Association of spontaneous abortion and *Ureaplasma parvum* detected in placental tissue

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Abstract

Spontaneous abortion is considered a public health problem having several causes, including infections. Among the infectious agents, bacteria of the vaginal microbiota and *Ureaplasma parvum* have been associated with abortion, but their participation needs to be further elucidated. This study aims to evaluate the influence of *Mollicutes* on the development of spontaneous abortion. Women who underwent spontaneous abortion and those with normal birth (control) were studied. Samples of cervical mucus (CM) and placental tissue were collected to identify *Mollicutes* using the quantitative polymerase chain reaction methodology. Eighty-nine women who had a miscarriage and 20 women with normal pregnancies were studied. The presence of *Mollicutes* in placental tissue increased the chance of developing miscarriage sevenfold. The prevalence of *U. parvum* in women who experienced spontaneous abortion was 66.3% in placental tissue. A positive association was observed between the detection of *U. parvum* in samples of placental tissue and abortion. There was a significant increase in microbial load in placental tissue for *M. hominis*, *U. urealyticum* and *U. parvum* compared to the control group. Detection of *U. parvum* in CM in pregnant women can ascend to the region of the placental tissue and trigger a spontaneous abortion.

Introduction

Recurrent spontaneous abortion (RSA) or induced abortion (IA) is an important worldwide health outcome, which occurs in about 14% of pregnancy-related deaths due to complications of spontaneous abortion and resulting in about 40 000 pregnancy-related annual deaths. [1]. RSA is defined as two or more successive abortions before 20 weeks from the last menstruation [2]. The possible causes for RSA include parental chromosomal abnormalities, untreated hypothyroidism, uncontrolled diabetes mellitus, certain uterine anatomic abnormalities and antiphospholipid antibody syndrome (APS). Other probable or possible aetiologies include additional endocrine disorders, heritable and/or acquired thrombophilias, immunologic abnormalities, environmental factors and infections. However, the role of infectious agents in recurrent loss is less clear, with a proposed incidence of 0.5–5% [3]. Septic abortion is an infection of the placental tissue and foetus. Placental tissue infection favours it spreading to the uterus, blood and organs. This sepsis causes most maternal deaths in developing countries mainly due to illegal and spontaneous abortions. The fatality rates of septic abortion are extremely variable, ranging from 5% to 20% in many developing countries [1].

Septic abortion is usually caused by bacteria of the vaginal microbiota [1]. Endometrial immunocytes are activated by recurrent bacteria and followed by an intense immune response disturbing the beginning of a pregnancy. This results in excessive Th1 cytokine production. Successful pregnancy is associated with a Th2 cytokine predominance, with Th1 cytokines being detrimental. Therefore, the bacterial toxins or undesirable local cytokines cause uterine contractions, placenta insufficiency, fetal death or life-threatening malformations and amnionitis, which causes abortion in the first trimester as well as preterm labour in the third trimester [4].

Potentially treatable genital tract infections account for 15% of early miscarriages and 66% of late miscarriages [5]. In pregnancy, bacterial vaginosis (BV) is a modification of the vaginal microbiota characterised by a diminished or absent microbiota of lactobacilli, which increases the vaginal pH and a significantly increased colonisation of several anaerobic or facultative microorganisms, mainly *Gardenerella vaginallis*, *Prevotella* sp, *Bacteroides* sp, *Mobiluncus* sp, gram positive cocci and genital mycoplasma (*Mycoplasma hominis* and *Ureaplasma urealyticum*) [6, 7]. Mycoplasmas are prokaryotes with no cell wall and are among the smallest
organisms with autonomous growth. They have smaller genomes than most bacteria and require very complex culture media. [8]. They are often present in vulvovaginal microbiota and can be associated with genital disorders [9, 10].

U. urealyticum and U. parvum are frequently found in the human urogenital tract in both healthy individuals and symptomatic patients [11] and have been isolated in the genital tract from healthy asymptomatic pregnant women and amniotic fluid [12]. M. genitalium is associated with male urethritis cervicitis and an increased risk of pelvic inflammatory disease (PID), endometritis and infertility. In the pregnant women, these species have been associated with chorioamnionitis [13]. The chronicity and pathogenesis of this association include evasion of the local host immune response. M. hominis can be isolated from endometrial tissue of healthy, nonpregnant women. This may disturb embryonic implantation and therefore, early pregnancy [7, 14].

There is growing evidence of an association between U. urealyticum, alone or in combination with M. hominis and obstetric complications such as premature rupture of membranes, preterm delivery and abortion. Indeed, maternal inflammatory responses are more intense in intra-amniotic infection with genital mycoplasmas than with other microorganisms [15]. However, there is controversy regarding the specific role of each mycoplasmas species in adverse pregnancy outcomes [9]. Due to the fact that Mollicutes infection often presents asymptomatically, being difficult to diagnosis and the literature reports an association of these microorganisms as spontaneous abortion, in addition to the variation in prevalence in different parts of the world and the absence of this in pregnant women in the studied area, the aim of the study was to evaluate the influence of Mollicutes on the development of spontaneous abortion.

Methods

Population

The cross-sectional case-control study included 89 women who had experienced spontaneous abortion and 20 women with no abortion experience. The clinical samples were obtained from July 2017 to August 2018 in a maternal and child referral centre in Vitória da Conquista Bahia, Brazil. Endocervical swabs before curettage and samples of the removed placental tissue after curettage were analysed. Women over the age of 18 were divided into two groups: with and without spontaneous abortion. Inclusion criteria were pregnancy between 08 and 20 weeks and no previous use of antibiotics for 2 weeks. Abortion due to anatomical abnormalities were also confirmed by ultrasound images and excluded. The control group consisted of women without spontaneous abortion and who had gestation from 38 to 42 weeks with vaginal delivery and no previous use of antibiotics for 2 weeks.

