## Original Paper

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# Prevalence of *Mollicutes* in pregnant women undergoing high-risk prenatal care at a maternal and child reference unit in Bahia, Brazil

Fabrícia Almeida Fernandes Santana[1], Jéssica Bomfim[2], Mariana Ferraz[2], Victória Cardoso[2], Jassy Borges[2], Danielle Souto de Medeiros[2], Maurício Grijó[2], Guilherme B. Campos[2] and Lucas Miranda Marques[1,2]

[1]Microbiology Department, State University of Santa Cruz (UESC), Ilhéus, Brazil and [2]Biointeraction Department, Multidisciplinary Institute of Health, Federal University of Bahia (UFBA), Vitória da Conquista, Brazil

## Abstract

During pregnancy, colonization by genital mycoplasmas may be associated with adverse outcomes. This study was conducted to investigate the prevalence of four species of *Mollicutes* (*Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma parvum*, and *Ureaplasma urealyticum*) in pregnant women receiving high-risk prenatal care and to evaluate possible associated factors. Data collection included the application of a questionnaire and the collection of cervical swabs from pregnant women. Species identification was performed by real-time PCR. The overall prevalence of *Mollicutes* was 60.97%. 55.9% of pregnant women were colonized by *Ureaplasma* spp., and 19.51% by *Mycoplasma* spp. The prevalence rates by species were 48.78% for *U. parvum*, 11.59% for *U. urealyticum*, 18.9% for *M. hominis*, and 1.22% for *M. genitalium*. Age, 12 years of schooling or more, age at first sexual intercourse up to 14 years, third trimester of pregnancy, having undergone infertility treatment, presence of STI, and groin lymph nodes were associated with a higher prevalence of microorganisms. The results presented are of utmost importance for understanding the prevalence of these microorganisms, the characteristics of colonized pregnant women, and planning screening strategies and interventions that minimize the negative impacts of these infections.

## Introduction

Infections by *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma genitalium*, and *Mycoplasma hominis*, species of the *Mollicutes* class and collectively referred to as genital mycoplasmas, have gained increasing attention due to their impacts on women's health. These microorganisms commonly colonize the urogenital tract, often without symptoms, but are associated with several clinical conditions such as urethritis, cervicitis, and pelvic inflammatory disease [1]. During pregnancy, colonization by mycoplasmas may be related to adverse outcomes, including spontaneous abortion, preterm birth, premature rupture of membranes, chorioamnionitis, and low birth weight [2]. Furthermore, vertical transmission of mycoplasmas during childbirth can cause neonatal infections, increasing neonatal morbidity and mortality [3].

The appropriate diagnosis and treatment of genital mycoplasma infections are challenging due to the insidious nature of these bacteria and their resistance to common antibiotics [4]. The most sensitive detection of these pathogens involves molecular biology techniques, which are not always available in resource-limited healthcare settings. In addition, the individual pathogenic potential of genital mycoplasma species, their synergy with other microorganisms, their interaction with the host immune system, and the effects that microbial load has on the change from mere colonization to infection with clinical repercussions are still unclear [5].

This study aims to contribute to the epidemiological knowledge of these infections, offering data that can guide prevention, diagnosis, and treatment. Although studies are showing the prevalence and adverse effects of genital mycoplasma infection in pregnant women, the data are still insufficient to clarify all aspects of this interaction, and it is crucial to investigate the prevalence of *Mollicutes* in pregnant women, evaluate the associated risk factors and the clinical implications of these infections [6]. Thus, the objective of the study was to investigate the presence of four species of *Mollicutes* (*M. hominis*, *M. genitalium*, *U. parvum*, and *U. urealyticum*) in the cervical region of pregnant women attended for high-risk prenatal care at a maternal and child referral unit in southwestern Bahia, Brazil, and to evaluate possible associated factors.

## Methods

### Study design

This cross-sectional study aimed to determine the prevalence of genital mycoplasma colonization in pregnant women. Researchers from the Federal University of Bahia and the State University of Santa Cruz collected data and biological material from pregnant women attending high-risk prenatal care at a maternal and child health unit in Vitória da Conquista, Bahia, Brazil.

### Population

Pregnant women aged 18 years or older, at any gestational age, and meeting the criteria for high-risk prenatal care by the Technical Manual for High-Risk Pregnancy of the Brazilian Ministry of Health (Brazil, 2022), such as obesity, repeated spontaneous abortion, pre-term birth in a previous pregnancy, hypertension or diabetes before pregnancy, infectious diseases during pregnancy and uterine mal-formations, took part in the study. Pregnant women who used antibiotics to treat infection in the last 14 days before collection were excluded. The sample consisted of 164 pregnant women treated between November 2021 and November 2022.

### Ethical procedures

This research complied with the provisions of Resolutions 466/12 and 510/2016 of the National Health Council, which regulate research involving human beings. It was carried out only after approval by the Ethics and Research Committee of the Multidisciplinary Institute of Health of the Federal University of Bahia – IMS/UFBA, under CAAE: 40117020.4.0000.5556. The researchers approached the participants empathetically, and the objectives of the study and the details regarding data collection and biological materials that would be collected were presented through the reading of the Free and Informed Consent Form (FICF); after consent, this was then signed by the participant, who kept a copy.

### Data and sample collection

Data collection consisted of applying a semi-structured questionnaire to identify the patient's epidemiological profile, which was divided into modules and adapted from the study by Campos et al. [7]. In addition, a cervical sample was collected by the gynecologists/obstetricians participating in the research and included in the routine of the health service. The order of collection of the cervical sample and questionnaire was conducted not to hinder the flow of clinical care in the health service. The cervical material was collected with a sterile cotton swab in the doctor's office, with the aid of a speculum, packaged in a Stuart transport medium, and transported in isothermal boxes to the Microbiology and Immunology laboratory of the Multidisciplinary Institute of Health, Federal University of Bahia. Upon arrival at the laboratory, the swabs were packaged in 15 ml Falcon tubes, incorrectly identified as containing 5 ml of PBS. The samples were then homogenized, aliquoted into 1.5 ml microtubes, and stored at −20 °C until processing.

