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**ORIGINAL ARTICLE****Basic Study**

- 1 Relationship between *Mollicutes* and spontaneous abortion: An epidemiological analysis

*Silva Oliveira MT, Oliveira CNT, da Silva LSC, Braga Martins Oliveira H, Sousa Freire R, Marques LM, Santos MLC, de Melo FF, Souza CL, Oliveira MV*

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## Basic Study

# Relationship between *Mollicutes* and spontaneous abortion: An epidemiological analysis

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## Abstract

### BACKGROUND

Abortion is of great importance in public health, as it is among the main causes of maternal morbidity and mortality. In addition to sociodemographic- and lifestyle-related factors, studies have associated infections of the genital tract with higher rates of abortion. Therefore, the exacerbated presence and rise of *Mollicutes* in the genitourinary tract may be related to higher rates of abortion.

### AIM

To perform an epidemiological analysis of women who had spontaneous abortions and placental colonization by *Mollicutes* in a maternity hospital.

### METHODS

This cross-sectional study involved the collection of data and biological material from women hospitalized due to spontaneous abortion or term delivery. The sample consisted of 89 women who miscarried and 20 women who had full term pregnancy. Data collection was carried out in three stages: (1) Conducting research on the information and clinical data in medical records of patients hospitalized due to abortion; (2) Application of a semi-structured questionnaire to identify the patient's epidemiological profile and (3) Collection of placental tissue. Placental samples were collected after the curettage procedure (abortion) and after placental expulsion (delivery), both performed by an obstetrician. Microbial identification in the fragments was performed by real-time polymerase chain

associated with the contributions by any of the senior authors or coauthors to this manuscript.

**Data sharing statement:** No additional data are available.

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reaction. In this study, the following explanatory variables were considered: (1) Sociodemographic variables; (2) Variables related to access to health services; (3) Variables related to lifestyle; and (4) Variables related to sexual and reproductive health, all of which were used to perform descriptive, univariate and multivariate analyses.

## RESULTS

In the final model, placental colonization by *Mollicutes* was independently associated with the variables age [odds ratio (OR) = 7.55; CI: 2.37-24.03] and menarche (OR = 3.43; CI: 1.03-11.44). In this investigation, the prevalence of *Mollicutes* colonization by at least one of the following three species: *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum* in women who had spontaneous abortion was 73.0%. When comparing colonization between the two groups of participating women, there was an 8.12-fold risk of placental colonization by at least one *Mollicutes* species in the women who had an abortion, compared to those who completed pregnancy. The final multivariate analysis model revealed a statistically significant association between placental colonization by *Mollicutes* in abortion with the following variables: age, as women up to 29 years old had a 7.55-fold risk of spontaneous abortion, compared to those who were older than 29 years; menarche, where women who had menarche up to 13 years old had a 3.43-fold risk of miscarriage compared to those who had menarche over 13 years old; and a change in eating habits, after the discovery of pregnancy, was a protective factor (OR = 0.16).

## CONCLUSION

These findings revealed a positive association between spontaneous abortion and placental colonization by *Mollicutes*. This indicates the need for further investigation of this issue, to guide decision-making for the prevention of abortion.

**Key Words:** Miscarriage; Epidemiology; *Mollicutes*; Primary prevention

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**Core Tip:** This study analyzed the association between spontaneous abortion and placental colonization by *Mollicutes* using a cross-sectional design, which involved the collection of data and biological material from women hospitalized due to spontaneous abortion or term delivery. Microbial identification in biological samples was performed by real-time polymerase chain reaction and the collected data were used to perform descriptive, univariate and multivariate analyses. The prevalence of *Mollicutes* among women who had spontaneous abortion was 73.0% and in women who had an abortion, an 8.12-fold risk of placental colonization by at least one species of *Mollicutes* was observed, compared to those who completed pregnancy.

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## INTRODUCTION

Abortion is considered the early termination of pregnancy at less than 22 wk and a fetus weighing less than 500 g. Approximately 10% to 15% of abortions occur spontaneously and mostly with an undetermined cause<sup>[1,2]</sup>. Abortion is of great importance in public health, as it is among the main causes of maternal morbidity and mortality, especially in developing countries such as Brazil<sup>[3]</sup>.

According to the World Health Organization, each year, approximately 500 thousand women die from causes related to pregnancy, with 98% of cases occurring in developing countries. Of these deaths, 15% is associated with abortion complications<sup>[4]</sup>. A global study revealed that in the period from 2010 to 2014, 25% of pregnancies worldwide resulted in abortion<sup>[5]</sup>.

Studies have associated sociodemographic factors (such as age, marital status, social context, socioeconomic conditions, and education) and factors related to lifestyle (such as alcohol consumption, smoking, pre-gestational nutritional status, reproductive history, environmental and occupational exposure of woman) with spontaneous abortion<sup>[6-9]</sup>.

Another important clinical aspect in pregnant women, is infections of the genital tract due to placental colonization with microorganisms related to bacterial vaginosis such as *Gardnerella vaginalis*, *Mollicutes* [*Mycoplasma hominis* (*M. hominis*), *Ureaplasma urealyticum* (*U. urealyticum*), *Ureaplasma parvum* (*U. parvum*)], *Mobiluncus spp*, *Chlamydia trachomatis*, among others<sup>[2,10]</sup> as a possible cause of spontaneous abortion.

