

Evaluation of the relationship between vaginal infection and recurrent miscarriage according to the Amsel and Nugent criteria

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ABSTRACT

Objective: The purpose of this research was to examine the relationship between recurrent miscarriages and vaginal infections using the Nugent scoring method for bacterial vaginosis (BV) diagnosis as well as Amsel's clinical criteria. **Patients and Methods:** The study included 120 women divided into three groups: 30 women with recurrent miscarriage, 30 pregnant women diagnosed with vaginitis, and 60 healthy controls. Vaginal swabs were collected and examined according to Amsel's criteria (pH, whiff test, clue cells, discharge) and Nugent's scoring system (Gram-stained smears for bacterial morphotypes). **Results:** According to Amsel's criteria, BV was diagnosed in 60% of women with recurrent miscarriage, 66.7% of pregnant women, and 53.3% of controls ($p = 0.574$). By contrast, Nugent scoring showed significantly higher accuracy: 73.3% in recurrent miscarriage cases, 60% in pregnant women, and 0% in controls ($p = 0.001$). The highest rate of recurrent miscarriage occurred in the third month of pregnancy (36.6%). No significant age differences were detected among the groups ($p = 0.086$). **Conclusions:** The findings highlight a strong association between vaginal infections—especially BV diagnosed using Nugent's method—and recurrent miscarriage. Nugent scoring proved more accurate than Amsel's criteria. Routine screening and early treatment of vaginal infections are recommended as preventive strategies to reduce the incidence of recurrent pregnancy loss and improve pregnancy outcomes.

Key words: Recurrent pregnancy loss , Nugent scoring , Amsel's criteria

تقييم العلاقة بين العدوى المهبليّة والإجهاض المتكرر وفقاً لمعايير أمسيل ونوجنت

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المخلص

الهدف: هدف هذا البحث إلى دراسة العلاقة بين الإجهاض المتكرر والالتهابات المهبليّة باستخدام طريقة نوجنت لتشخيص التهاب المهبل الجرثومي (BV)، بالإضافة إلى معايير أمسيل السريرية. المرضى والطرق: شملت الدراسة 120 امرأة، مقسمة إلى ثلاث مجموعات: 30 امرأة مصابة بالإجهاض المتكرر، و30 امرأة حامل مصابة بالتهاب المهبل، و60 امرأة سليمة. جُمعت مسحات مهبليّة وفُحصت وفقاً لمعايير أمسيل (الرقم الهيدروجيني، واختبار النفحة، وخلايا الدليل، والإفرازات) ونظام نوجنت للتسجيل (مسحات مصبوغة بغرام للأنماط الشكلية للبكتيريا). النتائج: وفقاً لمعايير أمسيل، شُخص التهاب المهبل الجرثومي لدى 60% من النساء المصابات بالإجهاض المتكرر، و66.7% من النساء الحوامل، و53.3% من النساء في



المجموعة الضابطة (قيمة الاحتمال = 0.574). في المقابل، أظهر نظام نوجنت للتسجيل دقة أعلى بكثير: 73.3% في حالات الإجهاض المتكرر، و60% لدى النساء الحوامل، و0% لدى المجموعة الضابطة (قيمة الاحتمال = 0.001). سُجِّل أعلى معدل للإجهاض المتكرر في الشهر الثالث من الحمل (36.6%). لم تُكتشف أي فروق عمرية ملحوظة بين المجموعات (قيمة الاحتمال = 0.086). الاستنتاجات: تُبرز النتائج وجود ارتباط قوي بين الالتهابات المهبليّة، وخاصة التهاب المهبل الجرثومي المُشخص باستخدام طريقة نوجنت، والإجهاض المتكرر. وقد ثبت أن تقييم نوجنت أكثر دقة من معايير أمسيل. ويُوصى بالفحص الدوري والعلاج المبكر للالتهاب المهبلي كاستراتيجيتين وقائيتين للحد من حالات الإجهاض المتكرر وتحسين نتائج الحمل.

الكلمات المفتاحية: الإجهاض المتكرر، تقييم نوجنت، معايير أمسيل

INTRODUCTION

Since there are few successful treatments for recurrent pregnancy loss (RPL) and its causes are frequently unclear It is among the most difficult problems in reproductive medicine.

(Bender *et al.*, 2018) . This medical condition mostly relates to two or more recurrent losses before 20 weeks, and it affects 1-3 percent of women who are pregnant (Ruth *et al.*, 2018) .

