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Association of *Gardnerella vaginalis* and *Lactobacillus* with recurrent abortion in a sample of Iraqi women

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ABSTRACT

Bacterial vaginosis is a prevalent genital condition in sexually active women globally, affecting 23–29%. It is characterized by an imbalance in the vaginal microbiota, with a rise in facultative organisms such as *Gardnerella* and a decrease in beneficial *Lactobacillus* spp. This study investigated the association between bacterial vaginosis and spontaneous miscarriage and assessed whether *Lactobacillus* influences outcomes using bacteriological techniques. The research was conducted at several hospitals and laboratories between December 2023 and August 2024. Fifty-five women who underwent legal abortion due to intrauterine fetal death were included. Vaginal swabs were collected from each patient and analyzed by Gram stain and culture on different agar media. *Gardnerella vaginalis* was present in 24 of 55 cases, with the highest occurrence in the second trimester (30.9%), followed by the third (7.3%), and the lowest in the first trimester; this difference was statistically significant ($p < 0.05$). In contrast, *Lactobacillus* spp. was identified in 19 samples; this was not statistically significant ($p > 0.05$). These findings suggest that the vaginal microbiome may contribute to miscarriage risk and highlight the need to clarify this relationship.

Keywords: *Abortion, Bacterial vaginosis, Gardnerella vaginalis, Lactobacillus*

1 INTRODUCTION

Recurrent spontaneous abortion (RSA) is a common pregnancy-related problem, affecting 2-5% of the population. Idiopathic etiology complicates clinical management and treatment in many patients. Common causes of RSA include chromosomal anomalies, metabolic and hormonal problems, autoimmune illness, and infection [1]. More than 80% of miscarriages occur in the first three months [2]. Miscarriage is a global reproductive health issue that can affect individuals' physical and mental health as well as their financial situation [3]. The microbiome is crucial for human development and immunity, and the health of the female reproductive system relies on maintaining a balanced state within the vaginal microbiota [4]. In women of reproductive age, an imbalance in vaginal bacteria is linked to several conditions, including reproductive disorders such as preterm birth [5] and intrauterine adhesions [6]. Missed abortion occurs when the embryo or fetus dies in the uterus and is not expelled in a timely manner, which may damage the endometrium and lead to complications such as endometrial fibrosis, intrauterine adhesions, and secondary infertility in women. The incidence of missed abortion is increasing due to factors such as environmental pollution, physiological changes in women, and hormonal fluctuations. Research on the causes of missed abortion has highlighted links at the chromosomal level [7–9]. However, the underlying etiology remains unclear [9–12].

There have been limited investigations into the relationship between the vaginal microbiome and miscarriage. The female genital tract (FGT) hosts numerous beneficial commensal bacteria; when this balance is disturbed, pathogens can establish infection, and disease may persist until the irregularities are corrected [13]. Pregnancy alters the vaginal microbiota; throughout gestation, *Lactobacillus* typically predominates [14].

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This dominance can hinder pathogen growth through the release of antimicrobial substances such as lactic acid, which helps maintain a low pH [15].

Bacterial vaginosis (BV) arises when the usual vaginal flora becomes imbalanced, with overgrowth of mixed pathogenic bacteria and a concomitant decrease in lactobacilli [16]. BV is associated with inflammation of the vaginal epithelial lining and an abnormal microbiota that may include aerobic and enteric bacteria [17]. Overall, the health of the female reproductive system is closely linked to the specific bacteria residing in the vagina. BV represents a frequent dysbiosis in which beneficial lactobacilli are displaced by diverse bacterial taxa [18].

Aerobic vaginitis is increasingly recognized as a global concern in the context of rising antimicrobial resistance, which complicates accurate diagnosis and effective therapy [19]. Although less common (7-12%) than BV, overlap in clinical features can lead to confusion between the two conditions. Aerobic vaginitis can be difficult to treat due to increasing antimicrobial resistance, underscoring the need for provider awareness to avoid misdiagnosis and ensure appropriate management [17, 20, 21].

Pathogenesis typically begins with bacterial adhesion to host cells, followed by proliferation and potential biofilm formation to enhance survival [22]. Factors such as mucin degradation may further promote biofilm development [23].

2 MATERIALS AND METHODS

2.1 Study setting and period

The research was conducted at Al Ramadi Teaching Hospital for Maternity and Children, Fallujah Teaching Hospital, and two private medical laboratories from December 2023 to August 2024. The study included 55 women who underwent legal abortion following intrauterine fetal death.