*Mollicutes* constitute a class of bacteria that differ from the other bacteria as they do not have a cell wall. The genera of interest in this study are *Mycoplasma* and *Ureaplasma*. The former are important opportunistic pathogens that colonize the male and female urogenital tract, and are responsible for triggering various pathologies including spontaneous abortion<sup>[11]</sup>. *Ureaplasma* species are one of the main pathogens associated with genital infection, for example non-gonococcal urethritis in men and, in women, gestational complications such as premature birth and abortion, among others<sup>[12]</sup>.

The presence of *Mollicutes* in the genitourinary tract and its pathogenic potential is related to age, hormones, sexual activity, pregnancies, low socioeconomic status, smoking, use of vaginal showers and the use of oral contraceptives<sup>[13]</sup>. The diagnosis of *Mollicutes* infection is quite difficult as they have low biosynthetic activity and require complex culture media, making the diagnosis using techniques such as Gram staining, complicated. They can also be diagnosed by immunoenzymatic reactions and by molecular biology, the latter being considered the gold standard for diagnosis<sup>[14]</sup>.

In pregnant women with modification of the vaginal microbiota, a reduction in *Lactobacillus* species is frequently found, triggering an increase in vaginal pH<sup>[15]</sup>. As a result, changes occur in the human vaginal microbiome, which contains a varied number of bacterial species, including members of the *Mollicutes* class, which may find conditions which allow entry into the uterine cavity<sup>[16]</sup>. In the uterus, they may be able to induce an immune response that can lead to excessive inflammation and consequently damage and death to the cellular components of the placental and fetal unit<sup>[17]</sup>.

Thus, in the present study we analyzed the association between spontaneous abortion and placental colonization by *Mollicutes*, in order to provide evidence capable of contributing to reduced health costs. In addition, the study of spontaneous abortion in women with placental colonization by *Mollicutes* has been little explored and may encourage further studies on this issue, thus increasing our understanding and management of spontaneous abortion. Therefore, we performed an epidemiological analysis of women treated for spontaneous abortion with placental colonization by *Mollicutes* in a maternity hospital in southwest Bahia, Brazil.

## MATERIALS AND METHODS

This is a cross-sectional study, derived from a larger project, entitled "Evaluation of the participation of *Mollicutes* and other microorganisms of genital interest in the etiopathogenesis of spontaneous abortions". The present study aimed to detect *Mollicutes* and other microorganisms of genital interest in the placental biopsy of women who underwent spontaneous abortion, to assess their probable relationship in etiopathogenesis. It involved the collection of data and biological material from two groups: patients hospitalized due to spontaneous abortion and patients who had term delivery and was carried out by researchers from the Universidade Federal da Bahia and the Universidade Estadual de Santa Cruz.

The main objective of the present investigation was to determine the association between patients hospitalized due to spontaneous abortion and placental colonization by *Mollicutes*. For the purpose of comparison and verification of this association, we assessed the prevalence of placental colonization by *Mollicutes* from the group of women who had term pregnancy.

The study was carried out in a public hospital, in a maternal and child health care



unit for the municipality of Vitória da Conquista, Bahia, Brazil and the southwestern macro-region of the state of Bahia, Brazil. Vitória da Conquista it is the third largest city in Bahia, with a total population of 348718 inhabitants<sup>[18]</sup>.

The participants were women aged 18 years or older, hospitalized due to spontaneous abortion, with a gestational age greater than or equal to 8 wk and less than 22 wk and a fetus weighing less than 500 g. In addition, a group of women who underwent vaginal delivery at term (gestational age between 38 and 42 wk), who voluntarily agreed to participate in the study after being approached by the researchers was also included.

The study excluded women who used antibiotics less than two weeks after the abortion occurred. Also excluded were those with confirmed immunodeficiency, and chronic diseases previously diagnosed (hypertension, diabetes, endocrine disorders) and a history of recurrent abortions due to anatomical abnormalities and/or congenital malformation.

In this study the sample consisted of 89 women who miscarried and were treated between July 2017 and August 2018 and 20 women who had full term pregnancy at the hospital.

Data collection took place in three stages, which are shown in **Figure 1**. The first stage involved the collection of information and clinical data from the medical records of the patients hospitalized due to abortion. The second stage was the application of a semi-structured questionnaire in order to identify the patient's epidemiological profile, divided into modules, as a result of the adaptation of the National Health Survey, and the instrument used in the study by Campos *et al.*<sup>[19]</sup>. The third stage consisted of collecting placental tissue<sup>[20]</sup>. Placental samples were collected after a curettage procedure using a sterile speculum performed by an obstetrician. The fragment was stored in a transport medium for *Mycoplasma* and transported in thermal boxes to the Microbiology and Immunology laboratory of the Instituto Multidisciplinar em Saúde, at the Universidade Federal da Bahia. For microbial identification of placental tissue, genomic deoxyribonucleic acid was initially extracted using the Invitrogen Purelink™ Genomic deoxyribonucleic acid Kit (Invitrogen, Waltham, MA, United States). Subsequently, real-time polymerase chain reaction was performed using StepOne Plus (Life Technologies) with a final volume of 25 µL and the use of Master Mix (Thermo Fisher Scientific, Waltham, MA, United States). To identify the *Mollicutes* species, TaqMan probes were used, following the basic amplification protocol for each species. The studied species were: *M. hominis*, *U. urealyticum* and *U. parvum*.