60% of human embryos are thought to die either before or shortly after implantation. According to Brosens *et al.* (2022), the endometrium's capacity for embryo biosensing and selection, as well as the inherent chromosomal instability in preimplantation embryos, are responsible for this high loss rate. The majority of pregnancy failures after implantation happen so early that they go undetected, while the remaining ones manifest as clinical losses. Preterm delivery , Risks include stillbirth, placental abruption, and fetal growth limitation.

associated with recurrent miscarriages, even though the majority of women will give birth to a live child. (Quenby and others, 2021). The chance of obstetric complications in a subsequent continuing pregnancy increases with each previous pregnancy loss. (Quenby *et al.*, 2021; Wu *et al.*, 2022).

The relationship between pregnancy outcomes and variations in vaginal microecology during pregnancy

is receiving more attention from researchers, who are doing in-depth studies of vaginal microecology (Gupta *et al.*, 2020). Microorganisms collectively referred to as the vaginal microbiota widely inhabit the vaginal tract, which serves as a crucial habitat for the growth of structured microbial communities (Petrova *et al.*, 2015). The body uses vaginal flora as its first line of defense against imported harmful germs and to keep a dynamic balance between mutual restriction and reliance. Throughout a woman's life

, her vaginal microbiota may alter due to changes in hormone levels, lifestyle, and physiology (Greenbaum *et al.*, 2019).



A common vaginal evacuation occurs during pregnancy due to altitude blood estrogen scale. As pregnancy continues, this discharge may get thicker and include more cervical mucus, which may put women at risk for bacterial vaginosis (BV) and other RTIs and STIs (Omole-Ohonsi & Nwokedi, 2011). Individual differences in sexual behavior, vaginal douching, intravaginal cleansing, geographical disparities, and other variables all contribute to the vaginal microbiota (Chen *et al.*, 2021).

RPL requires intensive medical intervention and careful pregnancy monitoring in subsequent pregnancies, as contrast to spontaneous pregnancy loss. It is now well acknowledged that RPL is a complex illness, with several variables being identified as its causative agents (Pei *et al.*, 2019). According to Quinby *et al.* (2021), 10.8%, 1.9%, and 0.7% Over 90% of the losses occur in the first trimester of pregnancy.

trimester of pregnancy. The frequency of RPL has been rising yearly globally, according earlier epidemiological research (Medicine, 2012).

Numerous bacterial species that have varying amounts and Women's health is significantly impacted by the relative proportions in the vagina (Obiero *et al.*, 2016). Healthy vaginal flora, dominated by *Lactobacillus* species, is crucial to preventing genital infections, which are believed to be a common cause of miscarriages. Alteration in the bacterial flora of the vagina is indicated by a relief in *Lactobacillus* and an increase in many facultative or anaerobic bacteria (Farr *et al.*, 2015).

Couples suffer greatly from the RPL, particularly in terms of poor quality of life in terms of physical and emotional roles, general health, mental health, energy, and social functioning (Tavoli *et al.*, 2018). They were forced to deal with anxiety, "subsyndromal depression," "depressive disorder," and "complicated grief" (Toffol *et al.*, 2013).

The treatment of individuals with RPL is multifaceted and should include particular therapy for any recognized reason, lifestyle change, and psychological support. Patients are encouraged to visit the RPL clinic as a pair. A thorough medical, obstetric, and family history should be included of the first consultation. It should also cover habits of both male and female partners, such as smoking, binge drinking, exercising excessively, and being underweight or overweight (Ou and Yu, 2019). A number of treatment approaches have been tested to lower the pregnancy loss rate (Green and O'Donoghue, 2019).

MATERIAL AND METHODS

This study was conducted in the Women and Children Teaching Hospital. Highly



distributed vaginal swab samples ; (30 women with recurrent miscarriage, 30 pregnant women patients diagnosed with vaginitis under the supervision of a gynecologist as vaginitis and 60 healthy individuals. Colposcope was used by gynecologists to provide a clear view of the vagina. Swabs were inserted into the posterior fornix, in the upper part of the vagina and rotated there before being withdrawn carefully to avoid any possible contamination. Swabs were placed in Amies transport media, sterile cotton swab and then brought to the laboratory. For wet and stained preparation as well as testing Amsle,s criteria , Nugent's criteria . which would detect the presence of vaginitis.