Clinical and demographic data

Initially, the research team worked with the team of the health clinics to identify possible eligible patients who presented a confirmation of spontaneous abortion by image examination (ultrasound). Subsequently, a questionnaire was administered to patients. Demographic data included age, ethnicity, marital status, religion, residency, education and income and lifestyle, pathological history, menstrual characteristics, sexual history, obstetric pregnancy and current symptoms of the last 3 months.

Samples

The patients were prepared for the curettage or childbirth by the hospital’s health team and the cervical mucus (CM) samples were swabbed from patients with and without spontaneous abortion (n = 109). Samples were collected before prophylactic use of antibiotics. After collecting cervical swab samples, all samples were placed in 5 ml transport media and kept at 4 °C, homogenised, aliquoted to 1 ml and stored at −20 °C. In the abortion group, the placental tissue samples were collected by speculum. In the control group, the placental samples were obtained after natural expulsion. Isopropyl alcohol (70% alcohol contents) was used to wipe the entire foetal part. Subsequently, amniotic membranes were ruptured with sterile scalpels and an internal fragment was collected to prevent contamination from the vaginal canal. Placental tissues from both groups were collected in duplicate and immediately refrigerated at −20 °C for quantitative polymerase chain reaction (qPCR) methodologies.

Real time PCR

Genomic DNA samples of CM and placental tissue were obtained according to the recommendations of Invitrogen Purelink™ Genomic DNA Kits (Invitrogen, Waltham, MA, USA). Real-time PCR assays were performed in a StepOne Plus real-time PCR cycler (Life Technologies) in a 25 ml final volume with the use of TaqMan Real-Time PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). Positive (DNA of M. genitalium, M. hominis, U. parvum, U. urealyticum and N. gonorrhoeae), negative control and samples were included. The PCR assays included a negative control (without DNA). Positive control and samples were electrophoresed in duplicate. Labelled probes in the TaqMan format were used to amplify the target gene of M. hominis [16], M. genitalium [17], Ureaplasma urealyticum [18], U. parvum [18] and Neisseria gonorrhoeae [19]. The standardisation of each microbial DNA for absolute quantitation was obtained from the Microbiology Laboratory of the University of São Paulo/Brazil. The DNA was extracted by the boiling method and quantified by spectrophotometry (NanoDrop ND 100). For each assay, a novel standard curve was used and the following quality parameters were adopted: $r^2 \geq 0.950$, reaction efficiency 95%–105% and slope $\sim 3.32$. The absolute quantification of microorganisms was done based on the standard curve.

Statistical analysis

Clinical and demographic data were analysed with IBM SPSS 21° and EpiInfo 7.2.2.16. software. Descriptive analysis of clinical and epidemiological data was performed by frequency comparison and using Pearson’s chi-square test considering $P < 0.05$ and a 95% confidence level. To assess the risk factors associated with bacterial infections, the odds ratio (OR) was calculated and all variables with $P$ value <0.20 in univariate analysis were included in multivariate analysis using logistic regression. In the final statistical model, only variables with $P < 0.05$ were considered significant. GraphPad Prism software (version 7) was used to analyse the quantification of microorganisms. First, the Kolmogorov–Smirnov test was applied to evaluate the normal distribution of data. Finally, data were analysed by means. Data were considered significant when $P < 0.05$. 
Results

Demographic data and prevalence of microorganisms

The mean age of the women was 27.3 years (study group: 28 ±7.3 years; control group: 24 ± 7.9 years). Most were married, lived in an urban area, were over 18 years old and had a low income. Both groups were not physically active, nor did they consume alcohol or tobacco. The mean age of menarche was 12.6 years (study group: 13 ± 1.4; control group: 12 ± 1.4). The mean age of onset of sexual activity was 16.5 years (study group: 17 ± 2.8; control group: 15 ± 2.5). It should be noted that 38 women did not have prenatal care during pregnancy and 75 women were pregnant for the first time (Table 1).

The prevalence of each microorganism for the type of sample in both groups and co-infection are presented in Table 2. All targeted DNA of microorganisms were detected at least once in the samples. The overall prevalence of Mollicutes in women experiencing spontaneous abortion was 95.5% in CM and 87.8% in the placental tissue with seven times greater chance of developing abortion in the presence of Mollicutes (Table 3). The prevalence of M. genitalium, M. hominis, U. urealyticum, U. parvum and N. gonorrhoeae in placental tissue was 41.6%, 10.1%, 11.2%, 66.3% and 2.2%, respectively. The control group was allowed for detecting M. genitalium and U. parvum in 55.0%, 25.0% respectively.

In the univariate analysis, no prenatal care, early coitus, the prevalence of Mollicutes and U. parvum in the placental tissue were significantly associated with spontaneous abortion, P < 0.05 (Table 3). Detection of M. hominis in the placental tissue was associated with the age <19, not being white, previous pregnancies and the presence of M. hominis in CM. The prevalence of M. genitalium was significantly associated with U. urealyticum in CM. The presence of U. urealyticum in placental tissue was associated with M. genitalium in CM. Finally, the presence of U. parvum was related to age <29 and bleeding during sexual intercourse. A positive association was observed between U. parvum detection in placental tissue samples and miscarriage (OR = 5.90). There were no significant differences in lifestyle, pathological history, sexual history, gestational history, menstrual and obstetric characteristics (Table 4).

In the final logistic regression model (Table 5), a positive association was found between spontaneous abortion and the presence of U. parvum in the placental tissue and early onset of sexual activity. Another positive association found was the relationship between M. hominis and U. urealyticum in CM and their respective placental tissue presence. As well as the association of the presence of M. genitalium in the placental tissue with U. parvum in the placental tissue and U. urealyticum in CM. No statistically significant difference was found in the final logistic regression model with the presence of U. parvum in the placental tissue.