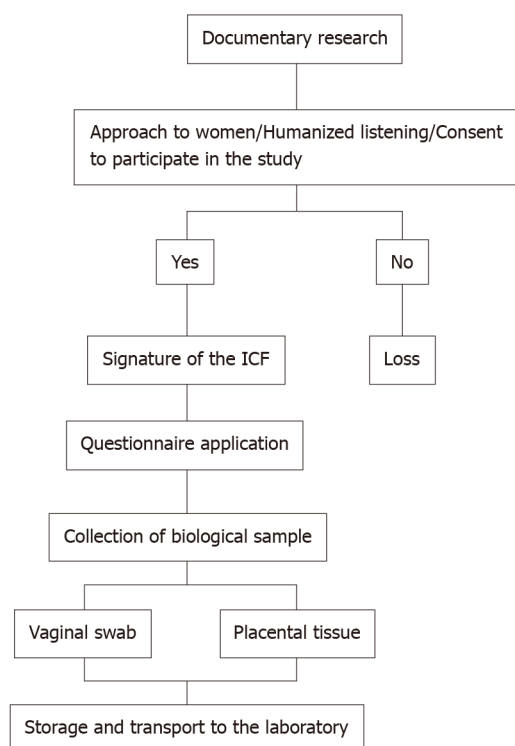
The identification of placental *Mollicutes* in patients with abortion constituted the outcome variable of this study. The following explanatory variables were considered: (1) Sociodemographic variables: Age group, income, marital status, education, and skin color; (2) Variables related to access to health services: Prenatal consultations; (3) Variables related to lifestyle: Type of food consumed, physical activity, alcohol and tobacco use; and (4) Variables related to sexual and reproductive health: Historical existence of sexually transmitted infection, presence of genital alterations (ulcers, blisters, lumps, discharge), presence of urinary tract infection, changes in the reproductive system such as: presence of polycystic fibroids and ovaries, number of previous pregnancies, number of abortions, menarche and first sexual intercourse.

A descriptive analysis of the variables was performed by comparing frequencies and using Pearson's Chi-square test with  $P < 0.05$  and a 95% confidence interval, using a Microsoft Office Excel database and analyzed using the statistical package EPI-INFO (version 7.2). To obtain measures of association of the variables, the odds ratio calculation was then used. All variables with  $P < 0.20$  in the univariate analysis were included in multivariate analysis using logistic regression, performed in SPSS version 21. In the final multivariate model, a significance index of  $P < 0.05$  was considered and the final model which obtained the best fit was evaluated by the Hosmer-Lemeshow test.

This study took place after approval by the teaching and research nucleus of the Health Foundation of Vitória da Conquista and approval by the Human Research Ethics Committee of the Instituto Multidisciplinar em Saúde (Universidade Federal da Bahia), opinion 1.764 332.

## RESULTS

After analyzing the placental tissue, the prevalence of colonization by *Mollicutes* with at least one of the three species: *M. hominis*, *U. urealyticum* and/or *U. parvum* among women who had a spontaneous abortion was 73.0%. In the larger study, in addition to



**Figure 1** Flowchart of the data collection procedure. ICF: Informed consent form.

studying women who had abortions, the presence of *Mollicutes* in the placenta of women who completed their term pregnancy was also investigated. In this group, the presence of at least one species of those listed above was detected in 25% ( $n = 5$ ) of women. When comparing colonization between the two groups of women, there was an 8.12-fold risk (CI: 2.66-24.78,  $P < 0.001$ ), for placental colonization by at least one *Mollicutes* species in women who had an abortion, compared to those who completed their pregnancy.

**Table 1** shows the descriptive analysis of women undergoing abortion who were included in the study ( $n = 89$ ). The average age of the participants was 28 years. Most women reported having a partner, were from an urban area, were non-white and had more than 8 years of education. 65.2% ( $n = 58$ ) of the study participants reported having a family income of up to one minimum wage, which at the time of the interview was R\$ 95400.

With regard to life habits, most of them reported not having changed their eating habits after the discovery of pregnancy, not smoking, not drinking alcohol and not taking part in physical activity. Regarding sexual and reproductive health, the majority declared that they did not have any gynecological disorders, such as fibroids, polycystic ovaries and never had a sexually transmitted infection. Of the interviewees, 65.1% ( $n = 56$ ) started their sexual life aged more than 15 years, and reported having had more than one sexual partner in her life and not using condoms during sexual intercourse. Just over half of the participants reported having had up to 2 pregnancies in their lifetime.

The average gestational age at which the abortion occurred was 12 wk, ranging from 8 to 22 wk. 58.4% ( $n = 52$ ) of the interviewees had at least one prenatal consultation and among all the women studied an average of 1.7 consultations occurred before the abortion. Most of the participants reported not having planned their pregnancy and having had some type of vaginal discharge in the last three months. A small number of women reported having had pelvic pain and dysuria.

**Table 2** shows the results of the univariate analysis between the variables selected for the study and placental colonization by at least one of the studied *Mollicutes* species. It was found that women up to 29 years old had a 7.26-fold risk (CI: 2.49-21.12) of placental colonization by at least one of the studied *Mollicutes* species, compared with those older than 29 years. Women who reported menarche age of up to 13 years had a 3.15-fold risk (CI: 1.13-8.79) of placental colonization by at least one of the studied *Mollicutes* species compared with those who had menarche over 13 years. Women who had up to 2 pregnancies had a 2.73-fold risk (CI: 1.04 -7.05) of



**Table 1** Descriptive analysis of women with spontaneous abortion participating in the study, Vitória da Conquista, Bahia, Brazil, 2018