### DNA extraction and identification by qPCR

For microbial identification of cervical samples, genomic DNA was initially extracted using the standardized boiling and PBS method [8]. DNA aliquots were then subjected to quantification and quality analysis by spectrophotometry in a NanoDrop (Thermo Scientific TM 5 NanoDrop 2000) at OD 260/280, also observing whether there was the presence of contaminants, such as lipids and proteins. Then, a real-time polymerase chain reaction was performed in StepOne Plus (Life Technologies) with a final volume of 25 μl and Master Mix (Thermo Fisher Scientific, Waltham, MA, United States). To identify the microbial species, TaqMan probes were used, following a basic amplification protocol for the species *U. urealyticum* [9], *U. parvum* [9], *M. hominis* [10], and *M. genitalium* [11]. Positive control (DNA extracted from the culture of microorganisms isolated and characterized in previous studies by the same research group and kept at −70 °C in PBS medium), negative control (no DNA), and samples were included.

### Statistical analysis

Initially, descriptive analysis of all variables was performed using absolute and relative frequencies (%). The prevalence of all outcomes was estimated, along with their 95% confidence intervals (95% CI). A bivariate analysis of the explanatory variables and outcomes was performed. Differences between categorical variables and the occurrence of each outcome were tested using Pearson's chi-squared test or Fisher's exact test. In all tests, $p < 0.05$ was considered significant. The prevalence ratio (PR) and its respective 95% confidence interval (95% CI) were estimated using Poisson regression with robust variance.

Multivariate analysis was performed for the following outcomes: presence of *Mollicutes*, presence of *Ureaplasma* spp., presence of *Mycoplasma* spp., presence of *U. urealyticum*, presence of *U. parvum*, and presence of *M. hominis*. For this stage, the variables that obtained a $p$-value <0.20 in the bivariate analysis were selected. The multivariate models were analyzed using the backward stepwise method for variable selection and compared using the Akaike information criterion (AIC). The adequacy of the final model was assessed using the chi-squared test (goodness-of-fit). Only variables with a $p$-value <0.05 remained in the final statistical model. The statistical software STATA version 16.1 (Stata Corporation, College Station, USA) was used for all analyses.

## Results

There was variation in the sample size, as collecting information from the questionnaire for five pregnant women was impossible. For this reason, the data presented in Table 1 refer to the sample number of 159 pregnant women who collected swabs and questionnaires. Most study participants were between 20 and 34 years old, had a partner at the time of the study, were from urban areas, reported being non-black, had 12 or more years of education, and had an income of less than one minimum wage. Most were in the 3rd trimester of pregnancy, did not practice physical activity (aerobic exercise or weight training at least three times a week), and did not drink alcohol. Regarding gynecological and obstetric history, most reported not having had fibroids, polycystic ovaries, or other gynecological alterations, had not had an ectopic pregnancy, and had not undergone infertility treatment. Regarding STI history (HIV, syphilis, hepatitis B and C, HPV, genital herpes, gonorrhea, chlamydia and trichomoniasis), most reported not having had an STI in the past, had not undergone treatment for STI, and current STI tests (performed at the health service and which did not include mycoplasmas for either the pregnant woman or the partner) were negative. Most had their first sexual intercourse after the age of

**Table 1.** Characteristics of pregnant women receiving high-risk prenatal care in southwestern Bahia (*n* = 159). Brazil, 2021–2022

| Variables | *n*[a] | % | CI 95%[b] |
|---|---|---|---|
| **Age** | | | |
| Up to 19 years | 22 | 13.8 | 09.2–20.2 |
| 20 to 34 years | 91 | 57.2 | 49.4–64.7 |
| 35 years and over | 46 | 28.9 | 22.4–36.5 |
| **Marital status** | | | |
| With partner | 145 | 91.2 | 85.6–94.7 |
| Without partner | 14 | 8.8 | 5.3–14.4 |
| **Origin** | | | |
| Urban area | 119 | 74.8 | 67.5–81.0 |
| Rural area | 40 | 25.2 | 19.0–32.5 |
| **Color** | | | |
| Black | 35 | 22.0 | 16.2 -29.2 |
| Non-black | 124 | 78.0 | 70.8–83.8 |
| **Education** | | | |
| Up to 11 years | 65 | 40.9 | 33.4–48.7 |
| 12 years or older | 94 | 59.1 | 51.3–66.5 |
| **Income** | | | |
| <1 minimum wage | 85 | 53.8 | 46.0–61.5 |
| 1 minimum wage or more | 73 | 46.2 | 38.5–54.1 |
| **Gestational age** | | | |
| 1st trimester | 14 | 09.0 | 05.4–14.7 |
| 2nd trimester | 66 | 42.6 | 35.0–50.5 |
| 3nd trimester | 75 | 48.4 | 40.5–56.3 |
| **Physical activity** | | | |
| No | 125 | 78.6 | 71.5–84.3 |
| Yes | 34 | 21.4 | 15.6–28.5 |
| **Use of alcoholic beverages** | | | |
| No | 150 | 94.3 | 89.4–97.0 |
| Yes | 9 | 5.7 | 2.9–10.7 |
| **Myoma** | | | |
| No | 143 | 89.9 | 84.1—93.8 |
| Yes | 16 | 10.1 | 6.2–15.8 |
| **Polycystic ovaries** | | | |
| No | 136 | 85.5 | 79.1—90.2 |
| Yes | 23 | 14.5 | 9.8–20.9 |
| **Other gynecological alteration** | | | |
| No | 146 | 91.8 | 86.4–95.2 |
| Yes | 13 | 8.2 | 4.8–13.6 |
| **Had any STIs[c]** | | | |
| No | 144 | 90.6 | 84.9–94.2 |
| Yes | 15 | 9.4 | 5.7–15.1 |
| **Underwent treatment for STI** | | | |
| No | 145 | 91.2 | 85.6–94.7 |

(*Continued*)