Quantification of microorganisms

A significant increase was observed between M. hominis load (mean 6.7 × 10^8, minimum 1.1 × 10^7 and maximum 5.9 × 10^9) U. parvum load (mean 4.7 × 10^10, minimum 1.2 × 10^9 and maximum 1.0 × 10^12) and U. urealyticum load (mean 3.3 × 10^7, minimum 2.3 × 10^5 and maximum 2.7 × 10^9) detection in placental tissue of women with miscarriage compared to the control group. A significant increase was also observed between U. parvum load (mean 1.1 × 10^{11}, minimum 1.5 × 10^5 and maximum 2.5 × 10^{12}) detection in CM of women with miscarriage compared to the control group (mean 8.3 × 10^6, minimum 2.2 × 10^5 and maximum 3.7 × 10^8) (Fig. 1).

Discussion

Spontaneous abortion is a common gestational complication and occurs in approximately 15–20% of pregnancies. This disturbance may also occur due to chromosomal abnormalities, endocrine, immunological changes and infections, which are mostly primarily from the female reproductive tract [20]. Infections in pregnancy cause aggressive inflammatory response mediated mainly by endometrial leukocytes causing spontaneous abortion [4]. The microorganisms associated with amniotic membrane rupture and premature birth are C. trachomatis, N. gonorrhoeae and M. genitalium. In addition, the presence of M. hominis, U. urealyticum and U. parvum in the vagina correlates with chorioamnionitis, premature birth and spontaneous abortion; however, a larger and more diverse group of women needs to be studied to better understand spontaneous abortion worldwide [21]. Therefore, it is necessary to understand the prevalence of spontaneous abortion and the risk factors associated with microorganisms, especially mycoplasmas. Although there are many studies in the literature on the association of mycoplasmas with adverse pregnancy events, the results are conflicting often due to the characteristics of the studied population. Therefore, this study investigates the presence of Mollicutes in placental tissue and CM in women with and without spontaneous abortion.

In the present study, we identified a protective association (OR = 0.21) in univariate analysis for abortion in women who started sexual activity before age 16. However, in the final logistic regression model, this association did not persist (OR = 6.12) and a higher risk of abortion was identified for women having sex before age 16. Similar results were reported by Machado et al., [22] who states that, with the early onset of sex, the time of exposure to sexual activities becomes longer, thereby increasing exposure to various pregnancies. Correia et al. [23] also affirm that the beginning of sexual activity in young people is associated with not using contraceptive methods in their first sexual intercourse and, as a consequence, a high number of unplanned pregnancies occur in the first year of sexual activity and spontaneous abortion can occur. A number of these women also underwent unsafe abortion practices possibly due to fear and shame, as well as difficulties in accessing health services or lack of financial resources. A positive association was observed between the occurrence of spontaneous abortion and the absence of prenatal care (OR = 13.5). Machado et al. [22] pointed out that not receiving early prenatal care is a risk factor. Not seeking early health services makes it difficult to identify possible treatable causes for spontaneous abortion.

In the present study, we concluded that women with detected Mollicutes had a seven times greater chance of developing spontaneous abortion. U. parvum was the most prevalent followed by M. genitalium, M. hominis and U. urealyticum. Other studies on spontaneous abortion mentioned a lower prevalence of Mollicutes [20, 24, 25]. In fact, the prevalence of Mollicutes in humans varies with age, socioeconomic factors, sexual activity, number of partners and lifestyle [7].

A positive association was observed between U. parvum detection in placental tissue samples and miscarriage in univariate analysis (OR = 5.90) and in the final logistic regression model (OR = 6.46). U. parvum was associated with age below 19 years (OR = 4.2) and age from 20 to 29 years (OR = 3.8). Studies on abortion
Table 1. Demographic and clinical data of women with spontaneous abortion and the control group, n (%)  

| Variables                      | Spontaneous abortion | Control |
|--------------------------------|-----------------------|---------|
|                                | n        | %     | n        | %     |
| **Age (y)**                    |          |       |          |       |
| ⩽19                            | 15       | 16.9  | 7        | 35    |
| 20–29                          | 37       | 41.6  | 7        | 35    |
| ⩾30                            | 37       | 41.6  | 6        | 30    |
| **Marital status**             |          |       |          |       |
| With mate                      | 68       | 78.2  | 16       | 80    |
| Without mate                   | 19       | 21.8  | 4        | 20    |
| **Provenance**                 |          |       |          |       |
| Countryside                    | 28       | 21.3  | 7        | 35    |
| Urban area                     | 61       | 76.4  | 13       | 35    |
| **Ethnicity**                  |          |       |          |       |
| White                          | 27       | 30.3  | 4        | 20    |
| Non-white                      | 62       | 69.7  | 16       | 80    |
| **Religion**                   |          |       |          |       |
| Catholic                       | 49       | 55.1  | 9        | 47.4  |
| Non-catholic                   | 26       | 29.2  | 5        | 26.3  |
| None                           | 14       | 15.7  | 5        | 26.3  |
| **Schooling**                  |          |       |          |       |
| None                           | 2        | 2.3   | 0        | 0     |
| Up to 7 years of study         | 29       | 33    | 7        | 36.8  |
| Over 8 years of study          | 57       | 64.8  | 12       | 63.2  |
| **Income**                     |          |       |          |       |
| Up to minimum wage             | 58       | 65.2  | 15       | 78.9  |
| Above minimum wage             | 30       | 33.7  | 4        | 21.1  |
| **Physical activity**          |          |       |          |       |
| Yes                            | 16       | 18.2  | 4        | 20    |
| No                             | 72       | 81.8  | 16       | 80    |
| **Alcohol consumption**        |          |       |          |       |
| Yes                            | 14       | 15.7  | 2        | 10    |
| No                             | 75       | 84.3  | 18       | 90    |
| **Smoking**                    |          |       |          |       |
| Yes                            | 4        | 4.5   | 1        | 5     |
| No                             | 85       | 95.5  | 19       | 95    |
| **Onset of sexual activity**   |          |       |          |       |
| <16 years                      | 30       | 34.9  | 10       | 55.6  |
| 16 to 17 years old             | 28       | 32.6  | 6        | 33.3  |
| ⩾18 years                      | 28       | 32.6  | 2        | 11.1  |
| **Dyspareunia**                |          |       |          |       |
| Yes                            | 26       | 29.5  | 3        | 15    |
| No                             | 62       | 70.5  | 17       | 85    |