Variables	n	(%)
Placenta colonized by <i>Mollicutes</i>		
Yes	65	73
No	24	27
Age		
Up to 29 yr	52	58.5
> 29 yr	37	41.5
Marital status		
With companion	68	78.2
Without companion	19	21.8
Year of study <sup>1</sup>		
None	2	2.3
Up to 7 yr	29	32.9
> 7 yr	57	64.8
Origin		
Rural area	28	31.5
Urban area	61	68.5
Skin color		
White	27	30.3
Non-white	62	69.7
Income <sup>1,2</sup>		
Up to the minimum wage	58	65.2
Above the minimum wage	30	33.6
Religion		
Catholic	49	55.1
Others <sup>5</sup>	26	29.2
None	14	15.7
Changes in eating habits during pregnancy <sup>3</sup>		
Yes	10	11.5
No	77	88.5
Physical activity <sup>1</sup>		
Yes	16	18.2
No	72	81.8
Alcohol consumption <sup>6</sup>		
Yes	14	15.7
No	75	84.3
Smoking <sup>6</sup>		
Yes	4	4.5
No	85	95.5
Gynecological disorders <sup>1,7</sup>		
Yes	9	88.7
No	79	10.3

Menarche		
Up to 13 yr	67	75.3
> 13 yr	22	24.7
First sexual intercourse <sup>4</sup>		
Up to 15 yr	30	34.9
> 15 yr	56	65.1
Number of partners <sup>1</sup>		
1	53	60.2
> 1	35	39.8
Previous history of STI		
Yes	5	5.6
No	84	94.4
Condom use during sexual intercourse <sup>1</sup>		
No	51	57.9
Sometimes	37	42.1
Pain during intercourse		
Yes	26	29.6
No	62	70.4
Pregnancies (including the current one)		
Up to 2 pregnancies	53	59.5
> 2 pregnancies	36	40.5
Premature births		
None	82	92.1
More than one	7	7.9
Prenatal care		
Yes	52	58.4
No	37	41.6
Pregnancy was planned <sup>3</sup>		
Yes	32	36.8
No	55	63.8
Number of live children		
1	59	66.3
> 1	30	33.7
Presence of vaginal discharge		
Yes	58	65.2
No	31	34.8
Dysuria		
Yes	16	18
No	73	82
Groin swelling		
Yes	4	4.5
No	85	95.5
Vaginal alteration <sup>8</sup>		

Yes	9	10.1
No	80	89.9
Vaginal itching		
Yes	27	30.3
No	62	69.7
Vaginal erythema		
Yes	18	20.2
No	71	79.8
Pelvic pain		
Yes	33	37.1
No	56	62.9
Vaginal wart		
Yes	2	2.2
No	87	97.8
Vaginal wound		
Yes	2	2.2
No	87	97.8

<sup>1</sup>Non-responding.<sup>2</sup>Minimum salary of R\$ 954.00.<sup>3</sup>Non-responders.<sup>4</sup>Non-responders.<sup>5</sup>Evangelical, spirit, candomblé.<sup>6</sup>Any quantity and frequency.<sup>7</sup>Uterine fibroids, ovarian cysts.<sup>8</sup>Any anatomical or physiological change.

STI: Sexually transmitted infection.

spontaneous abortion and placental colonization by at least one of the studied *Mollicutes* species, when compared to those who had more than 2 pregnancies. For those with up to one live child, there was a 3.33-fold (CI: 1.25-8.84) risk compared to those who had more than one live child.

The final multivariate analysis (Table 3) revealed a statistically significant association between placental microbial colonization by *Mollicutes* in abortion and the variables: Age (women up to 29 years of age had a 7.55-fold risk of having a miscarriage, compared to those older than 29 years); menarche (women who had menarche up to 13 years of age had a 3.43-fold risk of having a miscarriage than those who had menarche over 13 years of age). A change in eating habits after the discovery of pregnancy appeared to be a protective factor.

## DISCUSSION

Microbial colonization by *Mollicutes* in the urogenital tract may predispose to spontaneous abortion<sup>[11,21]</sup>. In this study, the prevalence of microbial colonization by at least one of three *Mollicutes* species (*U. urealyticum*, *U. parvum*, *M. hominis*) in the placenta of women with spontaneous abortion was 73.0%. The prevalence of *U. urealyticum* in endocervical material from patients who had an abortion was 16.5% in another study<sup>[22]</sup>. Previous studies were also carried out in other countries such as Germany, Switzerland and Australia, and showed evidence that the presence of *Mollicutes* and other microorganisms in the vaginal microbiota may be related to adverse pregnancy outcomes, including spontaneous abortion<sup>[21,23,24]</sup>.

In the present study, women who had up to one live child had a 3.33-fold risk (CI: 1.25-8.84) of abortion compared with those who had more than one live child. In addition, women who had 2 pregnancies had a 2.73-fold risk of abortion when colonized by *Mollicutes*, compared to those with more than 2 pregnancies. The

**Table 2 Univariate analysis of placental colonization by *Mollicutes* in spontaneous abortion and selected variables, Vitória da Conquista, Bahia, Brazil, 2018**

Variable	OR (IC)	$\chi^2$	P value
Age			
Up to 29 yr	7.26 (2.49-21.12)	15	0.00
> 29 yr	1		
Marital status			
With companion	1		
Without companion	2.33 (0.62-9.08)	1.69	0.19
Year of study <sup>1</sup>			
None	1		
Up to 7 yr	2.27 (0.12-39.64)	0.3	0.57
> 7 yr	3.21 (0.18-54.79)	0.72	0.39
Origin			
Rural area	1.15 (0.41-3.22)	0.08	0.77
Urban area	1		
Skin color			
White	1		
Non-white	1.43 (0.49-4.13)	0.44	0.5
Income <sup>1,2</sup>			
Up to the minimum wage	1.22 (0.46-3.26)	0.17	0.67
Above the minimum wage	1		
Religion			
Catholic	1		
Others <sup>5</sup>	0.98 (0.33-2.86)	0	0.97
None	0.90 (0.24-3.28)	0.01	0.87
Changes in eating habits during pregnancy <sup>3</sup>			
Yes	1		
No	3.05 (0.79-11.69)	2.84	0.09
Physical activity <sup>1</sup>			
Yes	1		
No	1.26 (0.33-4.12)	0.15	0.69
Alcohol consumption <sup>6</sup>			
Yes	5.75 (0.70-46.19)	3.31	0.06
No	1		
Smoking <sup>6</sup>			
Yes	1.11	0	0.92
No	1		
Gynecological disorders <sup>1,7</sup>			
Yes	3.28 (0.38-27.78)	1.32	0.25
No	1		
Menarche			
Up to 13 yr	3.15 (1.13-8.79)	5.07	0.02

