Gynecol 2000;183(2):431-7.

129. Naessens A, Foulon W, Cammu H, Goossens A, Lauwers S. Epidemiology and pathogenesis of ureaplasma urealyticum in spontaneous abortion and early preterm labor. Acta Obstet Gynecol Scand 1987;66(6):513-6.

130. Rosenstein IJ, Morgan DJ, Sheehan M, Lamont RF, Taylor-Robinson D. Bacterial vaginosis in pregnancy: distribution of bacterial species in different gram-stain categories of the vaginal flora. J Med Microbiol 1996;45(2):120-6.

131. Hitti J, Watts D. Bacterial Sexually Transmitted Infections in Pregnancy. In: Holmes K, editor. Sexually Transmitted Diseases 4th ed New York: McGraw-Hill Medical; 2008.

132. Witkin S, Kligman I, Grifo J, Rosenwaks Z. Chlamydia trachomatis detected by polymerase chain reaction in cervices of culture-negative women correlates with adverse in vitro fertilization outcome. J Infect Dis 1995;171(6):1657-9.

133. Sozio J, Ness RB. Chlamydial lower genital tract infection and spontaneous abortion. Infect Dis Obstet Gynecol 1998;6(1):8-12.

134. Licciardi F, Grifo JA, Rosenwaks Z, Witkin SS. Relation between antibodies to Chlamydia trachomatis and spontaneous abortion following in vitro fertilization. J Assist Reprod Genet 1992;9(3):207-10.

135. Witkin SS, Ledger WJ. Antibodies to Chlamydia trachomatis in sera of women with recurrent spontaneous abortions. Am J Obstet Gynecol 1992;167(1):135-9.

136. Rae R, Smith IW, Liston WA, Kilpatrick DC. Chlamydial serologic studies and recurrent spontaneous abortion. Am J Obstet Gynecol 1994;170(3):782-5.

137. Sugiura-Ogasawara M, Ozaki Y, Nakanishi T, Kumamoto Y, Suzumori K. Pregnancy outcome in recurrent aborters is not influenced by Chlamydia IgA and/or G. Am J Reprod Immunol 2005;53(1):50-3.

138. Osser S, Persson K. Chlamydial antibodies in women who suffer miscarriage. Br J Obstet Gynaecol 1996;103(2):137-41.

139. Paukku M, Tulppala M, Puolakkainen M, Anttila T, Paavonen J. Lack of association between serum antibodies to Chlamydia trachomatis and a history of recurrent pregnancy loss. Fertil Steril 1999;72(3):427-30.

140. Carey C, Klebanoff M, Hauth J, Hillier S, Thom E, Ernest J, et al. Metronidazole to Prevent Preterm Delivery in Pregnant Women with Asymptomatic Baceterial Vaginosis. The New England Journal of Medicine 2000;342(8):534-540.

141. Kekki M, Kurki T, Pelkonen J, Kurkinen-Raty M, Cacciatore B, Paavonen J. Vaginal clindamycin in preventing preterm birth and peripartal infections in asymptomatic women with bacterial vaginosis: a randomized, controlled trial. Obstet Gynecol 2001;97(5 Pt 1):643-8.

142. Elliott B, Brunham RC, Laga M, Piot P, Ndinya-Achola JO, Maitha G, et al. Maternal gonococcal infection as a preventable risk factor for low birth weight. J Infect Dis 1990;161(3):531-6.

143. Martius J, Krohn MA, Hillier SL, Stamm WE, Holmes KK, Eschenbach DA. Relationships of vaginal Lactobacillus species, cervical Chlamydia trachomatis, and bacterial vaginosis to preterm birth. Obstet Gynecol 1988;71(1):89-95.

144. Guerra B, Ghi T, Quarta S, Morselli-Labate AM, Lazzarotto T, Pilu G, et al. Pregnancy outcome after early detection of bacterial vaginosis. Eur J Obstet Gynecol Reprod Biol 2006;128:40-45.

145. Hay PE, Lamont RF, Taylor-Robinson D, Morgan DJ, Ison C, Pearson J. Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. Bmj 1994;308(6924):295-8.

146. McGregor JA, French JI, Parker R, Draper D, Patterson E, Jones W, et al. Prevention of premature birth by screening and treatment for common genital tract infections: results of a prospective controlled evaluation. Am J Obstet Gynecol 1995;173(1):157-67.