**Table 1.** (*Continued*)

| Variables | *n*[a] | % | CI 95%[b] |
|---|---|---|---|
| Yes | 14 | 8.8 | 5.3–14.4 |
| **Ectopic pregnancy** | | | |
| No | 153 | 96.2 | 91.8–98.3 |
| Yes | 6 | 3.8 | 1.7–8.2 |
| **Infertility treatment** | | | |
| No | 149 | 93.7 | 88.6–96.6 |
| Yes | 10 | 6.3 | 3.4–11.3 |
| **STI testing during prenatal care** | | | |
| Negative | 153 | 96.2 | 91.8–98.3 |
| Positive | 6 | 3.8 | 1.7–8.2 |
| **Age of first sexual intercourse** | | | |
| Up to 14 years | 26 | 17.3 | 12.0–24.3 |
| 15 years or older | 124 | 82.7 | 75.7–88.0 |
| **Had more than 1 sexual partner** | | | |
| No | 46 | 29.9 | 23.1–37.6 |
| Yes | 108 | 70.1 | 62.4–76.9 |
| **Pain during sexual intercourse** | | | |
| No | 28 | 18.1 | 12.7–25.0 |
| Yes | 127 | 81.9 | 75.0–87.3 |
| **Bleeding during intercourse** | | | |
| No | 148 | 95.5 | 90.8–97.9 |
| Yes | 7 | 4.5 | 2.1–9.2 |
| **Condom use** | | | |
| No/Sometimes | 121 | 78.1 | 70.8–84.0 |
| Yes | 34 | 21.9 | 16.0–29.2 |
| **Year of last gynecological examination** | | | |
| In the last year | 87 | 54.7 | 46.9–62.3 |
| More than 1 year | 72 | 45.3 | 37.7–53.1 |
| **Use of contraceptives** | | | |
| No | 77 | 49.0 | 41.2–56.9 |
| Yes | 80 | 51.0 | 43.1–58.7 |
| **Number of pregnancies** | | | |
| 1 | 51 | 32.1 | 25.2–39.8 |
| 2 | 45 | 28.3 | 21.8–31.8 |
| 3 or more | 63 | 39.6 | 32.3–47.5 |
| **Abortion** | | | |
| None | 112 | 70.9 | 63.3–77.5 |
| 1 or more | 46 | 29.1 | 22.5–36.7 |
| **Preterm birth** | | | |
| None | 128 | 81.0 | 74.0–86.4 |
| 1 or more | 30 | 19.0 | 13.6–25.9 |
| **Stillborn** | | | |
| None | 131 | 83.4 | 76.7–88.5 |
| 1 or more | 26 | 16.6 | 11.5–23.3 |

(*Continued*)

Table 1. (Continued)

| Variables | $n^a$ | % | CI 95%$^b$ |
|---|---|---|---|
| Expected prenatal appointments | | | |
| ≥6 consultations | 117 | 76.5 | 69.0–82.5 |
| <6 consultations | 36 | 23.5 | 17.4–30.9 |
| Use of antibiotics in the last 3 months | | | |
| No | 102 | 64.2 | 56.3–71.3 |
| Yes | 57 | 35.9 | 28.7–43.6 |
| Presence of symptoms during pregnancy | | | |
| None | 29 | 18.1 | 12.9–24.9 |
| 1 | 55 | 34.4 | 27.4–42.1 |
| 2 or more | 76 | 47.5 | 39.8–55.3 |
| Vaginal discharge | | | |
| No | 76 | 47.8 | 40.0–55.6 |
| Yes | 83 | 53.2 | 44.4–60.0 |
| Itching | | | |
| No | 136 | 85.5 | 79.1–90.2 |
| Yes | 23 | 14.5 | 9.8–20.9 |
| Vaginal erythema | | | |
| No | 154 | 96.9 | 92.6–98.7 |
| Yes | 5 | 3.1 | 1.3–7.4 |
| Pelvic pain | | | |
| No | 84 | 52.8 | 45.0–60.0 |
| Yes | 75 | 47.2 | 39.5–55.0 |
| Genital warts | | | |
| No | 158 | 99.4 | 95.6–99.9 |
| Yes | 1 | 0.6 | 0.0–4.3 |
| Genital vesicles | | | |
| No | 156 | 98.1 | 94.3–99.4 |
| Yes | 3 | 1.9 | 0.6–5.7 |
| Genital wounds | | | |
| No | 157 | 99.4 | 95.6–99.9 |
| Yes | 1 | 0.6 | 0.0–4.4 |
| Pain when urinating | | | |
| No | 133 | 83.7 | 77.0–88.6 |
| Yes | 26 | 16.4 | 11.3–23.0 |
| Swollen glands in the groin | | | |
| No | 151 | 94.4 | 89.5–97.0 |
| Yes | 9 | 5.6 | 2.93–10.5 |
| Other gynecological alteration | | | |
| No | 125 | 78.1 | 71.0–83.0 |
| Yes | 35 | 21.9 | 16.10–29.0 |
| Partner treatment for infertility | | | |
| No | 149 | 93.7 | 88.6–96.6 |
| Yes | 10 | 6.3 | 3.4–11.3 |

(Continued)

Table 1. (Continued)

| Variables | $n^a$ | % | CI 95%$^b$ |
|---|---|---|---|
| Partner treatment for STI | | | |
| No | 149 | 94.3 | 89.4–97.0 |
| Yes | 9 | 5.7 | 3.0–10.6 |

$^a$n = absolute frequency.
$^b$IC95%: confidence interval 95%.
$^c$STI = Sexually transmitted infection.

15, had more than one sexual partner during their lifetime, reported pain during sexual intercourse, reported bleeding during sexual intercourse, and did not use condoms or used them inconsistently. More than half had undergone a gynecological examination within 1 year before the survey and used contraceptives. Regarding obstetric history, the number of pregnancies was relatively homogeneous: 1, 2, or 3 or more. Most had no miscarriages, preterm births, or stillbirths. Regarding the number of expected prenatal consultations, most had six or more consultations and had not used antibiotics in the last 3 months. Most pregnant women had one or two or more symptoms at some point during pregnancy. More than half of the pregnant women had vaginal discharge as a symptom. Regarding information about their partners, most had not undergone treatment for infertility or STIs.

The overall prevalence of *Mollicutes* was 60.97% ($n = 100$). 55.9% ($n = 91$) of the pregnant women were colonized by *Ureaplasma* spp., and 19.51% ($n = 32$) were colonized by *Mycoplasma* spp. The prevalences by species were: 48.78% ($n = 80$) *U. parvum*, 11.59% ($n = 19$) *U. urealyticum*, 18.9% ($n = 31$) *M. hominis*, and 1.22% ($n = 2$) *M. genitalium* (Table 2). Coinfection between *Mollicutes* species occurred in 17.07% ($n = 28$), and isolated infection occurred in 43.9% ($n = 72$) of the participants (Table 2). Figure 1 shows the detection percentage for isolated infection and coinfection among positive cases.