(Continued)
Table 1. (Continued.)

| Variables                  | Spontaneous abortion | Control |
|----------------------------|-----------------------|---------|
|                            | n    | %    | n    | %    |
| Yes                        | 52   | 58.4 | 19   | 95   |
| No                         | 37   | 41.6 | 1    | 5    |
| Vaginal discharge          |       |      |      |      |
| Yes                        | 58   | 65.2 | 12   | 60   |
| No                         | 31   | 34.8 | 8    | 40   |
| Vaginal itching            |       |      |      |      |
| Yes                        | 27   | 30.3 | 6    | 30   |
| No                         | 62   | 69.7 | 14   | 70   |
| Pelvic pain                |       |      |      |      |
| Yes                        | 33   | 37.1 | 6    | 30   |
| No                         | 56   | 62.9 | 14   | 70   |
| Dysuria                    |       |      |      |      |
| Yes                        | 16   | 18   | 5    | 25   |
| No                         | 73   | 82   | 15   | 75   |
| Previous pregnancies       |       |      |      |      |
| Yes                        | 25   | 28.1 | 9    | 45   |
| No                         | 64   | 71.9 | 11   | 55   |

\[2^2\] non-respondent in the abortion group.  
\[1^1\] non-respondent in the control group.  
\[1^1\] non-respondent in the abortion group.  
\[1^1\] minimum wage of R$954.00 per month.  
\[1^1\] non-respondent in the abortion group.  
\[1^1\] non-respondent in the control group.

Table 2. Prevalence and co-infection of microorganisms and spontaneous abortion

| Individual prevalence                                                                 |
|----------------------------------------------------------------------------------------|
| **Cervical mucous**                                                                     |
| **Abortion**                                                                            |
| Variables                                | N     | %    | N     | %    |
| M. hominis                               | 11    | 12.4 | 3     | 15   |
| M. genitalium                            | 49    | 55.1 | 6     | 30   |
| U. parvum                                | 68    | 76.4 | 13    | 65   |
| U. urealyticum                           | 25    | 28.1 | 3     | 15   |
| N. gonorrhoeae                           | 0     | 0    | 1     | 5    |
| **Placental tissue**                                                                   |
| **Abortion**                             | N     | %    | N     | %    |
| M. hominis                               | 9     | 10.1 | 0     | 0    |
| M. genitalium                            | 37    | 41.6 | 11    | 55   |
| U. parvum                                | 59    | 66.3 | 5     | 25   |
| U. urealyticum                           | 10    | 11.2 | 0     | 0    |
| N. gonorrhoeae                           | 2     | 2.2  | 0     | 0    |

| Co-infection prevalence                  |
|-----------------------------------------|
| **Variable**                            |
| **n** | %      | **n** | %    |
| U. parvum + U. urealyticum             | 7     | 7.9   | M. hominis + M. genitalium | 6 | 6.7 |
| U. parvum + M. genitalium              | 20    | 22.5  | M. genitalium + N. gonorrhoeae | 1 | 1.1 |
| U. parvum + M. hominis                 | 5     | 5.6   | U. parvum + M. genitalium + U. urealyticum | 3 | 3.4 |
| U. urealyticum + M. genitalium         | 5     | 5.6   | U. parvum + M. genitalium + M. hominis | 4 | 4.5 |
Table 3. Univariate analysis of the occurrence or not of abortion and the presence of *Mollicutes*

| Variables              | Presence of abortion |          |          |          | Presence of *Mollicutes* |          |          |
|------------------------|----------------------|----------|----------|----------|--------------------------|----------|----------|
|                        | OR (IC 95%)          | $X^2$    | P        | OR (IC 95%)          | $X^2$    | P        |
| Age (y)                |                      |          |          |                      |          |          |
| £19                    | 0.35 (0.10–1.20)     | 0.90     | 0.09     | 2.55 (0.84–7.74)     | 2.79     | 0.09     |
| 20 to 29               | 0.86 (0.26–2.79)     | 0.06     | 0.80     | 2.54 (1.04–6.22)     | 4.30     | 0.04*    |
| #x2ATE;30              | 1.0                  |          | 1.0      |                      |          |          |
| Marital status         |                      |          |          |                      |          |          |
| Without mate           | 1.12 (0.33–3.74)     | 0.03     | 0.86     | 1.83 (0.66–5.13)     | 1.36     | 0.24     |
| With mate              | 1.0                  |          | 1.0      |                      |          |          |
| Colour                 |                      |          |          |                      |          |          |
| Non-white              | 0.57 (0.17–1.88)     | 0.86     | 0.35     | 0.80 (0.33–1.94)     | 0.23     | 0.63     |
| White                  | 1.0                  |          | 1.0      |                      |          |          |
| Provenance             |                      |          |          |                      |          |          |
| Countryside            | 0.85 (0.31–2.37)     | 0.09     | 0.76     | 1.10 (0.47–2.55)     | 0.05     | 0.82     |
| Urban area             | 1.0                  |          | 1.0      |                      |          |          |
| Religion               |                      |          |          |                      |          |          |
| None                   | 0.51 (0.15–1.78)     | 1.12     | 0.29     | 0.72 (0.25–2.08)     | 0.36     | 0.55     |
| Non-catholic           | 0.96 (0.29–3.15)     | 0.00     | 0.94     | 1.10 (0.44–2.79)     | 0.04     | 0.83     |
| Catholic               | 1.0                  |          | 1.0      |                      |          |          |
| Schooling              |                      |          |          |                      |          |          |
| None                   | †                     |          |          | 0.57 (0.03–9.48)     | 0.16     | 0.69     |
| Up to 7 years of study | 0.87 (0.31–2.45)     | 0.07     | 0.80     | 1.14 (0.48–2.65)     | 0.08     | 0.77     |
| Over 8 years of study  | 1.0                  |          | 1.0      |                      |          |          |
| Income                 |                      |          |          |                      |          |          |
| Up to minimum wage     | 0.52 (0.15–1.69)     | 1.23     | 0.27     | 0.81 (0.34–1.92)     | 0.21     | 0.64     |
| Above minimum wage     | 1.0                  |          | 1.0      |                      |          |          |
| Physical activity      |                      |          |          |                      |          |          |
| No                     | 1.13 (0.33–3.82)     | 0.03     | 0.85     | 1.58 (0.59–4.23)     | 0.84     | 0.36     |
| Yes                    | 1.0                  |          | 1.0      |                      |          |          |
| Alcohol consumption    |                      |          |          |                      |          |          |
| Yes                    | 1.68 (0.35–8.02)     | 0.43     | 0.51     | 2.74 (0.73–10.2)     | 2.37     | 0.12     |
| No                     | 1.0                  |          | 1.0      |                      |          |          |
| Onset of sexual activity|                      |          |          |                      |          |          |
| <16 years              | 0.21 (0.04–1.06)     | 4.06     | 0.04*    | 1.20 (0.44–3.25)     | 0.13     | 0.71     |
| 16–17 years old        | 0.33 (0.06–1.80)     | 1.76     | 0.19     | 1.21 (0.43–3.40)     | 0.13     | 0.71     |
| #x2ATE;18 years        | 1.0                  |          | 1.0      |                      |          |          |
| Dyspareunia            |                      |          |          |                      |          |          |
| Yes                    | 2.38 (0.64–8.81)     | 1.76     | 0.19     | 1.69 (0.67–4.30)     | 1.25     | 0.26     |
| No                     | 1.0                  |          | 1.0      |                      |          |          |
| Condom use             |                      |          |          |                      |          |          |
| No                     | †                     |          |          | 1.62 (0.09–27.2)     | 0.11     | 27.2     |
| Sometimes              | †                     |          |          | 1.33 (0.05–31.1)     | 0.03     | 0.85     |
| Less than half the time| †                     |          |          | 2.50 (0.13–45.0)     | 0.40     | 0.52     |