2.2 Inclusion criteria

Eligible participants were women with recurrent missed abortions who reported no intercourse during the index pregnancy and no use of hormones during that pregnancy. They had no history of HPV infection or of infections with *Trichomonas vaginalis*, toxoplasmosis, rubella, cytomegalovirus, herpes simplex, or HIV; no history of prior chromosomal abnormality leading to abortion; and no history of transvaginal medical manipulation. All participants provided written informed consent to join the research.

2.3 Exclusion criteria

Women were excluded if they had a history of hypertension or diabetes mellitus (during or outside pregnancy), a prior ectopic pregnancy, gynecologic malformations, or endocrine disorders such as polycystic ovary syndrome, hyperprolactinemia, hypothyroidism, or hyperthyroidism. Additional exclusions included a history of congenital abnormalities or any recent febrile or flu-like illness, as well as organic disease affecting the kidney, liver, or heart.

2.4 Control definition

Typical vaginal discharge is odorless and of normal color and is not accompanied by itching, dysuria, or dyspareunia; it is not associated with yeast infection, diabetes, hypertension, or bacterial infection. Controls in this study had no discharge. *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, and *Lactobacillus jensenii* were confirmed by VITIK2, and Nugent scores were 1-3. Participants had no hypertension or diabetes, no history of infants with congenital anomalies, and no history of abortion; all were multiparous.

2.5 Sample collection

Patients in the case group were evaluated immediately after a diagnosis of missed abortion. Vaginal discharge examinations and sampling were performed by gynecologists according to standard procedures for each patient. Two sterile vaginal swabs were used to collect an adequate amount of secretion by rotating the swabs three times in the same area of the posterior vaginal fornix. The first swab was processed for Gram staining and Nugent scoring, which yielded scores of 6–9. The second swab was cultured for identification and antimicrobial susceptibility on Blood agar, Chocolate agar, MacConkey agar, De Man–Rogosa–Sharpe (MRS) agar, and Nutrient agar. Organism confirmation was performed using the VITEK-2 system. Because results can be affected by sampling time and potential contamination, specimens were collected and processed promptly.

2.6 Sample size determination

The prevalence of medically induced abortion in Anbar, Iraq, is not well documented in publicly available sources. In Iraq, induced abortion is generally restricted and permitted primarily when the mother's life is at risk, which can lead to unsafe practices and associated health risks for women [24]. To contextualize the study setting, hospital records from Al-Ramadi Teaching Hospital for

Maternity and Children and Fallujah Teaching Hospital were reviewed for the preceding three years. The mean numbers of medically induced abortions recorded were 73 cases at Al-Ramadi and 31 cases at Fallujah. Based on these data, the target sample size was calculated using Cochran's formula, assuming a 5% margin of error, a standard normal deviate of 1.96, and a 95% confidence level, yielding an estimated sample size of 83 patients. Application of the prespecified exclusion criteria resulted in the exclusion of 28 cases according to hospital records.

2.7 Vaginal sample collection and examination

Two sterile cotton-tipped swabs were used to collect samples from the lateral vaginal wall. Vaginal pH was measured using a narrow-range pH strip by touching the moistened swab to the strip and comparing the color change with the manufacturer's scale. One swab was applied to a clean glass slide for Gram staining. For the amine test, the swab was promptly mixed with two drops of 10% KOH. Bacterial vaginosis (BV) was diagnosed clinically when three or more findings were present, including homogeneous discharge and pH ≥ 4.5. After air-drying, the smear was heat-fixed and Gram-stained [25].

2.8 Gram staining

Gram-stained slides were examined under oil-immersion light microscopy at a maximum magnification of 100×. Grading followed the standardized quantitative morphological classification (Nugent score) [26]. Alterations in the vaginal microbiota consistent with BV were identified using the Nugent scoring system, with scores of 0-3 indicating normal, 4-6 intermediate, and 7-10 indicative of BV [27].

2.9 Culture

Swabs were inoculated onto blood agar, Chocolate agar, MacConkey agar, Nutrient agar, and De Man-Rogosa-Sharpe (MRS) agar to isolate non-fastidious aerobic bacteria associated with aerobic vaginitis. Plates were incubated aerobically at 37° C for 24-48 hours. Colonies were evaluated by Gram staining and identified using the VITEK-2 system [28, 29].