Laboratory investigation

The premier swab was used for the smell test, wet mount , and phi measurement. Gram's staining was applied to the second swab in a similar manner. Litmus paper was used to measure vaginal pH; the color shift of the litmus paper was noticed and monitored. For the clue cells, the wet mount preparation was done. The smell test included adding a few drops of a 10% (KOH) to the discharge on a slide that had been cleaned. The test was deemed successful if there was a strong fishy odor. BV was diagnosed based on Amsel's criteria. When at least three of the four following criteria were satisfied, it was deemed to be a case of BV according to Amsel's criterion [10].

Vaginal discharge that is thin, consistent, and grayish white, with a vaginal pl1 more than 4.5

A fishy or amine-like scent appears after adding 10% KOH. Microscopic analysis revealed 20% of the clue cells.

A clinical microbiologist used Gram staining to calculate the Nugent's score, which took into account the number of distinct bacterial morphologies: *Prevotellal Bacteroides* , *Mobiluncus*, *Gardnerella vaginalis*, and *Lactobacillus* (big uniform Gram-positive bacilli) mimic each other. A score of 4-6 was seen as moderate, a score of 7-10 as consistent with BV, and a score of 0–3 as negative for BV.

RESULTS

Demographic characteristics:

The age-specific frequency distribution of the patient and control group revealed that. The mean age of women who had repeated abortions was 29.03 ± 6.96 years, pregnant women were 25.93 ± 5.18 years, and healthy women subjects were 28.96 ± 6.20 years (table 1). The mean age of patients and control subjects did not vary significantly ($P = 0.086$). According to age group, there was no discernible variation in the frequency distribution of pregnant women, recurrent abortion

women, and healthy women subjects ($P = 0.349$). Patients in the age group under 30 had a greater frequency distribution than patients in other age groups.

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Table (1): Distribution of patients and control subjects according to age

Characteristic	Recurrent abortion $n = 30$	Pregnant women $n = 30$	Healthy women $n = 60$	P
Age (years)				
Mean $\pm$ SD	29.03 ± 6.96	25.93 ± 5.18	28.96 ± 6.20	0.086 ¥
Range	16 – 45	19– 35	20- 46	
< 30, n (%)	16 (53.3 %)	21 (70.0%)	36 (60.0 %)	0.349 ¥ NS
30-39, n (%)	11 (36.7 %)	9 (30.0 %)	22 (36.7%)	
≥ 40 , n (%)	3 (10.0 %)	0	2 (3.3%)	

n : number of cases; **SD**: standard deviation; ¥: one way ANOVA; ¥: Chi-square test; **NS**: not significant at $P > 0.05$

Occurrence of bacterial vaginosis based on Amsel's and Nugent criteria:

The frequency distribution of bacterial vaginosis using the Nugent scoring system (NSS) and Amsel's clinical criteria (ACC) revealed the existence of "clue cells" with mixed flora that included a lot of microscopic gram-negative rods, gram-variable rods, and coccobacilli (Table4). There are either none at all or very few *lactobacilli* morphotypes (Figure 1). More than 20% of the vaginal secretion's cells are clue cells, or bacterially coated epithelial cells, as shown by Gram stain microscopy. On saline mount or Gram stain, clone cells may be identified as

distinct from normal vaginal epithelial cells. The adhering tiny coccobacilli in clue cells cover the cell boundaries. A crucial part of the Amsel's clinical criteria for bacterial vaginosis include clue cells, together with vaginal pH, the KOH "whiff test," clue cells, and the appearance of the vaginal discharge.

The whiff test results revealed a positive test of the vaginal secretion treated with 10 % KOH result in production of a fishy odor for patients with bacterial vaginosis .

In details, the ACC identified (54) specimens (18 recurrent abortion women, 20 pregnant women and 16 healthy women) positive specimens. The results of vaginal fluid examination was homogenous, thin and milky that shown on cotton swabs or by help women's physician, the fluid (discharge) was from age started blog (menstruating) until menopause. The clear fluid (transparent) refers to presence of normal flora (*Lactobacillus spp.*), the pH was normal (about 4) as a result of fermentation sugar to glycogen. However, since lactobacilli diminish and do not create as many chemicals that reduce the pH in the vagina, the vaginal pH was higher than 4.5 in instances of bacterial vaginosis. Glycogen, a crucial food supply for microbes, is produced by the thickening vaginal epithelium's cells. The environment rich in glycogen favors organisms that ferment glucose, primarily lactobacilli. The vagina's pH shifts from almost physiologic to acidic when the glycogen is broken down. and move the vaginal media to an acidic state, although some discharge the same cheesy indication (*Candida vaginitis*).