> 13 yr	1		
First sexual intercourse <sup>4</sup>			
Up to 15 yr	2.17 (0.71-6.65)	1.93	0.16
> 15 yr	1		
Number of partners <sup>1</sup>			
1	2.78 (0.16-46.33)	0.55	0.45
> 1	1		
Previous history of STI			
Yes	1.5 (0.16-14.21)	0.13	0.71
No	1		
Condom use during sexual intercourse <sup>1</sup>			
No	1		
Sometimes	2.14 (0.78-5.87)	2.24	0.13
Pain during intercourse			
Yes	1.36 (0.47-3.95)	0.32	0.56
No	1		
Pregnancies (including the current one)			
Up to 2 pregnancies	2.73 (1.04-7.15)	4.36	0.03
> 2 pregnancies	1		
Premature births			
None	1		
More than one	2.17 (0.45-10.50)	0.97	0.32
Prenatal care			
Yes	1		
No	1.26 (0.48-3.09)	0.22	0.63
Pregnancy was planned <sup>3</sup>			
Yes	1		
No	1.33 (0.50-3.48)	0.34	0.55
Number of live children			
1	3.33 (1.25-8.84)	6.15	0.01
> 1	1		
Presence of vaginal discharge			
Yes	2.42 (0.92-6.33)	3.33	0.06
No	1		
Dysuria			
Yes	1.13 (0.32-3.92)	0.03	0.84
No	1		
Groin swelling			
Yes	1.11 (0.11-11.24)	0	0.92
No	1		
Vaginal itching			
Yes	1.27 (0.46-3.50)	0.22	0.63
No	1		

Vaginal erythema			
Yes	0.95 (0.29-3.02)	0	0.93
No	1		
Pelvic pain			
Yes	1.61 (0.58-4.44)	0.88	0.34
No	1		

<sup>1</sup>Non-responding.<sup>2</sup>Minimum salary of R\$ 95450.<sup>3</sup>Non-responders.<sup>4</sup>Non-respondents.<sup>5</sup>Evangelical, spirit, candomblé.<sup>6</sup>Any quantity and frequency.<sup>7</sup>Uterine fibroids, ovarian cysts.

**Table 3 Final regression model by groups of selected variables and the presence of *Mollicutes* in placental tissue of spontaneous abortion, Vitória da Conquista, Bahia, Brazil, 2018**

Variable	OR, IC (95%)	P value
Age		
Up to 29 yr	7.55 (2.37-24.03)	0.01
> 29 yr	1.0	
Menarche		
Up to 13 yr	3.43 (1.03-11.44)	0.04
> 13 yr	1.0	
Dietary change after pregnancy		
Yes	0.16 (0.03-0.06)	0.03
No	1.0	

Model adequacy test-Hosmer Lemeshow,  $\chi^2 = 2.37$   $P = 0.49$ . OR: Odds ratio.

literature demonstrates conflicting data on this issue, some studies have shown a protective effect against spontaneous abortion in women with more than one live child<sup>[7]</sup>, and some have indicated an increased risk in women with more than one live child<sup>[8,21]</sup>.

Results also vary with parity: the difficulty of becoming pregnant or the existence of complications in previous pregnancies may be the reason for past spontaneous losses<sup>[6,7,20]</sup>. In other studies, women with more live children indicated a higher number of pregnancies, and had the greatest risk of abortion<sup>[8,25]</sup>.

Age up to 29 years was independently associated (OR = 7.55; CI: 2.37-24.03) with the presence of *Mollicutes* in the placenta of women with abortion. Age between 20 and 30 years was also associated with abortion and the presence of infectious diseases such as STORCH syndrome (syphilis, toxoplasmosis, rubella, cytomegalovirus and herpes virus)<sup>[1]</sup> or the presence of *Ureaplasma spp* in the female genital tract<sup>[26]</sup>. These findings are in line with other studies, where it was shown that young age (women under 29 years old) is a risk factor for the occurrence of spontaneous abortion, and younger pregnant women may have less access to health services. This implies fewer possibilities for early detection of infections associated with abortion<sup>[1,6,22]</sup>.

Findings from studies that evaluated prenatal programs and cervical screening using Pap smear, which has often shown a higher prevalence of the use of these services by older women, corroborates these findings<sup>[27,28]</sup>. However, other studies diverge from these findings where the occurrence of spontaneous abortion occurs in a greater proportion of women over 35 years of age<sup>[8,25]</sup>, these studies are usually population-based and self-reported, and only point out that the older the age, the longer the time of exposure to pregnancy and abortion.