2.10 Statistical analysis

Data were entered into SPSS software (version 20) for analysis. Assuming an effect size of 0.5, a chi-square test was used for comparisons, with a two-sided significance threshold of $p < 0.05$ and statistical power of 0.80. Under these assumptions, the analysis indicated that a minimum of 55 participants was required.

2.11 Ethical approval

This study received approval from the Medical Ethics Committee at the University of Al-Anbar, Ramadi, Iraq, on 2024-02-22. Parents also provided signed informed consent for the research.

3 RESULTS

Bacterial vaginosis was associated with a 15% higher risk of spontaneous abortion. Additional factors, including abnormal vaginal discharge, vaginal pH > 4.5, positive clue cells, and a positive whiff test, were also associated with spontaneous abortion in adjusted analyses.

3.1 Detection of defense factors of *gardnerella vaginalis*

Gardnerella vaginalis was detected in 24 of 55 patients with abortion. The highest proportion occurred in the second trimester (30.9%), followed by the third trimester (7.3%), with the lowest in the first trimester (5.5%), as shown in Table 1 and Figure 1.

Table 1 Association between *Gardnerella vaginalis* and stage of abortion

Gardnerella		**Stage of abortion**			**Total**
		First trimester	Second trimester	Third trimester	
No	Count	10	2	19	31
	% of Total	18.2%	3.6%	34.5%	56.4%
Yes	Count	3	17	4	24
	% of Total	5.5%	30.9%	7.3%	43.6%
Total	Count	13	19	23	55
	% of Total	23.6%	34.5%	41.8%	100.0%

Note. Statistical significance was set at $p < 0.05$. Findings for *Gardnerella vaginalis* by trimester were significant ($p < 0.05$).

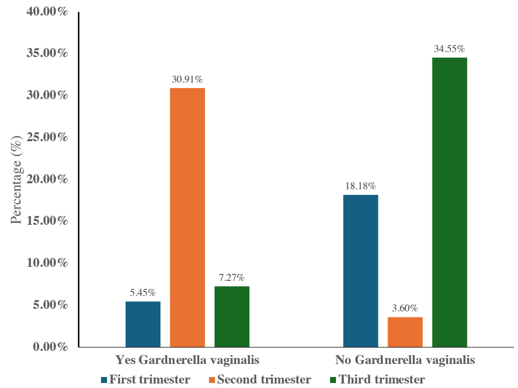

Fig. 1 Number of cases of *Gardnerella* with abortion

3.2 Detection of defense factors of *lactobacillus*

Lactobacillus spp. were identified in 19 of 55 patients, Table 2 and Figure 2. The highest proportion occurred in the third trimester (20.0%), followed by the second trimester (9.1%), with the lowest in the first trimester (5.5%).

Table 2 Association between *Lactobacillus* and stage of abortion

Lactobacillus		**Stage of abortion**			**Total**
		First trimester	Second trimester	Third trimester	
No	Count	10	14	12	36
	% of Total	18.2%	25.5%	21.8%	65.5%
Yes	Count	3	5	11	19
	% of Total	5.5%	9.1%	20.0%	34.5%
Total	Count	13	19	23	55
	% of Total	23.6%	34.5%	41.8%	100.0%

Note. Statistical significance was set at $p < 0.05$. Findings for *Lactobacillus* by trimester were non-significant ($p > 0.05$).

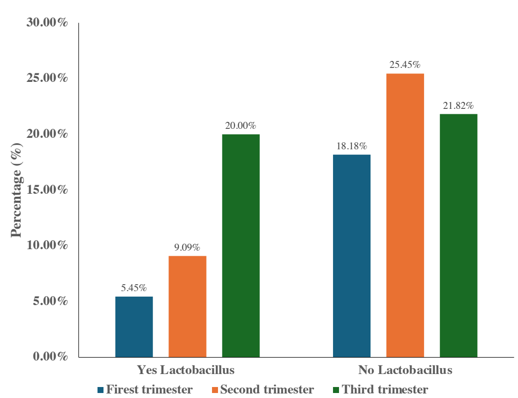

Fig. 2 Number of cases of *Lactobacillus* with abortion

3.3 Association between *lactobacilli* and *gardnerella vaginalis* in abortion

Table 3 shows that *Lactobacillus* spp. was associated with a lower detection of *Gardnerella vaginalis.*

Table 3 Association between Lactobacilli and Gardnerella vaginalis in abortion

Lactobacillus		Gardnerella		Total
		No	Yes	
No	Count	15	21	36
	% of Total	27.3%	38.2%	65.5%
Yes	Count	16	3	19
	% of Total	29.1%	5.5%	34.5%
Total	Count	31	24	55
	% of Total	56.4%	43.6%	100.0%

*Note.*Statistical significance was set at $p < 0.05$. Association was significant ($p < 0.05$) .