Table 3 presents the results that showed statistical significance in the bivariate analysis for the prevalence of *Mollicutes*, *Mycoplasma* spp., and *Ureaplasma* spp. Table 4 shows the statistical significance of the bivariate analysis for *Ureaplasma* spp., *U. parvum*, and *U. urealyticum*. Table 5 presents the statistical significance data from the bivariate analysis for *Mycoplasma* spp., *M. genitalium*, and *M. hominis*. The complete data from the bivariate analysis can be found in Supplementary Tables S1, S2, and S3, which are included in the Supplementary Materials.

After multivariate analysis, the following factors were associated with a higher prevalence of *Mollicutes*: 12 or more years of schooling (PR = 1.36, CI95% = 1.04–1.78), age at first sexual intercourse up to 14 years (PR = 1.41, CI95% = 1.07–1.84), and infertility treatment (PR = 0.32, CI95% = 0.14–0.75). For *Ureaplasma* spp.: age at first sexual intercourse up to 14 years (PR = 1.37, CI95% = 1.03–1.81) and infertility treatment (PR = 0.27, CI95% = 0.09–0.76). For *Mycoplasma* spp., being between 20 and 34 years of age (PR = 2.61, CI95% = 1.10–6.20). For *U. urealyticum*: being in the 3rd trimester of pregnancy (PR = 0.24, CI95% = 0.08–0.77), having had STIs (PR = 2.58, CI95% = 1.09–6.12), and having groin lymph nodes (PR = 3.07, CI95% = 1.04–9.04). For *U. parvum*: 12 years of schooling or more (PR = 1.60, CI95% = 1.11–2.30), age at first sexual intercourse up to 14 years (PR = 1.60, CI95% = 1.13–2.26), and infertility treatment (PR = 0.30, CI95% = 0.11–0.82). For *M. hominis*: age between 20 and 34 years (PR = 2.57, CI95% = 1.08–6.11). These data are presented in Table 6.

**Table 2.** Prevalence of *Mollicutes* colonization in pregnant women receiving high-risk prenatal care in southwestern Bahia (*n* = 164). Brazil, 2021–2022

| Variables | *n*[a] | % | CI 95%[b] |
|---|---|---|---|
| *Mycoplasma* spp. | 32 | 19.5 | 14.1–26.3 |
| *Mycoplasma genitalium* | 2 | 1.2 | 0.3–4.8 |
| *Mycoplasma hominis* | 31 | 18.9 | 13.6–25.7 |
| *Ureaplasma* spp. | 91 | 55.9 | 47.7–63.0 |
| *Ureaplasma urealyticum* | 19 | 11.6 | 7.5–17.5 |
| *Ureaplasma parvum* | 80 | 48.8 | 41.2–56.4 |
| *Mollicutes* infection | | | |
| None | 64 | 39.2 | 31.8–46.7 |
| Isolated | 72 | 43.9 | 36.4–51.6 |
| Co-infection | 28 | 17.1 | 12.0–23.7 |

[a]*n* = absolute frequency.
[b]CI95%: confidence interval 95%.

## Discussion

The prevalence of genital mycoplasmas in pregnant women has been reported in studies conducted in other countries and, to a lesser extent, in Brazil [12–19]. Some of these studies have attempted to associate risk factors for infection with adverse outcomes during pregnancy. However, it is still unclear whether all species are directly involved in the infection, whether the bacterial load is decisive, whether the crucial point is the interaction with other microorganisms, or whether it depends on the host response [6]. In Brazil, *Mollicutes* infections are not part of the standard STI

screening protocol for pregnant women, and, in the absence of rapid diagnostic tests and difficulty in accessing molecular tests, syndromic treatment has been the practice. This was the first study to assess the prevalence of *Mollicutes* in pregnant women receiving high-risk prenatal care in the interior of Bahia.

The overall prevalence of *Mollicutes* found in our study (60.97%) was higher than the 9.3% reported in the survey by Peretz et al. [16] carried out with 214 pregnant women in Israel, in which vaginal swab samples and detection by culture and PCR were used. The use of vaginal swab in the study above may explain the differences mentioned, since a higher rate of colonization by *Mollicutes* in the cervical region is expected, as occurred in our study. The prevalence was also higher than that observed in the study by Lee et al. [13], who detected 571 cases of MH and UU among 1035 participants in South Korea. However, this study used culture as the detection method, and molecular detection, compared to culture, is expected to provide a higher detection rate due to the higher sensitivity of the technique. By species, we detected a higher prevalence of *U. parvum*, which is in agreement with the study by Payne et al. [15] carried out in Australia, which found a higher prevalence of *U. parvum* (between 35% and 63%) at three different times during pregnancy. This higher prevalence of *U. parvum* was not observed in studies such as that of Abdelaziz et al. [12], which showed a higher prevalence of *M. hominis*, and that of Lee et al. [13], which found a higher prevalence of *U. urealyticum*.