After final adjustment, women who had their first menstruation up to 13 years of age had a 3.43-fold risk (CI: 1.03-11.44) of developing spontaneous abortion with placental colonization by *Mollicutes* when compared to women who menstruated after 13 years. Early menarche was associated with spontaneous abortion<sup>[6]</sup>, and in Brazilian girls this age has decreased further, implying precocious reproduction, which may be related to a greater number of pregnancies among young women. This, together with other factors, can lead to an increase in the occurrence of spontaneous abortion<sup>[29,30]</sup>. Thus, it can be inferred that sex education, and the provision of family planning by health services, should be started early, in order to avoid early pregnancies.

Prenatal care was also one of the variables examined in this study. Even though there was no statistical significance, it still deserves to be mentioned, as most of the participants stated that they had prenatal care before the abortion occurred. However the average number of consultations was 1.7 per woman, a very low rate, considering that the average gestational age at abortion was 12 wk. According to technical norms of the Ministry of Health, prenatal care must occur as early as possible. Right at the first consultation it is necessary to request laboratory tests and to perform a gynecological examination (specular and cytopathological examination). Also, at this time, the existence of urinary tract infections and the presence of bacterial vaginosis should be investigated. The latter is defined as infection of the female genital tract, where among several pathogens, *Mollicutes* can be the causative agents. This can provide an opportunity for the early diagnosis and treatment of these infections, preventing gestational adverse effects such as the occurrence of spontaneous abortion<sup>[28,31,32]</sup>.

Socioeconomic factors have been identified as important elements related to spontaneous abortion. Some studies have shown a significant association between low income levels, low education level and spontaneous abortion<sup>[3,7,8]</sup>. In this study, income was not significantly associated with spontaneous abortion, but due to the homogeneity in this variable, most participants 65.2% ( $n = 58$ ) reported income of up to one minimum wage, resulting in low power for comparison purposes.

Schooling has been reported in the literature to be an important factor related to the occurrence of spontaneous abortion. In countries such as Brazil, women who had up to 8 years of education had a 1.66-fold greater risk (CI: 1.11-2.49) of having a miscarriage, than those with more than 8 years of education<sup>[6]</sup>, and similar results have been found in other studies<sup>[7,8]</sup>. These findings were not observed in this study, most women reported having more than 7 years of education; thus, education level was not significant. Among the women studied, the problem was access to health services than the lack of information.

In summary, it should be noted that this is a pioneering study in the southwestern region of Bahia, Brazil, which revealed a positive association between spontaneous abortion and placental colonization by *Mollicutes*. The study also showed an association between abortion and women up to 29 years of age, who had their first menstruation up to 13 years old. These findings require more detailed studies within this area, in order to provide a better epidemiological profile of women undergoing abortion and to support decision-making by health managers. Decision-making in abortion can have an important impact on reducing health costs, as managing the complications of abortion and its occurrence is much more costly than preventing it.

## CONCLUSION

This is a pioneering study in the southwestern region of Bahia, which revealed a positive association between spontaneous abortion and placental colonization by *Mollicutes*, and abortion was also associated with women up to 29 years of age, who had their first menstruation up to 13 years of age. These findings are important, but point to the need for more detailed studies within this area, in order to provide a better epidemiological profile of women undergoing abortion to support decision-making by health managers, with a view to decreasing health costs, since the prevention of abortion is much less costly than managing its occurrence and complications.

## ARTICLE HIGHLIGHTS

### Research background

Abortion is of great importance in public health, as it is among the main causes of

maternal morbidity and mortality. In addition to sociodemographic- and lifestyle-related factors, studies have demonstrated that infections of the genital tract are associated with higher rates of abortion. The increased presence *Mollicutes* in the genitourinary tract may be related to higher rates of abortion. In addition, the diagnosis of this infection requires complex culture media, making the diagnosis more difficult, thus knowledge of the local epidemiological rates of these infections can guide clinical practice.

### Research motivation

The importance of understanding the factors that may be related to spontaneous abortions is inadequate in clinical practice in order to avoid such outcomes and to ensure better public health principles.

### Research objectives

To perform an epidemiological analysis of women with spontaneous abortion and placental colonization by *Mollicutes* in a maternity hospital.

### Research methods

This is a cross-sectional study which involved the collection of data and biological material from women hospitalized due to spontaneous abortion or for term delivery. The sample analyzed consisted of 89 women who miscarried and 20 women who had full term pregnancy. Data collection took place in three stages: (1) Conducting research to identify information and clinical data in the medical records of patients hospitalized due to abortion; (2) Application of a semi-structured questionnaire to identify the patient's epidemiological profile; and (3) Collection of placental tissue. Placental samples were collected after the curettage procedure (abortion) and after placental expulsion (delivery), both performed by an obstetrician. Microbial identification of the fragments was performed by real-time polymerase chain reaction. In this study, the following explanatory variables were considered: (1) Sociodemographic variables; (2) Variables related to access to health services; (3) Variables related to lifestyle; and (4) Variables related to sexual and reproductive health, all of which were used to perform descriptive, univariate and multivariate analyses.

### Research results

Colonization by *Mollicutes* was observed in 73.0% of the study participants. In the final model, placental colonization by *Mollicutes* was independently associated with the variables age [odds ratio (OR) = 7.55; CI: 2.37-24.03] and menarche (OR = 3.43; CI: 1.03-11.44). In this investigation, 73.0% of women who had a spontaneous abortion had colonization by *Mollicutes*. When comparing colonization between the two groups of participating women, there was an 8.12-fold risk of placental colonization by at least one *Mollicutes* species in those who had an abortion, compared to those who completed pregnancy. The final multivariate analysis model revealed a statistically significant association between placental microbial colonization by *Mollicutes* and abortion in relation to the variables: age, where women up to 29 years old had a 7.55-fold risk of spontaneous abortion, compared to those older than 29 years; menarche, where women who had menarche up to 13 years old had a 3.43-fold risk of miscarriage compared to those who had menarche over 13 years old; and change in eating habits, after the discovery of pregnancy, was a protective factor (OR = 0.16).