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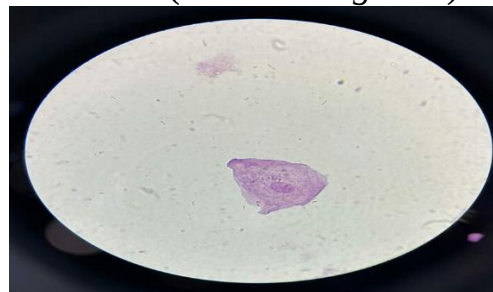


Figure (1) :Gram stianed smear showed clue cells (magnification power 100X).

The findings of the Nugent score examinations indicated the relative abundance of three bacterial morphotypes: curved rods (*Mobiluncus species*), (*Gardnerella*

vaginalis and *Bacteroides species*), and large Gram-positive rods (*Lactobacillus species*) (Table 2). A score of 7 to 10 indicates bacterial vaginosis, whereas a score of 0 to 3 indicates normal vaginal flora. The Nugent score is a measure of the evaluated bacterial morphotypes. A vaginal flora score of 4 to 6 is considered intermediate, meaning that it is aberrant but not consistent with bacterial vaginosis (figure 2).

Table (2) Laboratory examination of vaginal smears and the determination of the Nugent Score N Score = The sum of the scores for each bacterial morphotype listed below.

Normal flora	score	small bacilli gram-negative	score	curved gram-negative	Score	Sum
or > 30	0	0	0	0	0	0
5-30	1	<1	1	<1	1	3
1-4	2	1-4	2	1-4	1	5
<1	3	5-30	3	5-30	2	8
0	4	30 or >	4	30 or >	2	10

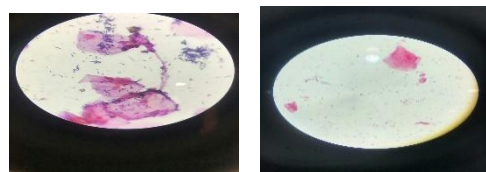


Figure (2): Gram stained smear showed clue cells with nugent score criteria of bacterial vaginosis (A= bacterial vaginosis, B= normal flora) (magnification power 100X).

The interpretation of Nugent score and its relationship with clue cell present means that smear stained with Grams stain not consistent with BV when score is 4-

6 bacterial without clue cell , while the smear was considered as consistent with BV when clue cell is present with bacterial score is 4-6 or more, Table (3).

Table (3): Relationship between Nugent score and clue cell present for gram stained smear of vaginal specimens.

Nugent Score (bacteria number)	Clue cell	Smear Result
0-3		Not BV*
4-6	Not present	
4-6	Are present	BV*
>or = 7		

(*) Bacterial vaginosis

Table (4) : Bacterial vaginosis outcomes according to Amsel's clinical criteria and Nugent score system for positive RA, PW and HW

Characteristic	Recurrent abortion <i>n</i> =30	Pregnant women <i>n</i> = 30	Healthy women <i>n</i> = 60	<i>P</i>
Amsel's clinical criteria				
Positive	18 (60.0)	20 (66.7)	32 (53.3)	0.574 ¥ NS
Negative	12 (40.0)	10 (33.3)	28 (46.7)	
Nugent score system				
Positive	22 (73.3)	18 (60.0)	0	0.001 ¥ S
Negative	8 (26.7%)	12 (40.0)	60 (100.0)	

n: number of cases; **¥:** Chi-square test; **NS:** not significant at $P > 0.05$, **S:** Significant difference at $p < 0.05$

The widely used "Nugent score" scoring system, which uses microscopic examination of a Gram-stained vaginal discharge , is a recognized technique for



detecting bacterial vaginosis (Muzny *et al.*, 2014). This score, which is based on a Gram-stained vaginal smear analysis, is often used in studies. The nugent score for the diagnosis of bacterial vaginosis has a strong agreement with the amsel clinical criteria. The Amsel criteria, wet mount microscopy, and the Nugent score on Gram-stained preparations are the three most widely used diagnostic methods, despite being often used in scientific projects

—remain underused in routine BV diagnosis (Menard *et al.*, 2008). Wet mount microscopy and the Amsel criteria are often used inappropriately in clinical practice because physicians are not proficient in detecting clue cells and typical BV flora using wet mount preparation microscopy.