The present study also identified a high co-infection by at least two species, emphasizing co-infection between *M. hominis* and *U. urealyticum*. A retrospective cohort study carried out by Jeon et al. [20] in South Korea included 1381 pregnant women admitted to a high-risk unit and demonstrated that the group with genital colonization by mycoplasmas had a higher risk of premature
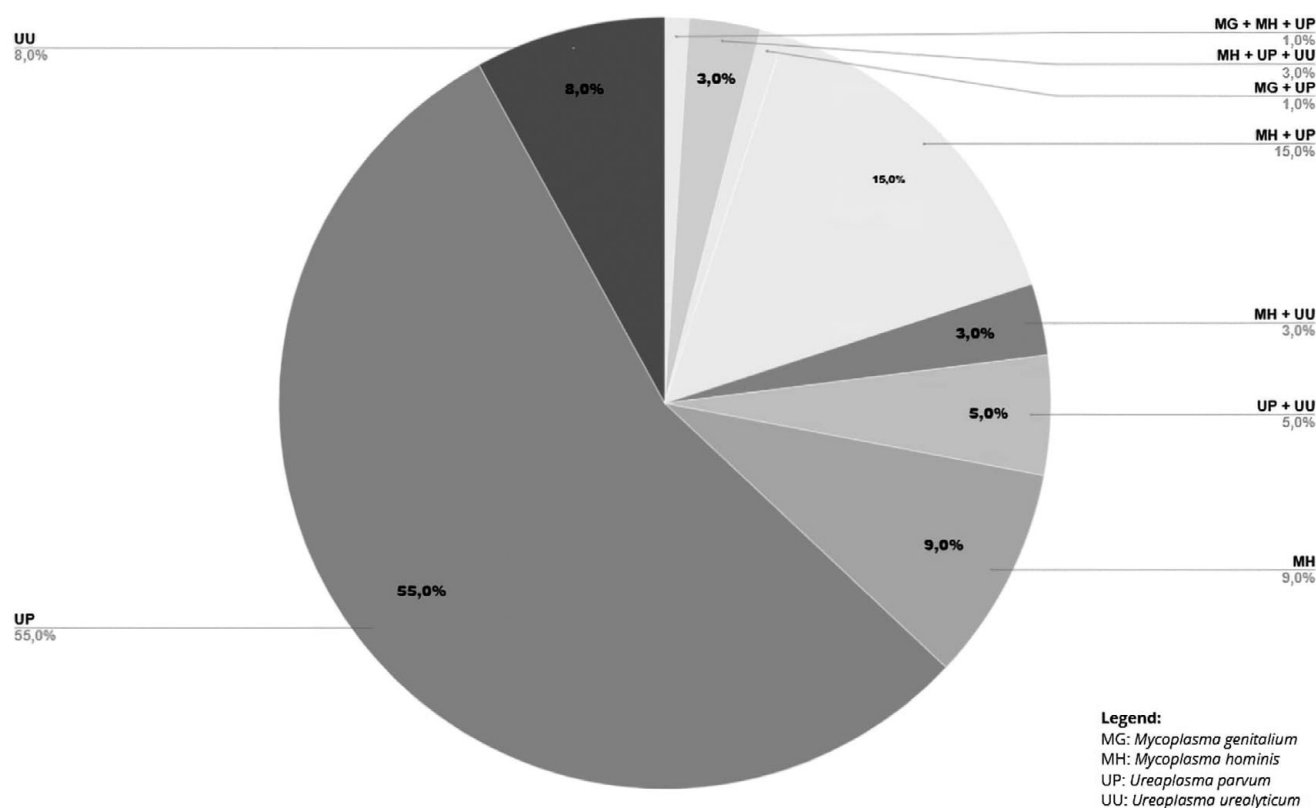


**Figure 1.** Coinfection between Mollicutes species in pregnant women receiving high-risk prenatal care in southwesternBahia (n = 100). Brazil, 2021-2022.

**Table 3.** Bivariate analysis for the prevalence of *Mollicutes*, *Mycoplasma* spp. and *Ureaplasma* spp. in pregnant women receiving high-risk prenatal care in southwestern Bahia (*n* = 164). Brazil, 2021–2022

| Variables | Mollicutes | | | | | Mycoplasma spp. | | | | | Ureaplasma spp. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $n$[a] | P (%)[b] | p-value | PR[c] | CI 95%[d] | $n$[a] | P (%)[b] | p-value | PR[c] | CI 95% | $n$[a] | P (%)[b] | p-value | PR[c] | CI 95% |
| Age | | | **0.031** | | | | | **0.001** | | | | | 0.237 | | |
| Up to 19 years old | 19 | 86.40 | | 1.00 | – | 11 | 50.00 | | 1.00 | – | 16 | 72.73 | | 1.00 | – |
| 20–34 | 51 | 56.00 | | 0.65 | 0.51–0.83 | 13 | 14.29 | | 2.88 | 1.35–6.14 | 48 | 52.75 | | 0.73 | 0.90–1.85 |
| 35 or more | 29 | 63.00 | | 0.73 | 0.55–0.96 | 8 | 17.39 | | 0.82 | 0.37–1.84 | 26 | 56.52 | | 0.78 | 0.68–1.29 |
| Marital status | | | 0.459 | | | | | **0.037**[e] | | | | | 0.241 | | |
| With partner | 89 | 61.38 | | 1.00 | – | 32 | 22.07 | | 1.00 | – | 80 | 55.17 | | 1.00 | – |
| Without partner | 10 | 71.43 | | 1.16 | 0.81–1.66 | ** | ** | ** | ** | ** | 10 | 71.43 | | 1.29 | 0.90–1.86 |
| Gestational age | | | 0.749 | | | | | **0.024** | | | | | 0.356 | | |
| 1st trimester | 10 | 71.43 | | 1.00 | – | ** | ** | | ** | ** | 10 | 71.43 | | 1.00 | – |
| 2 nd trimester | 40 | 60.61 | | 0.85 | 0.57–1.24 | 10 | 15.15 | | ** | ** | 39 | 59.09 | | 0.83 | 0.56–1.22 |
| 3rd trimester | 47 | 62.67 | | 0.88 | 0.60–1.28 | 21 | 28.00 | | ** | ** | 39 | 52.00 | | 0.73 | 0.49–1.08 |
| Myoma | | | **0.031** | | | | | 0.335[e] | | | | | 0.104 | | |
| No | 93 | 65.03 | | 1.00 | – | 30 | 20.98 | | 1.00 | – | 84 | 58.74 | | 1.00 | – |
| Yes | 6 | 37.50 | | 0.58 | 0.30–1.10 | 2 | 12.50 | | 0.60 | 0.16–2.27 | 6 | 37.5 | | 0.64 | 0.33–1.22 |
| Polycystic ovaries | | | **0.013** | | | | | 0.488[e] | | | | | **0.022** | | |
| No | 90 | 66.18 | | 1.00 | – | 28 | 20.59 | | 1.00 | – | 82 | 60.29 | | 1.00 | – |
| Yes | 9 | 39.13 | | 0.59 | 0.35–1.00 | 4 | 17.39 | | 0.84 | 0.33–2.19 | 8 | 34.78 | | 0.58 | 0.32–1.03 |
| Age of first sexual intercourse | | | 0.176 | | | | | **0.030** | | | | | 0.117 | | |
| ≥15 years | 73 | 58.87 | | 1.00 | – | 20 | 16.13 | | 1.00 | – | 65 | 52.42 | | 1.00 | – |
| Up to 14 years | 19 | 73.08 | | 1.24 | 0.94–1.63 | 9 | 34.62 | | 2.15 | 1.10–4.18 | 18 | 69.23 | | 1.32 | 0.97–1.80 |
| Vaginal discharge | | | 0.665 | | | | | 0.780 | | | | | 0.995 | | |
| Partner treatment for infertility | | | 0.034 | | | | | 0.495[e] | | | | | **0.000** | | |
| No | 95 | 63.76 | | 1.00 | – | 29 | 20.42 | | 1.00 | – | 87 | 61.27 | | 1.00 | – |
| Yes | 3 | 30.00 | | 0.47 | 0.18–1.23 | 3 | 16.67 | | 0.82 | 0.28–2.42 | 3 | 16.67 | | 0.27 | 0.10–0.77 |