3.4 Detection of defense factors of microorganisms

There was no significant association between microorganism and stage of abortion. Table 4 displays the distribution of microorganisms by trimester.

4 DISCUSSION

An inverse relationship was observed between *Gardnerella* and *Lactobacillus* when compared in women with abortion. In our cohort, 43.6% of women had bacterial vaginosis (BV) detected by cytological methods, and *G. vaginalis* was isolated microbiologically; other BV-associated organisms were also recovered. A significant association was found between a history of abortion and BV in the study group (p<0.05) [30].

We noted differences in *Lactobacillus* composition between fertile women with successful pregnancies and women with infertility or a history of spontaneous abortions. Higher *Lactobacillus* levels, lower Nugent scores, and lower vaginal pH were associated with healthier flora, whereas lower *Lactobacillus* abundance was linked to dysbiosis, consistent with prior studies [31, 32]. Several authors have reported that BV in early pregnancy may be associated with second-trimester miscarriage and preterm labor [30, 33–36]. In our data, women with first-trimester abortion had a lower frequency of *G. vaginalis* than those with *Lactobacillus*, whereas BV was more common among women with second-trimester miscarriage; the association between BV and second-trimester miscarriage was statistically significant. Rai et al. suggested that untreated infections persisting sub-clinically may contribute to pregnancy loss [30, 37]. Other studies examined BV in relation to recurrent pregnancy loss in a sample of 55 women, reporting higher BV prevalence among those with recurrent losses [30, 38].

Bacterial vaginosis may contribute to spontaneous abortion and second-trimester miscarriage. Both clinical and subclinical BV present before pregnancy have been linked to increased risk of spontaneous abortion in a prospective study [39], and BV has also been implicated in early pregnancy loss [40]. Across gestation, vaginal microbial composition changes; decreases in beneficial *Lactobacillus* with increased diversity may raise the risk of spontaneous abortion [41].

Studies further indicate that reduced *Lactobacillus* abundance and greater vaginal bacterial diversity can increase the likelihood of first-trimester miscarriage, with alterations detectable prior to clinical recognition [42,43]. In our cohort, the proportion of *Lactobacillus* isolates was 3.4%. A higher *Lactobacillus* presence generally reflects a healthy vaginal environment, whereas low levels accompanied by more pathogens suggest dysbiosis; *Lactobacillus* can inhibit pathogens by producing hydrogen peroxide, bacteriocins, and lactic acid [43–45].

The prevalence of *G. vaginalis* in our study aligns with reports from Basrah (6.5% among women undergoing abortion) [43, 46] and is similar to rates in other developing settings such as South Africa (6.5%) [43, 47] and Iraq (7.7%) [43, 48]. *G. vaginalis* is frequently identified as the most common pathogen in samples from individuals with vaginal infections [49].

Limitations. This study could not experimentally confirm causal mechanisms underlying missed abortion (e.g., in vivo or in vitro models). Future work across biochemistry, microbiology, and clinical research is needed. Most participants were from Al-Ramadi and Fallujah, which may limit generalizability, though this does not alter the interpretation of the current findings.

5 CONCLUSION

This research found an association between *Lactobacillus* and *Gardnerella vaginalis* in cases of abortion. The presence of *Lactobacillus* was associated with lower detection of *G. vaginalis*, consistent with known defensive mechanisms such as production of lactic acid, hydrogen peroxide, and bacteriocins. Overall, the findings support a role for the vaginal microbiome in miscarriage risk and underscore the importance of further elucidating this relationship. Our recommendation is screening for bacterial vaginosis in women with a history of miscarriage to help mitigate the risk of spontaneous abortion, particularly in early pregnancy.

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FUNDING SOURCE

No funds received.

DATA AVAILABILITY

Study data will be available upon request to the corresponding author.