147. Oakeshott P, Hay P, Hay S, Steinke F, Rink E, Kerry S. Association between bacterial vaginosis or chlamydial infection and miscarriage before 16 weeks' gestation: prospective community based cohort study. Bmj 2002;325(7376):1334.

148. Ralph SG, Rutherford AJ, Wilson JD. Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. Bmj 1999;319(7204):220-3.

149. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. N Engl J Med 1995;333(26):1737-42.

150. Holst E, Goffeng AR, Andersch B. Bacterial vaginosis and vaginal microorganisms in idiopathic premature labor and association with pregnancy outcome. J Clin Microbiol 1994;32(1):176-86.

151. Kurki T, Sivonen A, Renkonen OV, Savia E, Ylikorkala O. Bacterial vaginosis in early pregnancy and pregnancy outcome. Obstet Gynecol 1992;80(2):173-7.

152. Meis PJ, Goldenberg RL, Mercer B, Moawad A, Das A, McNellis D, et al. The preterm prediction study: significance of vaginal infections. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Am J Obstet Gynecol 1995;173(4):1231-5.

153. Hauth JC, Goldenberg RL, Andrews WW, DuBard MB, Copper RL. Reduced incidence of preterm delivery with metronidazole and erythromycin in women with bacterial vaginosis. N Engl J Med 1995;333(26):1732-6.

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

155. McDonald HM, O'Loughlin JA, Vigneswaran R, Jolley PT, Harvey JA, Bof A, et al. Impact of metronidazole therapy on preterm birth in women with bacterial vaginosis flora (Gardnerella vaginalis): a randomised, placebo controlled trial. Br J Obstet Gynaecol 1997;104(12):1391-7.

156. Carey JC, Klebanoff MA, Hauth JC, Hillier SL, Thom EA, Ernest JM, et al. Metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis. National

Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. N Engl J Med 2000;342(8):534-40.

157. Public Health Service, Center for Health Statistics. National survey of family growth. Hyattsville, Maryland: US Department of Health and Human Services. 1995.

158. Westrom L. Effect of acute pelvic inflammatory disease on fertility. Am J Obstet Gynecol 1975;121:707-713.

159. Kiviat NB, Wolner-Hanssen P, Eschenbach DA, Wasserheit JN, Paavonen JA, Bell TA, et al. Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis. Am J Surg Pathol 1990;14(2):167-75.

160. Simms I, Mallinson H, Peeling R, Thomas K, Gokhale R, Rogers P. Risk factors associated with pelvic inflammatory disease: a UK study. Int J STD & AIDS 2002;13:18.

161. Haggerty CL, Hillier SL, Bass DC, Ness RB. Bacterial vaginosis and anaerobic bacteria are associated with endometritis. Clin Infect Dis 2004;39(7):990-5.

162. Williams J, VanDerPol B, Batteiger B, al. E. Mycoplasma genitalium symptoms and concordance in sexual dyads. International Society for STD Research Book of Abstracts 2007;abstract O-034.

163. Eckert L, Thwin S, Hillier S, Kiviat N, Eschenbach D. The antimicrobial treatment of sub-acute endometritis: a proof of concept study. Am J Obstet Gynecol 2004;190(2):305-313.

164. Wiesenfeld H, Hillier S, Krohn M, al. e. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. Obstet Gynecol 2002;100:456-463.

165. Henry-Suchet J, Utzmann C, De Brux J, Ardoin P, Catalan F. Microbiologic study of chronic inflammation associated with tubal factor infertility: role of Chlamydia trachomatis. Fertil Steril 1987;47(2):274-7.

166. Moore DE, Spadoni LR, Foy HM, Wang SP, Daling JR, Kuo CC, et al. Increased frequency of serum antibodies to Chlamydia trachomatis in infertility due to distal tubal disease. Lancet 1982;2(8298):574-7.

167. Sellors JW, Mahony JB, Chernesky MA, Rath DJ. Tubal factor infertility: an association with prior chlamydial infection and asymptomatic salpingitis. Fertil Steril 1988;49(3):451-7.

168. Haggerty CL, Peipert JF, Weitzen S, Hendrix SL, Holley RL, Nelson DB, et al. Predictors of chronic pelvic pain in an urban population of women with symptoms and signs of pelvic inflammatory disease. Sex Transm Dis 2005;32(5):293-9.