### Research conclusions

Our study revealed a positive association between spontaneous abortion and placental colonization by *Mollicutes*. This signals the need for more investigations on this issue, in order to guide decision-making for the prevention of abortion.

### Research perspectives

The information provided by this study is applicable for the development of guidelines for the prevention of abortion.

## REFERENCES

- 1 Barbaresco AA, da Costa TL, Avelar JB, Rodrigues IM, do Amaral WN, de Castro AM. [Vertical transmission from abortive material and blood with emphasis on *Toxoplasma gondii*]. *Rev Bras Ginecol Obstet* 2014; **36**: 17-22 [PMID: 24554225 DOI: 10.1590/S0100-72032014000100005]

- 2 **Nigro G**, Mazzocco M, Mattia E, Di Renzo GC, Carta G, Anceschi MM. Role of the infections in recurrent spontaneous abortion. *J Matern Fetal Neonatal Med* 2011; **24**: 983-989 [PMID: [21261443](#) DOI: [10.3109/14767058.2010.547963](#)]
- 3 **Adesse L**, Silveira K, Bonan C, Fonseca VM. Complicações do abortamento e assistência em maternidade pública integrada ao Programa Nacional Rede Cegonha. *Saúde Debate* 2015; **39**: 694-706 [DOI: [10.1590/0103-1104201510600030011](#)]
- 4 **World Health Organization**. Complications of Abortion: Technical and Managerial Guidelines for Prevention and Treatment. 1995 [cited 6 January 2021]. In: World Health Organization [Internet]. Available from: <https://xueshu.baidu.com/usercenter/paper/show?paperid=b8dc8fab4ea99cc703b4024413dc4358>
- 5 **Guttmacher institute**. Aborto Inducido a nível mundial - incidência y tendencias mundiales. Datasheet. New York: Guttmacher institute. 2018. Available from: <https://www.guttmacher.org/es/fact-sheet/aborto-in>
- 6 **Correia LL**, Rocha HAL, Leite ÁJM, Campos JS, Silva AC, Machado MMT, Rocha SGM, Gomes TN, Cunha AJLA. Tendência de abortos espontâneos e induzidos na região semiárida do Nordeste do Brasil : uma série transversal. *Rev Bras Saúde Mater Infant* 2018; **18**: 133-42 [DOI: [10.1590/1806-93042018000100006](#)]
- 7 **Machado CJ**, Lobato AC, Melo VH, Guimarães MD. [Spontaneous and voluntary fetal losses in Brazil in 1999-2000: a study of associated factors]. *Rev Bras Epidemiol* 2013; **16**: 18-29 [PMID: [23681319](#) DOI: [10.1590/s1415-790x2013000100002](#)]
- 8 **Cecatti JG**, Guerra GV, Sousa MH, Menezes GM. [Abortion in Brazil: a demographic approach]. *Rev Bras Ginecol Obstet* 2010; **32**: 105-111 [PMID: [20512256](#) DOI: [10.1590/s0100-72032010000300002](#)]
- 9 **Hure AJ**, Powers JR, Mishra GD, Herbert DL, Byles JE, Loxton D. Miscarriage, preterm delivery, and stillbirth: large variations in rates within a cohort of Australian women. *PLoS One* 2012; **7**: e37109 [PMID: [22629355](#) DOI: [10.1371/journal.pone.0037109](#)]
- 10 **Krauss-Silva L**, Almada-Horta A, Alves MB, Camacho KG, Moreira ME, Braga A. Basic vaginal pH, bacterial vaginosis and aerobic vaginitis: prevalence in early pregnancy and risk of spontaneous preterm delivery, a prospective study in a low socioeconomic and multiethnic South American population. *BMC Pregnancy Childbirth* 2014; **14**: 107 [PMID: [24641730](#) DOI: [10.1186/1471-2393-14-107](#)]
- 11 **Bayraktar MR**, Ozerol IH, Gucluer N, Celik O. Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women. *Int J Infect Dis* 2010; **14**: e90-e95 [PMID: [19515594](#) DOI: [10.1016/j.ijid.2009.03.020](#)]
- 12 **Maleki S**, Motamedi H, Moosavian S M, Shahbaziyan N. Frequency of *Mycoplasma hominis* and *Ureaplasma urealyticum* in Females With Urogenital Infections and Habitual Abortion History in Ahvaz, Iran; Using Multiplex PCR. *Jundishapur J Microbiol* 2013; **6**: e10088 [DOI: [10.5812/jjm.10088](#)]
- 13 **Milanezi F**, Falconi A, Schnabel B, R. Ricardi L, M. Monfredini P, Ziliotto A, Lopes V, Machado S, Oliveira M, Centrone C, Nakano V. Prevalence of *Mycoplasma hominis* and *Ureaplasma* spp. in Routine Gynecological Care in Sao Paulo City, Brazil. *Arch Clin Infect Dis* 2016; **11**: e36668 [DOI: [10.5812/archcid.36668](#)]
- 14 GS. *Mycoplasma hominis* e *Ureaplasma* sp. em amostras do trato genitourinário e sua relação com sintomas de infecção genital. *Rev Bras Anal Clin* 2007; **39**: 295-298
- 15 **Giakoumelou S**, Wheelhouse N, Cuschieri K, Entrican G, Howie SE, Horne AW. The role of infection in miscarriage. *Hum Reprod Update* 2016; **22**: 116-133 [PMID: [26386469](#) DOI: [10.1093/humupd/dmv041](#)]
- 16 **Foschi C**, Salvo M, D'Antuono A, Gaspari V, Banzola N, Cevenini R, Marangoni A. Distribution of genital *Mollicutes* in the vaginal ecosystem of women with different clinical conditions. *New Microbiol* 2018; **41**: 225-229 [PMID: [29620787](#)]
- 17 **Mor G**, Aldo P, Alvero A. The unique immunological and microbial aspects of pregnancy. *Nat Rev Immunol* 2017; **17**: 469-482 [PMID: [28627518](#) DOI: [10.1038/nri.2017.64](#)]
- 18 **IBGE**. Panoramas: Vitória da Conquista – Bahia [cited 6 January 2021]. In: Instituto Brasileiro de Geografia e estatísticas [Internet]. Available from: <https://cidades.ibge.gov.br/brasil/ba/vitoria-da-conquista/panorama>
- 19 **Campos GB**, Lobão TN, Selis NN, Amorim AT, Martins HB, Barbosa MS, Oliveira TH, dos Santos DB, Figueiredo TB, Miranda Marques L, Timenetsky J. Prevalence of *Mycoplasma genitalium* and *Mycoplasma hominis* in urogenital tract of Brazilian women. *BMC Infect Dis* 2015; **15**: 60 [PMID: [25886914](#) DOI: [10.1186/s12879-015-0792-4](#)]
- 20 **Brasil**. Ministério da Saúde. Percepção Do Estado De Saúde, Estilos De Vida E Doenças Crônicas [cited 6 January 2021]. Instituto Brasileiro de Geografia e Estatística (IBGE) [Internet]. 2014: 1-181 Available from: <ftp://ftp.ibge.gov.br/PNS/2013/pns2013.pdf>
- 21 **Kikhney J**, von Schöning D, Steding I, Schulze J, Petrich A, Hiergeist A, Reisch U, Moter A, Thomas A. Is *Ureaplasma* spp. the leading causative agent of acute chorioamnionitis in women with preterm birth? *Clin Microbiol Infect* 2017; **23**: 119.e1-119.e7 [PMID: [27756710](#) DOI: [10.1016/j.cmi.2016.10.010](#)]
- 22 **Ahmadi A**, Khodabandehloo M, Ramazanzadeh R, Farhadifar F, Nikkhoo B, Soofizadeh N, Rezaii M. Association between *Ureaplasma urealyticum* endocervical infection and spontaneous abortion. *Iran J Microbiol* 2014; **6**: 392-397 [PMID: [25926956](#)]