(Continued)
| Variables                        | Presence of abortion | Presence of Mollicutes |
|---------------------------------|----------------------|------------------------|
|                                 | OR (IC 95%)          | $\chi^2$ | $P$   | OR (IC 95%) | $\chi^2$ | $P$   |
| More than half of the time      | ↑                    |          |      | 1.66 (0.07–37.7) | 0.10 | 0.75 |
| Yes                             | ↑                    |          |      | 1.0 |          |      |      |
| Gestational planning            |                      |          |      |                |      |      |
| No                              | 0.57 (0.19–1.72)     | 0.99     | 0.32 | 1.09 (0.48–2.49) | 0.05 | 0.83 |
| Yes                             | 1.0                  |          |      | 1.0 |          |      |      |
| Prenatal care                   |                      |          |      |                |      |      |
| No                              | **13.52 (1.7–105)**  | 9.62     | 0.00*| 1.93 (0.81–4.58) | 2.27 | 0.13 |
| Yes                             | 1.0                  |          |      | 1.0 |          |      |      |
| Vaginal discharge               |                      |          |      |                |      |      |
| Yes                             | 1.25 (0.46–3.37)     | 0.19     | 0.66 | 2.0 (0.88–4.50) | 2.84 | 0.09 |
| No                              | 1.0                  |          |      | 1.0 |          |      |      |
| Vaginal erythema                |                      |          |      |                |      |      |
| Yes                             | 2.28 (0.48–10.7)     | 1.13     | 0.29 | 1.37 (0.48–3.92) | 0.35 | 0.55 |
| No                              | 1.0                  |          |      | 1.0 |          |      |      |
| Dysuria                         |                      |          |      |                |      |      |
| Yes                             | 0.66 (0.21–2.07)     | 0.52     | 0.47 | 1.14 (0.41–3.12) | 0.06 | 0.79 |
| No                              | 1.0                  |          |      | 1.0 |          |      |      |
| Blood in sexual intercourse     |                      |          |      |                |      |      |
| Yes                             | ↑                    |          |      | 0.36 (0.06–2.24) | 1.30 | 0.25 |
| No                              | ↑                    |          |      | 1.0 |          |      |      |
| Genital alteration              |                      |          |      |                |      |      |
| Yes                             | 0.63 (0.16–2.60)     | 0.40     | 0.53 | 3.09 (0.64–14.8) | 2.14 | 0.14 |
| No                              | 1.0                  |          |      | 1.0 |          |      |      |
| Previous pregnancies            |                      |          |      |                |      |      |
| 1 gestation                     | 0.48 (0.18–1.29)     | 2.17     | 0.14 | 1.51 (0.63–3.61) | 0.87 | 0.35 |
| +1 pregnancy                    | 1.0                  |          |      | 1.0 |          |      |      |
| Presence of Mollicutes in the placental tissue | **8.13 (2.66–24.8)** | **16.4** | 0.00* | ↑ |          |      |      |
| No                              | 1.0                  |          |      | ↑ |          |      |      |
| *M. hominis in CM               |                      |          |      |                |      |      |
| Yes                             | 0.80 (0.20–3.17)     | 0.10     | 0.75 | ↑ |          |      |      |
| No                              | 1.0                  |          |      | ↑ |          |      |      |
| *U. parvum in CM                |                      |          |      |                |      |      |
| Yes                             | 1.74 (0.61–4.93)     | 1.11     | 0.29 | ↑ |          |      |      |
| No                              | 1.0                  |          |      | ↑ |          |      |      |
| *U. urealyticum in CM           |                      |          |      |                |      |      |
| Yes                             | 2.21 (0.60–8.22)     | 1.47     | 0.23 | ↑ |          |      |      |
| No                              | 1.0                  |          |      | ↑ |          |      |      |
| *U. parvum in the placental tissue | **5.90 (1.9–17.8)** | **11.5** | 0.00* | ↑ |          |      |      |
| No                              | 1.0                  |          |      | ↑ |          |      |      |