[a]$n$ = Absolute frequency.
[b]P = Prevalence.
[c]PR = Crude prevalence ratio.
[d]CI95%: Confidence interval 95%.
[e]Fisher's exact test.
\*\*Statistical analysis was not possible.
Statistically significant values are highlighted in bold.

rupture of membranes and chorioamnionitis compared to the group without colonization (42.4% vs. 35.6%, *p* = 0.013). In an extensive multicenter study with 3643 participants in Austria, Rittenschober-Böhm et al. [21] investigated *U. parvum* serovars in the vaginal region and their relationship with spontaneous preterm birth. In this study, in the samples positive for UP (37%, *n* = 1347), serovar 3 was the most common isolate, and there was a significantly increased risk of preterm birth at low (<32 weeks) and extremely low (<28 weeks) gestational age. The high prevalence of genital mycoplasmas in pregnant women deserves attention since several studies have shown an association of these microorganisms with adverse pregnancy outcomes.

Still, about premature birth, the study by Vouga et al. [22] in Switzerland evaluated colonization by mycoplasmas and subsequent treatment in 5.377 pregnant women. 2.259 women (42%) had a positive culture for *Ureaplasma* spp. or *M. hominis*, representing significant colonization (percentage of positive cultures),

even using a less sensitive method. Women colonized by genital mycoplasmas and treated demonstrated lower premature birth rates (*Ureaplasma* spp., *p* 0.024; *M. hominis*, *p* 0.001). Some studies have shown that genital mycoplasma infection may play an essential role in the etiology of spontaneous abortion [18, 19, 23–25, 26], premature rupture of membranes [27–29], chorioamnionitis [30–32], intra-amniotic infection [33–35] and other obstetric complications [36–38].

The prevalence of *Mollicutes*, *Ureaplasma* spp., and *U. parvum* in the present study was associated with pregnant women who had their first sexual intercourse at up to 14 years of age. Although we did not find studies in the literature that associated this same risk factor for infection with the *Mollicutes* class in pregnant women, the study by Lee et al. [13] found a high prevalence (88.2%) of *Mollicutes* in pregnant women aged between 15 and 19 years, which may be a reflection of the early onset of sexual activity, drawing attention to the need for more excellent care with pregnant adolescents.

**Table 4.** Bivariate analysis for the prevalence of *Ureaplasma* spp., *Ureaplasma parvum* and *Ureaplasma urealyticum* in pregnant women receiving high-risk prenatal care in southwestern Bahia (*n* = 164). Brazil, 2021–2022

| Variables | *Ureaplasma* spp. | | | | | *U. urealyticum* | | | | | *U. parvum* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $n$[a] | P (%)[b] | p-value | PR[c] | CI 95%[d] | $n$[a] | P (%)[b] | p-value | PR[c] | CI 95%[d] | $n$[a] | P (%)[b] | p-value | PR[c] | CI 95%[d] |
| Polycystic ovaries | | | **0.022** | | | | | 0.498[e] | | | | | 0.039 | | |
| No | 82 | 60.29 | | 1.00 | | 16 | 11.76 | | 1.00 | | 73 | 53.68 | | 1.00 | |
| Yes | 8 | 34.78 | | 0.58 | 0.32–1.03 | 2 | 8.70 | | 0.74 | 0.18–3.02 | 7 | 30.43 | | 0.58 | 0.30–1.07 |
| STI Treatment | | | 0.241 | | | | | **0.033** | | | | | 0.274 | | |
| No | 10 | 71.43 | | 1.00 | – | 4 | 28.57 | | 1.00 | – | 9 | 64.29 | | 1.00 | – |
| Yes | 80 | 55.17 | | 0.77 | 0.54–1.11 | 14 | 9.66 | | 0.34 | 0.13–0.89 | 71 | 48.97 | | 0.76 | 0.50–1.17 |
| Ectopic pregnancy | | | 0.527[e] | | | | | **0.020**[e] | | | | | **0.014**[e] | | |
| No | 87 | 56.86 | | 1.00 | – | 15 | 9.80 | | 1.00 | – | 80 | 52.29 | | 1.00 | – |
| Yes | 3 | 50.00 | | 0.88 | 0.39–1.99 | 3 | 50.00 | | 5.1 | 2.00–13.00 | ** | ** | | ** | ** |
| Use of contraceptives | | | 0.119 | | | | | 0.678 | | | | | **0.031** | | |
| No | 48 | 62.34 | | 1.00 | – | 8 | 10.39 | | 1.00 | – | 45 | 58.44 | | 1.00 | – |
| Yes | 40 | 50.00 | | 0.80 | 0.61–1.06 | 10 | 12.50 | | 1.20 | 0.50–2.90 | 33 | 41.25 | | 0.71 | 0.51–0.98 |
| Vaginal discharge | | | 0.995 | | | | | **0.028** | | | | | 0.940 | | |
| No | 43 | 56.58 | | 1.00 | – | 13 | 17.11 | | 1.00 | – | 38 | 50.00 | | 1.00 | – |
| Yes | 47 | 56.63 | | 1.00 | 0.76–1.32 | 5 | 6.02 | | 0.35 | 0.13–0.94 | 42 | 50.60 | | 1.01 | 0.74–1.38 |
| Partner treatment for infertility | | | **0.000** | | | | | 0.102[e] | | | | | **0.003** | | |
| No | 87 | 61.27 | | 1.00 | – | 18 | 12.68 | | 1.00 | – | 77 | 54.23 | | 1.00 | - |
| Yes | 3 | 16.67 | | 0.27 | 0.10–0.77 | ** | ** | | ** | ** | 3 | 16.67 | | 0.31 | 0.11–0.88 |

[a]$n$ = Absolute frequency.
[b]P = Prevalence.
[c]PR = Crude prevalence ratio.
[d]CI95%: Confidence interval 95%.
[e]Fisher's exact test; **Statistical analysis was not possible.
Statistically significant values are highlighted in bold.