- 23 **Capoccia R**, Greub G, Baud D. *Ureaplasma urealyticum*, *Mycoplasma hominis* and adverse pregnancy outcomes. *Curr Opin Infect Dis* 2013; **26**: 231-240 [PMID: [23587772](#) DOI: [10.1097/QCO.0b013e328360db58](#)]
- 24 **Sweeney EL**, Dando SJ, Kallapur SG, Knox CL. The Human *Ureaplasma* Species as Causative Agents of Chorioamnionitis. *Clin Microbiol Rev* 2017; **30**: 349-379 [PMID: [27974410](#) DOI: [10.1128/CMR.00091-16](#)]
- 25 **Noguez PT**, Muccillo-baisch AL, Cezar-vaz MR, Cristina M. Aborto espontâneo em mulheres residentes nas proximidades do parque industrial do município do rio grande - RS. *Texto Context Enferm* 2008; **17**: 435-46 [DOI: [10.1590/S0104-07072008000300004](#)]
- 26 **Marovt M**, Keše D, Kotar T, Kmet N, Miljković J, Šoba B, Matičič M. *Ureaplasma parvum* and *Ureaplasma urealyticum* detected with the same frequency among women with and without symptoms of urogenital tract infection. *Eur J Clin Microbiol Infect Dis* 2015; **34**: 1237-1245 [PMID: [25717022](#) DOI: [10.1007/s10096-015-2351-8](#)]
- 27 **Gomes Kde O**, Reis EA, Guimarães MD, Cherchiglia ML. [Use of health services by quilombo communities in southwest Bahia State, Brazil]. *Cad Saude Publica* 2013; **29**: 1829-1842 [PMID: [24068228](#) DOI: [10.1590/0102-311X00151412](#)]
- 28 **Santos GH**, Martins Mda G, Sousa Mda S, Batalha Sde J. [Impact of maternal age on perinatal outcomes and mode of delivery]. *Rev Bras Ginecol Obstet* 2009; **31**: 326-334 [PMID: [19838577](#)]
- 29 **Klug DP**, Fonseca PHS da. Análise da maturação feminina: um enfoque na idade de ocorrência da menarca. *Rev da Educ Física* 2006; **17**: 139-147
- 30 **Abeyseena C**, Jayawardana P, Seneviratne R. Risk Factors for Spontaneous Abortion. *J Coll Community Physicians Sri Lanka* 2009; **14** [DOI: [10.4038/jccpsl.v14i1.2943](#)]
- 31 **Ministério da Saúde**. Manual Técnico de Gestação de Alto Risco [cited 6 January 2021]. Editora Ms. 2012
- 32 **FEBRASGO**. Manual de Assistência Pré-natal 2014 [cited 6 January 2021]. Federação Brasileira das Associações de Ginecologia e Obstetrícia. São Paulo, 2014. Available from: [www.abenforj.com.br/site/arquivos/manuais/304\\_Manual\\_Pre\\_natal\\_25SET.pdf](http://www.abenforj.com.br/site/arquivos/manuais/304_Manual_Pre_natal_25SET.pdf)



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