† Not statistically evaluated; CM, cervical mucous; significant value $P < 0.5$. 
Table 4. Univariate analysis of the occurrence of abortion and presence of M. hominis, M. genitalium, U. urealyticum and U. parvum

| Variables          | Presence of M. hominis | Presence of M. genitalium | Presence of U. urealyticum | Presence of U. parvum |
|--------------------|------------------------|---------------------------|----------------------------|-----------------------|
|                    | OR (IC 95%)             | $X^2$                      | $P$                         | OR (IC 95%)           | $X^2$                      | $P$                         | OR (IC 95%) | $X^2$                      | $P$                         |
| Age (y)            |                        |                           |                             |                       |                           |                             |                           |                        |                             |                             |
| ≤19                | 9.0 (0.85–94.90)        | 4.49                      | 0.03*                       | 1.26 (0.45–3.54)       | 0.20                      | 0.66                         | 2.06 (0.40–10.59)        | 0.77                      | 0.38                         | 4.22 (1.02–17.47)          | 4.30                      | 0.04*                       |
| 20–29              | 5.62 (0.62–50.72)       | 2.90                      | 0.08                        | 0.87 (0.37–2.05)       | 0.10                      | 0.76                         | 0.73 (0.15–3.50)         | 0.16                      | 0.69                         | 3.82 (1.39–10.54)          | 7.06                      | 0.007*                      |
| ≥30                | 1.0                    |                           |                             | 1.0                    |                           |                             | 1.0                       |                           |                             |                           |                             |
| Marital status     |                        |                           |                             |                       |                           |                             |                           |                        |                             |                             |                             |
| Without mate       | 3.36 (0.80–14.04)       | 3.00                      | 0.08                        | 2.29 (0.89–5.88)       | 3.04                      | 0.08                         | 1.63 (0.38–7.04)         | 0.44                      | 0.51                         | 1.18 (0.40–3.51)           | 0.09                      | 0.76                         |
| With mate          | 1.0                    |                           |                             | 1.0                    |                           |                             | 1.0                       |                           |                             |                           |                             |
| Colour             |                        |                           |                             |                       |                           |                             |                           |                        |                             |                             |                             |
| Non-white          | 4.83 (0.91–25.6)        | 3.91                      | 0.05*                       | 0.78 (0.34–1.80)       | 0.33                      | 0.56                         | 1.02 (0.24–4.28)         | 0.00                      | 0.98                         | 0.76 (0.29–2.03)           | 0.29                      | 0.59                         |
| White              | 1.0                    |                           |                             | 1.0                    |                           |                             | 1.0                       |                           |                             |                           |                             |
| Provenance         |                        |                           |                             |                       |                           |                             |                           |                        |                             |                             |                             |
| Countryside        | 0.59 (0.11–3.05)        | 0.40                      | 0.52                        | 0.78 (0.34–1.77)       | 0.34                      | 0.56                         | 2.44 (0.64–9.21)         | 1.80                      | 0.18                         | 1.41 (0.53–3.73)           | 0.48                      | 0.49                         |
| Urban area         | 1.0                    |                           |                             | 1.0                    |                           |                             | 1.0                       |                           |                             |                           |                             |
| Religion           |                        |                           |                             |                       |                           |                             |                           |                        |                             |                             |                             |
| None               | 1.19 (0.21–6.69)        | 0.04                      | 0.83                        | 3.81 (1.26–11.5)       | 6.03                      | 0.01*                        | 0.55 (0.06–5.01)         | 0.29                      | 0.59                         | 1.05 (0.30–3.60)           | 0.00                      | 0.94                         |
| Non-catholic       | 0.29 (0.03–2.52)        | 1.41                      | 0.23                        | 1.27 (0.52–3.10)       | 0.28                      | 0.60                         | 0.93 (0.21–4.09)         | 0.01                      | 0.93                         | 1.58 (0.56–4.47)           | 0.74                      | 0.39                         |
| Catholic           | 1.0                    |                           |                             | 1.0                    |                           |                             | 1.0                       |                           |                             |                           |                             |
| Schooling          |                        |                           |                             |                       |                           |                             |                           |                        |                             |                             |                             |
| None               | †                      |                           |                             | †                      |                           |                             |                           |                        |                             |                             |                             |
| Up to 7 years of study | 2.12 (0.48–9.18)    | 2.20                      | 0.06                        | 0.53 (0.10–2.73)       | 0.59                      | 0.44                         | 0.95 (0.37–2.44)         | 0.01                      | 0.92                         |
| Over 8 years of study | 1.0                      |                           |                             | 1.0                    |                           |                             | 1.0                       |                           |                             |                           |                             |