169. Ness RB, Soper DE, Peipert J, Sondheimer SJ, Holley RL, Sweet RL, et al. Design of the PID Evaluation and Clinical Health (PEACH) Study. Control Clin Trials 1998;19(5):499-514.

170. Dutro S, Hebb J, Garin C, Hughes J, Kenny G, Totten P. Development and performance of a microwell-plate-based polymerase chain reaction assay for Mycoplasma genitalium. Sexually Transmitted Diseases 2003;30:756-63.

171. Nugent R, Krohn M, Hillier S. Reliability of diagnosing bacterial vaginosis is improved by a standardized Gram stain interpretation. Journal of Clinical Microbiology 1991;29:297-301.

172. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983;74(1):14-22.

173. Moller BR, Taylor-Robinson D, Furr PM, Freundt EA. Acute upper genital-tract disease in female monkeys provoked experimentally by Mycoplasma genitalium. Br J Exp Pathol 1985;66(4):417-26.

174. Haggerty C, Totten P, Astete S, Lee S, Hoferka S, Kelsey S, et al. Persistent Mycoplasma genitalium and endometritis among women treated for pelvic inflammatory disease. International Society for STD Research Book of Abstracts 2007;abstract O-036.

175. Short VL, Totten PA, Ness RB, Astete SG, Kelsey SF, Haggerty CL. Mycoplasma genitalium has a less severe clinical presentation than Neisseria gonorrhoeae among women with clinically suspected pelvic inflammatory disease Clinical Infectious Diseases 2009;48(1):41-7.

176. Lindstrand A, Bergstrom S, Bugalho A, Zanconato G, Helgesson AM, Hederstedt B. Prevalence of syphilis infection in Mozambican women with second trimester miscarriage and women attending antenatal care in second trimester. Genitourin Med 1993;69(6):431-3.

177. Schulz KF, Cates W, Jr., O'Mara PR. Pregnancy loss, infant death, and suffering: legacy of syphilis and gonorrhoea in Africa. Genitourin Med 1987;63(5):320-5.

178. Haggerty CL, Totten PA, Astete SG, Ness RB. Mycoplasma genitalium among women with nongonococcal, nonchlamydial pelvic inflammatory disease. Infect Dis Obstet Gynecol 2006;2006:30184.

179. Jensen J, Borre M, Dohn B. Detection of *Mycoplasma genitalium* by PCR amplification of the 16S rRNA gene. J Clin Microbiol 2003;41:261–266.

180. Jensen J, Uldum S, Sondergard-Anderson J, Vuust J, Link K. Polymerase chain reaction for detection of Mycoplasma genitalium in clinical samples. J Clin Micorbiol 1991;29:46-50.

181. Jensen J, Bjornelius E, Dohn B, Lidbrink P. Comparison of first void urine and urogenital swab specimens for detection of Mycoplasma genitalium and Chlamydia trachomatis by polymerase chain reaction in patients attending a sexually transmitted disease clinic. Sex Transm Dis 2004;31(8):499-507.

182. Loeffelholz M, Lewinski C, Silver S. Detection of Chlamydia trachomatis in endocervical specimens by polymerase chain reaction. J Clin Microbiol 1992;30:2847-2851.

183. Pollard D, Tyler S, Ng C-W, Rozee K. A polymerase chain reaction (PCR) protocol for the specific detection of Chlamydia spp. Mol Cell Probes 1989;3:383-389.

184. Storgaard M, Ostergaard L, Jensen J. Chlamydia pneumoniae in children with otitis media. Clin Infect Dis 1997;25:1090-1093.

185. Nelson D, Grisso J, Joffe M, Brensinger C, Ness R, McMahon K, et al. Violence does not influence early pregnany loss. Ferility and Sterility 2003;80(5):1205-1211.

186. Hillis SD, Joesoef R, Marchbanks PA, Wasserheit JN, Cates W, Jr., Westrom L. Delayed care of pelvic inflammatory disease as a risk factor for impaired fertility. Am J Obstet Gynecol 1993;168(5):1503-9.

187. Hay PE. Bacterial vaginosis and miscarriage. Curr Opin Infect Dis 2004;17(1):41-4.