The ACC and NSS results can be as reliable as the results from BV diagnosis techniques. Mengistie *et al* (2013) had made a comparison between the results of ACC and gram staining methods and found that, especially pH of vagina and clue cells, ACC can be useful in making early diagnosis in the absence of the gram staining tool. Moreover, the NSS was shown to be as a golden standard technique in which other criteria can be compared to as a universal controlling scoring system (Chawla *et al.*, 2013).

Distribution of recurrent abortion women according to month of pregnancy:

The frequency distribution of recurrent abortion women according to month of pregnancy were shown in table (5). The frequency distribution of patients according to month of pregnancy was as following: 1 (3.3 %) patients in first month, 8 (26.7 %) of patients in second month , 11 (36.6 %) of patients in third month, 6 (20.0 %) of patients in forth month and 2 (6.7 %) of patients in third fifth and sixth month. The present results showed higher recurrent abortion occur in third month in compare with other months and the difference was significant ($p=0.007$).

Table (5): The frequency distribution of recurrent abortion women according to months of pregnancy

Characteristic	Patients <i>n</i> = 30	<i>P</i>
Month of pregnancy		
1 month, <i>n</i> (%)	1 (3.3 %)	0.007 ¥ S
2 month, <i>n</i> (%)	8 (26.7 %)	



3 month, n (%)	11 (36.6 %)	
4 month, n (%)	6 (20.0 %)	
5 month, n (%)	2 (6.7 %)	
6 month, n (%)	2 (6.7 %)	

n: number of cases; ¥: Chi-square test; *S*: significant at $P \leq 0.05$

The present results showed the mean age of recurrent abortion, pregnant women and healthy women were 29.03 ± 6.96 , 25.93 ± 5.18 , 28.96 ± 6.20 respectively , Aliyi *et al.*, (2022), found the mean age of pregnant woman was $26.29 (\pm 4.54)$ years ranging from 17–43 years . Another study of Aduloju *et al.*, (2019), in which found the mean age of pregnant woman ($n = 60$) was 26.24 ± 6.14 . while Issakhanova *et al.*, (2023), revealed the mean age of the 70 recurrent pregnant lose was 33.6 ± 7.0 years and control 78 was 35.7 ± 7.0 .

These outcome in consistence with outcome of Ryosuke Mori *et al.*,(2023), Which stated no disparity in the mean (SD) age $32.9 (4.3)$ and $30.8 (5.2)$ between patients and controls , While McQueen *et al.*, (2021), found that mean age was 33.3 years for controls ($N = 26$) and 35.2 years for women with recurrent pregnant loss ($N = 50$) . The statistical analysis performed on Mean $\pm$ SD of the RPLW and HW were not significant $P = (0.086)$.While McQueen *et al.*, (2021), found reverse the result significant $P = (0.06)$.

The age range of patients with recurrent pregnant loss at diagnosis was wide (between 15-45 years) . These results found that the highest percentage of 16 (53.3 %) fall in the age group (<30 years), followed by the age group between 30-39 years 11 (36.7 %),while the lowest percentage was 3 (10.0 %) of them those aged less than (≥ 40) years, table (3-1) . The present results indicated the prevalence of recurrent pregnant loss peaks < 30 years of age and decrease in the subsequent years of age . These outcome were rather comparable to a study findings done by Aliyi *et al.*, (2022), which revealed that the highest percentage (60.5%) of the patients with PRL fall in the age group (25–34) . Whereas , Zhao *et al.*, 2021, repoted that 28(43.1) was the highest percentage of the patients with PRL in the age group (30–35 years) . while the lowest percentage was 12(18.4) of them those aged less than (≥ 36) years and 13(72.2) was the highest percentage of the control



in the age group (≥ 36 years) while the lowest percentage was 1(5.6) of them those aged less than (≤ 29) years .

In the current study age range of pregnant woman group and control group was (19– 35 , 20- 46 respectively) in these age group we found the highest percentage of 21(70.0%) in the pregnant woman with BV fall in the age group <30 years and the highest percentage of 36 (60.0 %) of healthy woman . In study conducted by Govender *et al.*, (2023), found that 110 (39.2) was the highest percentage of the pregnant woman (n=281) with BV in the age group (≥ 25 years) . A Pervious study of Aduloju *et al.*, (2019) , showed that the percentage was 24 (40.0%) ranging from 25-29 years old of pregnant woman was similar rather to the current that study .