| Income             |                        |                           |                             |                       |                           |                             |                           |                        |                             |                             |                             |
| Up to minimum wage | †                      |                           |                             | 1.41 (0.61–3.23)       | 0.66                      | 0.45                         | 0.75 (0.19–2.89)         | 0.17                      | 0.68                         | 0.95 (0.37–2.41)           | 0.01                      | 0.91                         |
| Above minimum wage | †                      |                           |                             | 1.0                    |                           |                             | 1.0                       |                           |                             |                           |                             |
| Physical activity  |                        |                           |                             |                       |                           |                             |                           |                        |                             |                             |                             |
| No                 | 0.75 (0.14–4.02)        | 0.11                      | 0.74                        | 1.19 (0.44–3.21)       | 0.12                      | 0.75                         | 1.88 (0.22–16.2)         | 0.34                      | 0.56                         | 1.20 (0.39–3.70)           | 0.10                      | 0.75                         |
| Yes                | 1.0                    |                           |                             | 1.0                    |                           |                             | 1.0                       |                           |                             |                           |                             |
| Alcohol consumption|                        |                           |                             |                       |                           |                             |                           |                        |                             |                             |                             |
| Yes                | 0.64 (0.07–5.60)        | 0.16                      | 0.69                        | 0.37 (0.11–1.23)       | 2.76                      | 0.10                         | 1.40 (0.26–7.39)         | 0.15                      | 0.69                         | 3.57 (0.78–17.15)          | 2.80                      | 0.09                         |
| No                 | 1.0                    |                           |                             | 1.0                    |                           |                             | 1.0                       |                           |                             |                           |                             |
| Condition                          | Yes | No | Sometimes | More than half of the time | Less than half the time | No | Yes | Sometimes | More than half of the time | Less than half the time | No | Yes | Sometimes | More than half of the time | Less than half the time | No | Yes |
|-----------------------------------|-----|----|-----------|-----------------------------|-------------------------|----|-----|-----------|-----------------------------|-------------------------|----|-----|-----------|-----------------------------|-------------------------|----|-----|
| Onset of sexual activity         |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| <16 years                         | 5.40 (0.59–49.47) | 2.68 | 0.10 | 1.50 (0.58–3.91) | 0.69 | 0.41 | 0.51 (0.11–2.37) | 0.75 | 0.39 | 2.38 (0.79–7.15) | 2.45 | 0.12 |
| 16–17 years old                   | 1.80 (0.39–8.32)  | 0.58 | 0.45 | 1.18 (0.44–3.21) | 0.11 | 0.74 | 0.17 (0.02–1.57) | 2.99 | 0.08 | 2.60 (0.84–8.07) | 2.80 | 0.09 |
| 18 years                          | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Dyspareunia                       |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| Yes                               | 3.45 (0.85–14.09) | 3.26 | 0.07 | 0.84 (0.36–1.99) | 0.15 | 0.70 | 0.65 (0.13–3.39) | 0.26 | 0.61 | 1.24 (0.46–3.30) | 0.18 | 0.67 |
| No                                | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Condom use                        |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| No                                | 0.11 (0.01–2.02)  | 3.10 | 0.08 | 0.70 (0.04–11.7)  | 0.06 | 0.80 | ↑ | 1.68 (0.09–28.5)  | 0.13 | 0.72 |
| Sometimes                         | ↑ | 0.40 (0.01–10.0) | 0.32 | 0.57 | ↑ | ↑ |
| Less than half the time           | 0.14 (0.01–2.81)  | 2.12 | 0.15 | 1.33 (0.08–23.5) | 0.03 | 0.84 | ↑ | 3.17 (0.17–58.7) | 0.65 | 0.42 |
| More than half of the time        | ↑ | 0.60 (0.02–13.6) | 0.10 | 0.75 | ↑ | ↑ | 0.50 (0.02–12.9) | 0.18 | 0.67 |
| Yes                               | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Gestational planning              |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| No                                | 4.52 (0.53–38.55) | 2.23 | 0.13 | 1.47 (0.65–3.31) | 0.85 | 0.36 | 1.41 (0.34–5.88) | 0.22 | 0.64 | 0.93 (0.37–2.33) | 0.02 | 0.88 |
| Yes                               | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Prenatal Care                     |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| No                                | 0.68 (0.16–2.90)  | 0.28 | 0.60 | 1.23 (0.55–2.71) | 0.26 | 0.61 | 1.47 (0.39–5.49) | 0.33 | 0.57 | 1.10 (0.45–2.7) | 0.04 | 0.82 |
| Yes                               | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Vaginal discharge                 |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| Yes                               | 4.80 (0.57–40.29) | 2.48 | 0.12 | 0.54 (0.24–1.19) | 2.37 | 0.12 | 2.32 (0.46–11.67) | 1.09 | 0.30 | 2.16 (0.87–5.38) | 2.79 | 0.09 |
| No                                | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Vaginal erythema                  |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| Yes                               | 0.46 (0.05–3.96)  | 0.54 | 3.96 | 0.6 (0.23–1.73)  | 0.81 | 0.37 | 3.09 (0.77–12.44) | 2.73 | 0.10 | 0.75 (0.26–2.19) | 0.27 | 0.60 |
| No                                | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Dysuria                           |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| Yes                               | 2.58 (0.57–11.64) | 1.60 | 0.21 | 0.57 (0.21–1.56) | 1.21 | 0.27 | 2.18 (0.50–9.53) | 1.10 | 0.29 | 0.82 (0.27–2.51) | 0.13 | 0.72 |
| No                                | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Blood in sexual intercourse       |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| Yes                               | 2.34 (0.23–23.59) | 0.55 | 0.45 | 1.93 (0.31–12.0) | 0.51 | 0.47 | ↑ | 0.11 (0.01–1.07) | 4.97 | 0.02* |
| No                                | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Genital alteration                |     |    |           |                             |                         |    |     |           |                             |                         |    |     |           |                             |                         |    |     |
| Yes                               | 2.90 (0.52–17.19) | 1.62 | 0.20 | 0.90 (0.27–3.02) | 0.03 | 0.86 | 5.21 (1.07–25.52) | 4.90 | 0.03* | 4.54 (0.54–38.2) | 2.29 | 0.13 |
| No                                | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