Table 4 Microorganisms and stage of abortion n: Count, %: Percentage

Bacteria		Stage of abortion			Total
		First trimester	Second trimester	Third trimester	
E. coli, S. aureus, Gardnerella	n	1	0	0	1
	%	1.8%	0.0%	0.0%	1.8%
Gardnerella, E. coli	n	1	0	0	1
	%	1.8%	0.0%	0.0%	1.8%
Streptococcus epidermidis, Gardnerella	n	0	0	1	1
	%	0.0%	0.0%	1.8%	1.8%
E. coli, S. saprophyticus	n	0	0	1	1
	%	0.0%	0.0%	1.8%	1.8%
E. coli,S. aureus,Gardnerella	n	0	1	1	2
	%	0.0%	1.8%	1.8%	3.6%
E. coli , S. epidermidis	n	0	0	1	1
	%	0.0%	0.0%	1.8%	1.8%
E. coli , Streptococcus pyogenes, Gardnerella	n	1	1	0	2
	%	1.8%	1.8%	0.0%	3.6%
E. coli , S. saprophyticus, Gardnerella	n	0	0	1	1
	%	0.0%	0.0%	1.8%	1.8%
E. coli, Gardnerella	n	1	0	0	1
	%	1.8%	0.0%	0.0%	1.8%
E. coli, S. saprophyticus, Lactobacillus	n	0	1	0	1
	%	0.0%	1.8%	0.0%	1.8%
Klebsiella, Gardnerella	n	1	1	0	2
	%	1.8%	1.8%	0.0%	3.6%
Klebsiella, S. saprophyticus	n	2	1	0	3
	%	3.6%	1.8%	0.0%	5.5%
Klebsiella, S. aureus	n	0	0	2	2
	%	0.0%	0.0%	3.6%	3.6%
Klebsiella, S. aureus, Gardnerella	n	0	1	0	1
	%	0.0%	1.8%	0.0%	1.8%
P. aeruginosa, Gardnerella	n	0	3	0	3
	%	0.0%	5.5%	0.0%	5.5%
P. aeruginosa, Lactobacillus	n	0	0	1	1
	%	0.0%	0.0%	1.8%	1.8%
P. aeruginosa, S. epidermidis	n	0	0	1	1
	%	0.0%	0.0%	1.8%	1.8%
P. aeruginosa, Streptococcus pyogenes	n	0	1	1	2
	%	0.0%	1.8%	1.8%	3.6%
P. aeruginosa,Streptococcus pyogenes,Gardnerella	n	0	1	0	1
	%	0.0%	1.8%	0.0%	1.8%
P. aeruginosa, S. saprophyticus	n	1	0	0	1
	%	1.8%	0.0%	0.0%	1.8%
S. saprophyticus, Gardnerella	n	0	2	0	2
	%	0.0%	3.6%	0.0%	3.6%
S. saprophyticus, Lactobacillus	n	0	2	2	4
	%	0.0%	3.6%	3.6%	7.3%
S. aureus,Gardnerella	n	0	1	0	1
	%	0.0%	1.8%	0.0%	1.8%
S. aureus, Lactobacillus	n	1	1	1	3
	%	1.8%	1.8%	1.8%	5.5%

S. epidermidis ,Gardnerella	n	0	0	1	1
	%	0.0%	0.0%	1.8%	1.8%
S. saprophyticus, Gardnerella	n	0	1	0	1
	%	0.0%	1.8%	0.0%	1.8%
S. saprophyticus, Lactobacillus	n	1	0	2	3
	%	1.8%	0.0%	3.6%	5.5%
S. epidermidis, Gardnerella	n	0	0	1	1
	%	0.0%	0.0%	1.8%	1.8%
Streptococcus agalactiae, Gardnerella	n	1	0	1	2
	%	1.8%	0.0%	1.8%	3.6%
Streptococcus agalactiae, Lactobacillus	n	1	1	4	6
	%	1.8%	1.8%	7.3%	10.9%
Streptococcus pyogenes, Lactobacillus	n	1	0	1	2
	%	1.8%	0.0%	1.8%	3.6%
Total	n	13	19	23	55
	%	23.6%	34.5%	41.8%	100.0%

DECLARATIONS

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent to publish

All authors consent to the publication of this work. Written informed consent for publication was obtained from the participants.

Ethical approval

This study received approval from the Medical Ethics Committee at the University of Al-Anbar, Ramadi, Iraq, on 2024-02-22. Parents also provided signed informed consent for the research.

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