Studies indicate a strong association between the early onset of sexual activity and STIs [39], which implies increased costs for the management of these infections in health services.

Our study observed an association between infertility treatment and the prevalence of *Mollicutes*, *Ureaplasma* spp., and *U. parvum*. Regarding infertility, many studies show that genital mycoplasmas influence male and female fertility. The inflammatory processes triggered by these pathogens can lead to pelvic inflammatory disease, deterioration of spermatogenesis, obstruction of the seminal tract, agglutination of motile sperm, induction of apoptosis, production of immobilization factors, and impairment of the acrosome region [40–42] and some mycoplasma strains resistant to macrolide treatment have already been reported [43]. By correlating this information with the results obtained in our study, it is possible to assume that having undergone treatment implies that, at some point before pregnancy, there was an investigation for infertility, with one of the potential causes being Mycoplasma infection. Once the treatment was performed, the infection was resolved. The woman was able to become pregnant. However, mycoplasma infection is only one of the possible causes of infertility, and we did not have information on the reason for the women who underwent treatment.

There are also conflicting studies in the literature, such as that by Günyeli et al. [44], which indicate no differences between fertile and infertile couples regarding Mycoplasma infection. Even so, the association between infertility treatment and *Mollicutes* infection should be monitored in more depth since professionals working in reproductive medicine are aware of the high rate of couples with difficulty getting pregnant due to infection by some microorganisms, including mycoplasmas. In cases such as couples with infertility or pregnant women with complications in previous pregnancies in which *Mollicutes* were isolated, screening and treatment protocols for these infections could be implemented, reducing the negative impacts on health and costs. Although some studies have not found evidence to screen and treat colonized patients without complications, there is still a need for more studies with specific patient groups, such as pregnant women.

Regarding the association between *Mollicutes* and women having previously had STIs, there are studies in the literature that reveal that mycoplasmas are more frequent in HIV-infected patients, as shown in the systematic review by Boujemaa, Singh-Suri, and Kaur [45]. In our study, HIV infection was not reported in any of the participants. The study by Koch et al. [46] showed that in both men and women, infection by *Trichomonas vaginalis* increases colonization by *M. hominis* and *U. urelyticum*. A high prevalence of co-infection by HPV and *U. parvum* [47] and *U. urealyticum* [48] was also observed. Infection by *M. genitalium* is considered an emerging STI with controversial management [49]. In recent decades, there has been a growing association between Mycoplasma infections and other STIs;

**Table 5.** Bivariate analysis for the prevalence of *Mycoplasma* spp., *Mycoplasma genitalium* and *Mycoplasma hominis* in pregnant women receiving high-risk prenatal care in southwestern Bahia (*n* = 164). Brazil, 2021–2022

| Variables | *Mycoplasma* spp. | | | | | *M. genitalium* | | | | | *M. hominis* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n*[a] | P (%)[b] | *p*-value | PR[c] | CI 95%[d] | *n*[a] | P (%)[b] | *p*-value | PR[c] | CI 95%[d] | *n*[a] | P (%)[b] | *p*-value | PR[c] | CI 95%[d] |
| Age | | | **0.001** | | | | | 0.341[e] | | | | | **0.000** | | |
| Up to 19 years old | 11 | 50.00 | | 1.00 | – | 1 | 4.55 | | 1.00 | – | 11 | 50.00 | | 1.00 | – |
| 20–34 | 13 | 14.29 | | 2.88 | 1.35–6.14 | 1 | 1.10 | | ** | ** | 12 | 13.19 | | 2.88 | 1.35–6.14 |
| 35 or more | 8 | 17.39 | | 0.82 | 0.37–1.84 | ** | ** | | ** | ** | 8 | 17.39 | | 0.76 | 0.33–1.73 |
| Gestational age | | | **0.024** | | | | | 0.585[e] | | | | | **0.036** | | |
| 1st trimester | ** | ** | | ** | ** | ** | ** | | ** | ** | ** | ** | | ** | ** |
| 2 nd trimester | 10 | 15.15 | | ** | ** | ** | ** | | ** | ** | 10 | 15.15 | | ** | ** |
| 3rd trimester | 21 | 28.00 | | ** | ** | 2 | 2.67 | | ** | ** | 20 | 26.67 | | ** | ** |
| Physical activity | | | **0.298** | | | | | 0.617[e] | | | | | 0.247 | | |
| No | 23 | 18.40 | | 1.00 | – | 2 | 1.60 | | 1.00 | – | 22 | 17.60 | | 1.00 | – |
| Yes | 9 | 26.47 | | 1.44 | 0.73–2.82 | ** | ** | | ** | ** | 9 | 26.47 | | 1.50 | 0.76–2.96 |
| Age of first sexual intercourse | | | **0.030** | | | | | 0.682[e] | | | | | **0.026**[e] | | |
| ≥15 years | 20 | 16.13 | | 1.00 | – | 2 | 1.61 | | 1.00 | – | 19 | 15.32 | | 1.00 | – |
| Up to 14 years | 9 | 34.62 | | 2.15 | 1.10–4.18 | ** | ** | | ** | ** | 9 | 34.62 | | 2.26 | 1.15–4.43 |

[a]*n* = Absolute frequency
[b]P = Prevalence.
[c]PR = Crude prevalence ratio.
[d]CI95%: Confidence interval 95%.
[e]Fisher's exact test.
**Statistical analysis was not possible.
Statistically significant values are highlighted in bold.

however, epidemiological surveillance data provided by the Brazilian Ministry of Health [50] are limited to HIV/AIDS, hepatitis, and syphilis. Estimating the prevalence of these infections worldwide is necessary to clarify diagnostic gaps, design control programs, and allocate health resources.