(Continued)
Table 4. (Continued.)

| Variables | Presence of *M. hominis* |  | Presence of *M. genitalium* |  | Presence of *U. urealyticum* |  | Presence of *U. parvum* |  |
|-----------|--------------------------|---|-----------------------------|---|----------------------------|---|------------------------|---|
|           | OR (IC 95%) | X²  | P          | OR (IC 95%) | X²  | P          | OR (IC 95%) | X²  | P          | OR (IC 95%) | X²  | P          |
| Previous pregnancies | | | | | | | | | | | | |
| 1 gestation | 6.42 (1.46–28.17) | 7.38 | 0.006* | 1.19 (0.52–2.70) | 0.18 | 0.67 | 1.84 (0.47–7.17) | 0.79 | 0.37 | 2.56 (0.85–7.71) | 2.92 | 0.09 |
| +1 pregnancy | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| *M. hominis* in CM | | | | | | | | | | | | |
| Yes | 30 (5.73–157.05) | 27.3 | 0.000* | 1.83 (0.59–5.70) | 1.12 | 0.29 | 0.77 (0.09–6.71) | 0.05 | 0.81 | 1.41 (0.35–5.76) | 0.23 | 0.63 |
| No | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| *M. genitalium* in CM | | | | | | | | | | | | |
| Yes | 0.98 (0.32–3.0) | 0.00 | 0.97† | 2.63 (1.0–6.5) | 4.56 | 0.03* | 0.46 (0.19–1.13) | 2.88 | 0.09 |
| No | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| *U. parvum* in CM | | | | | | | | | | | | |
| Yes | 1.09 (0.21–5.70) | 0.01 | 0.92 | 0.72 (0.30–1.71) | 0.54 | 0.46 | 1.27 (0.25–6.49) | 0.08 | 0.78 | 2.18 (0.80–5.95) | 2.38 | 0.12 |
| No | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| *U. urealyticum* in CM | | | | | | | | | | | | |
| Yes | 2.25 (0.55–9.17) | 1.33 | 0.25 | 2.49 (1.0–6.0) | 4.25 | 0.04* | 7.91 (1.85–33.75) | 9.80 | 0.001* | 0.68 (0.26–1.78) | 0.62 | 0.43 |
| No | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| *U. parvum* in the placental tissue | | | | | | | | | | | | |
| Yes | 1.02 (0.24–4.39) | 0.00 | 0.98 | 0.45 (0.20–0.98) | 4.12 | 0.04* | 1.21 (0.29–5.06) | 0.07 | 0.79 | † | | |
| No | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | † | | |

† Not statistically evaluated; CM, cervical mucus; significant value P < 0.5.
In this study, we investigated the role of *mollicutes* and co-infection with the spontaneous abortion. This is the first study to show such high placental tissue and CM colonisation and premature delivery also presented comparable data. The ureaplasmal DNA in the placental tissue is related to inflammation, histological changes and elevated cytokine levels, regardless of gestational period [27].

A positive association was also observed in univariate analysis and in the final logistic regression model between the presence of *M. hominis* and *U. urealyticum* with their respective presence in the CM. However, a direct relationship with the development of abortion was not observed. In other studies, the results were also similar [24, 28]. These microorganisms are often found in the genital tract of pregnant women, where they can invade the placenta and contribute to the development of abortion. *U. urealyticum* in asymptomatic pregnant women, in combination with other factors, may be a contributing factor to premature birth and spontaneous abortion [27].

In the present study, there was no association between *M. genitalium* and *N. gonorrhoeae* and spontaneous abortion. Similar results were reported by Ramazanadeh et al. [29] who did not associate *M. genitalium* with spontaneous abortion. These microorganisms cause mucopurulent cervicitis in women and can migrate from the vagina to the uterus. However, an association was observed between the presence of *M. genitalium* in the placental tissue with *U. urealyticum* in CM and *U. parvum* in the placental tissue. In addition, the presence of mycoplasmas with other genital bacteria during preconception and pregnancy may be associated with gestational adverse events including spontaneous abortion [29] and adverse pregnancy outcomes are more severe in patients with more than one organism compared to those with only one [30].

Regarding microbial load, a significant increase was observed in the placental tissue between the amount of *M. hominis* (*P < 0.00*), *U. urealyticum* (*P < 0.00*), and *U. parvum* (*P < 0.01*) from the abortion group compared to the control group. This same association was observed in CM only for *U. parvum* (*P < 0.01*). Other studies [27, 31–35] also associated the presence of chorioamnionitis, intense inflammatory reaction and gestational adverse events with the bacterial density of *mollicutes*. However, Contini et al. [20] did not associate *M. hominis*, *U. parvum* and *U. urealyticum* density with adverse events. The relationship between *mollicutes* and abortion may be related to their presence in the upper genital tract microbiota and is associated with other risk factors, it may favour massive colonisation, generating aggressive inflammatory response and resulting in spontaneous abortion [26].

The present study has some limitations. First, the smaller number of participants in the control group was due to numerous refusals of patients in active vaginal labour (transient condition of pain, discomfort and concerns centred on the birth of their children), due to the fact that the collection of information was performed before childbirth. However, in women in the abortion group, no matter how much the situation caused grief, there was an absence of physical pain and a desire to know possible causes associated with abortion. Second, the information about spontaneous abortion was self-reported. And third, in the regression model, there may be a lack of consideration of bacterial vaginosis, due to the fact that *U. parvum*, together with other genital mycoplasmas, is highly associated with BV.

### Conclusion

In this study, we investigated the role of *mollicutes* and co-infection with the spontaneous abortion. This is the first study to show such high placental tissue and CM colonisation...
rates by *M. hominis*, *U. urealyticum* and *U. parvum* in women with spontaneous abortion. In addition, *U. parvum* was associated with spontaneous abortion. Moreover, lower genital colonisation in pregnancy by *M. hominis* and *U. urealyticum* was associated with placental tissue infection in women with spontaneous abortion. Thus, it is important to unravel, more broadly, the participation of mollicutes in the genesis of abortion.

**Acknowledgements.** The authors thank the women who even under difficult conditions agreed to participate in the study and Jim Hesson who edited the manuscript (https://www.academicenglishsolutions.com). This study was supported by State of Bahia Research Foundation (FAPESB/PPSUS SUS0044/2018) and financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brasil (CAPES) - Finance code 001.

**Conflict of interest.** The authors declare that they have no competing interests.

**Ethical standards.** The study was performed following human ethical precepts after approval by the Ethics Committee of Research with Human Beings of the Multidisciplinary Health Institute campus Anisio Teixeira under CAAE n° 56639916.8.0000.5556.

**Data.** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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