Bhujel *et al.*, (2021) interpreted that Nugent score of 7–10 was as consistent with BV and a score of 4–6 as intermediate, while a score of 0–3 was interpreted as negative for BV . The present results showed there was a relationship between nugent score and clue cell present for gram's staine smear of vaginal specimens . The commonly used scoring system known as the "Nugent score" is a recognized method for identifying bacterial vaginosis that involves microscopic analysis of a Gram-stained vaginal discharge smear (Muzny *et al.*, 2014). Based on an examination of a Gram-stained vaginal smear, this score is often utilized in research. There is a high correlation between the amsel clinical criteria and the nugent score for diagnosing bacterial vaginosis. Despite being often employed in research investigations, the three most popular diagnostic techniques—the Amsel criteria, wet mount microscopy, and the Nugent score on Gram-stained preparations—remain underused in routine BV diagnosis (Menard *et al.*, 2008). Wet mount microscopy and the Amsel criteria are often used inappropriately in clinical practice because physicians are not proficient in detecting clue cells and typical BV flora using wet mount preparation microscopy.

Based on Nugent score examinations, the present results revealed that 40 specimens (22 recurrent abortion women and 18 pregnant women) were positive to BV, (table 3-4). BV is the highly frequent common source of unusual vaginal release of discharge for childbearing-age-categorized females. The health condition can overwhelm the affected women especially in severe symptomatic cases showing discharge with offensive odor that can frequently re-happen between time and time; however, some cases may not face the appearance of clinical symptoms (Hay, 2017) .



According to a research conducted in Ethiopia, 51/248 (20.6%) of the 248 study participants had bacterial vaginosis, with a Nugent score of 7–10, and 37/248 (14.9%) had intermediate bacterial vaginosis, with a Nugent score of 4–6. It's unclear what "intermediate flora" means, although it might lead to either BV or normal flora. 48/248 (19.35%) of the pregnant women had bacterial vaginosis, according to Amsel's criteria (Aliei *et al.*, 2022). whereas past studies demonstrated the link between the Amsel criteria and the Nugent's method of diagnosis, among the pregnant women who were recruited, 246 (73.83%) received a negative diagnosis based on the Amsel criteria, whereas 87 (26.13%) had a positive diagnostic. 86 (25.82%) obtained positive results from the gram stain using Nugent's technique, 79 (23.72%) had intermediate results (scores between 4 and 6), and 168 (50.45%) had normal results. However, the normal and intermediate results were considered negative, and the abnormal Nugent's score of 7–10 and the diagnosis based on Amsel's criteria were significantly correlated (Ezeigwe *et al.*, 2023).

Over the course of six months, from November 2014 to May 2015, 160 non-pregnant (healthy) women with symptomatic vaginal discharge visited KIST Medical College Teaching Hospital. According to Nugent's scoring system, the overall prevalence of BV in this study was 24.4%(Ranjit *et al.*, 2018). While according to the Nugent score and Amsels, BV was positive diagnosed in 5 (29.4%) women (PRL) and BV was negative in 26(59.1%) women (44) .while in the (healthy) control group Bv was positive in 19 (13.7) and Bv was negative in 120 (86.3). (Isik.,2017) .

The recent findings indicated that the third month had a greater risk of repeated abortions than any other month. Hussien *et al.*, (2020) found that 77.27% of women had an abortion during the first trimester of pregnancy, while the third trimester had the lowest abortion rate (3%).

In agreement with these finding, Sundari *et al.*,(2019), revealed that the first trimester of pregnancy was when the bulk of the sample was reported. A study conducted in Tunisia discovered that the recurrent pregnant loss group had an average of three pregnancy losses, with the majority occurring before to 14 weeks (70.3%) and 29.7% occurring between 14 and 20 weeks (Ncib *et al.*, 2022).

Conclusions

This study demonstrates that vaginal infections, particularly bacterial vaginosis diagnosed by Nugent's criteria, are significantly associated with recurrent miscarriage. The third month of pregnancy represents the period of greatest



vulnerability. Nugent's scoring system is more sensitive than Amsel's clinical criteria and should be adopted in routine antenatal care. Early screening and treatment of vaginal infections are essential strategies to minimize recurrent miscarriage and improve maternal-fetal outcomes.

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