In pregnant women, data on STIs and mycoplasmas are even more limited, which is a concern since these infections, when undetected and untreated during pregnancy, are associated with adverse maternal and neonatal outcomes. The pregnancy condition is known to be immunotolerant, which may contribute to susceptibility to pathogens. In addition, pregnant women report lower rates of condom use, although less sexual activity and less risky sexual behaviors may offset this risk [51]. Although there are reports of *M. hominis* and herpes simplex infection in pregnant women [52] and some studies, such as that by Stafford et al. [53], show the association of *M. genitalium* with *Chlamydia trachomatis*, the existing data are not yet sufficient to demonstrate the cause-and-effect relationship in these associations, especially regarding other species of genital mycoplasmas. In our study, we observed that pregnant women who had some STIs had a higher risk of infection by *U. urealyticum*. This finding opens the possibility for further research to try to determine which specific STIs are associated with infection by *U. urealyticum* and other genital mycoplasmas.

Our study found a positive association between having 12 or more years of education and infection by *Mollicutes* and *U. parvum*. This finding differs from that found in the study by Jeon et al. [20], which showed that a lower level of education tends to be associated with genital colonization by mycoplasmas in high-risk pregnant women. For the gestational age variable, we observed in our

research that pregnant women in the 3rd trimester of pregnancy had a lower chance of infection by *U. urealyticum*, which contrasts with the finding of the work by Abdelaziz et al. [12] in which of the 200 pregnant women, 176 (88%) presented positivity for some bacteria in the vaginal region, most of them in the 3rd trimester of pregnancy (71.6%) and also contrasts with the study by Payne et al. [15] which demonstrated that the prevalence of UU and UP were similar in the three moments of pregnancy (1st, 2nd and 3rd trimesters), allowing us to understand that the colonization status is preserved during pregnancy. In the present study, we also observed that the presence of groin lymph nodes is positively associated with infection by *U. urealyticum*. However, we did not find other studies demonstrating this association for comparison purposes.

The results we present on the detection of *Mollicutes* in pregnant women have important implications for helping to understand the prevalence profile of these microorganisms in this population group. Since it has been demonstrated that mycoplasma infection has negative impacts on maternal and neonatal health, studies such as this provide data for the health system that help in the development of health policies aimed at screening and treating these infections, reducing health costs with the management of complications resulting from these infections. The high prevalence found in our study, associated with data from other studies, leads us to believe that if there were screening for genital mycoplasmas during prenatal care and subsequent treatment, adverse pregnancy outcomes would probably be reduced. Further research will be needed to confirm the prevalence and causal relationship and identify risk factors for *Mollicutes* infections in pregnant women. Studies must be well controlled and with different groups, which demonstrate the bacterial load, vaginal pH, quantity of *Lactobacillus*, elevation of

**Table 6.** Multivariate analysis for the prevalence of *Mollicutes*, *Mycoplasma* spp., *Ureaplasma* spp., *Ureaplasma urealyticum*, *Ureaplasma parvum* and *Mycoplasma hominis* in pregnant women receiving high-risk prenatal care in southwestern Bahia (*n* = 164). Brazil, 2021–2022

| Mollicutes | | | |
|---|---|---|---|
| Variables | PR[a] | CI 95%[b] | *p*-value |
| Education | | | 0.026 |
| 12 years or older | 1.36 | 1.04–1.78 | |
| Up to 11 years | 1.00 | – | |
| Age of first intercourse | | | 0.013 |
| Up to 14 years | 1.41 | 1.07–1.84 | |
| ≥15 years | 1.00 | – | |
| Infertility treatment | | | 0.009 |
| Yes | 0.32 | 0.14–0.75 | |
| No | 1.00 | – | |
| *Ureaplasma* ssp. | | | |
| Variables | PR[a] | CI 95%[b] | *p*-value |
| Age of first intercourse | | | 0.032 |
| Up to 14 years | 1.37 | 1.03–1.81 | |
| ≥15 years | 1.00 | – | |
| Infertility treatment | | | 0.013 |
| Yes | 0.27 | 0.09–0.76 | |
| No | 1.00 | – | |
| *Mycoplasma* ssp. | | | |
| Variables | PR[a] | CI 95%[b] | *p*-value |
| Age | | | 0.029 |
| 20–34 years old | 2.61 | 1.10–6.20 | |
| Up to 19 years old | 1.00 | – | |
| Age of first intercourse | | | 0.228 |
| Up to 14 years | 1.51 | 0.77–2.98 | |
| ≥15 years | 1.00 | – | |
| *U. urealyticum* | | | |
| Variables | PR[a] | CI 95%[b] | *p*-value |
| Gestational age | | | 0.016 |
| 3rd trimester | 0.24 | 0.08–0.77 | |
| 1st trimester | 1.00 | – | |
| Had STIs | | | 0.031 |
| Yes | 2.58 | 1.09–6.12 | |
| No | 1.00 | – | |
| Groin lymph nodes | | | 0.042 |
| Yes | 3.07 | 1.04–9.04 | |
| No | 1.00 | – | |
| *U. parvum* | | | |
| Variables | PR[a] | CI 95%[b] | *p*-value |
| Education | | | 0.011 |

(Continued)

**Table 6.** (*Continued*)

| *U. parvum* | | | |
|---|---|---|---|
| Variables | PR[a] | CI 95%[b] | *p*-value |
| 12 years or older | 1.60 | 1.11–2.30 | |
| Up to 11 years | 1.00 | – | |
| Age of first intercourse | | | 0.008 |
| Up to 14 years | 1.60 | 1.13–2.26 | |
| ≥15 years | 1.00 | – | |
| Infertility treatment | | | 0.018 |
| Yes | 0.30 | 0.11–0.82 | |
| No | 1.00 | – | |
| *M. hominis* | | | |
| Variables | PR[a] | CI 95%[b] | *p*-value |
| Age | | | 0.033 |
| 20–34 years old | 2.57 | 1.08–6.11 | |
| Up to 19 years old | 1.00 | – | |
| Age of first intercourse | | | 0.193 |
| Up to 14 years | 1.58 | 0.80–3.14 | |
| ≥15 years | 1.00 | – | |

[a]PR = Adjusted prevalence ratio.
[b]CI95%: Confidence interval 95%.

cytokines, and clinical conditions of pregnant women. It is worth noting that many studies in the literature did not present consistent data and used methods that were not very sensitive for detecting microorganisms. Although we had limitations in our study, we associated important epidemiological variables and used the gold standard method for detecting mycoplasmas.

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