ABSTRACT
Context: Bacterial vaginosis (BV) is related to the increased risk of miscarriage, preterm labor, and postpartum endometritis.
Aims: The aim of this study was to evaluate the association between BV and the history of spontaneous abortion and recurrent pregnancy losses. We also examined periods of gestation, including the first and second trimester miscarriages.
Materials and Methods: The study population consisted of 200 fertile women. Sixty one (30.5%) of 200 women had the history of a spontaneous abortion in the last six months (N = 30) and at least three recurrent pregnancy losses (N = 31). BV was diagnosed either by using Papanicolaou staining, Gram staining, or by culturing with BV-associated bacteria, Gardnerella vaginalis.
Results: The presence of BV was statistically associated with the history of a spontaneous abortion in the last 6 months (P < 0.05), whereas there was no significant relationship between BV and recurrent pregnancy losses (P > 0.05). These women were also evaluated in view of periods of gestation. Forty-seven (77%) of 61 women had first trimester miscarriage (≤12 weeks) and 14 (23%) of 61 women had second trimester miscarriage (>12 weeks). There was a statistically significant relationship between BV and second trimester miscarriage (P < 0.05). Positive BV findings were not associated with discharge, itching, and pain (P > 0.05).
Conclusion: BV may contribute to spontaneous abortion and second trimester miscarriage.
Key words: Bacterial vaginosis (BV); Gardnerella vaginalis; recurrent pregnancy losses; spontaneous abortion

Introduction
Vaginitis is the most common gynecological infection among women of fertile age.1,2 Bacterial vaginosis (BV) comprises the 50% of the all cases of vaginitis.3 To understand the pathological events related to vaginitis, it is necessary to understand the normal vaginal flora. In normal vaginal flora, there are Lactobacillus species in 95% and facultative anaerobic and anaerobic microorganisms, including Gardnerella vaginalis, Staphylococcus epidermis, Mycoplasma hominis, Streptococcal species, Bacterioides species, Prevotella bivius, Peptostreptococci species, in 5%.2,3 Lactobacillus species protect the vaginal flora from genital pathogens by producing lactic acid, H₂O₂, and antimicrobial proteins. In case of a decrease in the number of Lactobacillus species, these are replaced by anaerobic and facultative anaerobic microorganisms.

The presence of BV during pregnancy attracts the attention of physicians due to adverse pregnancy outcomes. These
adverse outcomes related to BV are the increased risk of late miscarriage, preterm labor, low-birth-weight infants, chorioamnionitis, postpartum endometritis, and postabortion pelvic inflammatory disease.\[^{4,9}\]

BV-associated microorganisms in amniotic fluid and the placenta coming from the cervicovaginal mucosa were found in association with abortion and preterm labor.\[^{10-12}\]\ Ralph et al. indicated when BV is identified before 16 weeks of gestation, the highest rates of preterm labor was detected, and BV was responsible for twofold risk of miscarriage in the first trimester.\[^{13}\]\ Similar to these results, Ugwumadu et al. found threefold increase in the risk of miscarriage in the first trimester.\[^{14}\]\ Contrarily, BV is found to be related to the late miscarriage in comparison with the first trimester pregnancy loss.\[^{15,16}\]\ In a large study that is conducted with 10,397 women, BV had caused low-birth-weight infant in more than 40% of women without BV in the second trimester of pregnancy.\[^{6}\]\ These previous studies were performed in pregnant women. Llahf-Camp et al. indicated that BV is not related to the history of recurrent pregnancy losses in fertile women.\[^{15}\]\ Except for this report, there is no previous documentation enlightening the relationship between BV and the history of abortion in fertile women. Therefore, we aimed to understand the relationship between BV and the history of abortion using Papanicolaou staining for cytological investigation, Gram staining, and culture of \textit{G. vaginalis} for microbiological examination. Presence of BV was correlated with the history of a spontaneous abortion and recurrent pregnancy losses as well as gestation periods, including the first and second trimester miscarriages.

Materials and Methods

Case selection
In our study, 200 fertile women with varied gynecological complaints were seen at the outpatient clinic of the gynecology and obstetrics. Pregnant women were not included in this study. This study was applied according to the principles of The Declaration of Helsinki. Before pelvic examination, data on age, menstruation date, pregnancy outcomes, contraception methods, gravidity, and clinical symptoms were enrolled.

Cytological examination
For cytological examination, the cervicovaginal fluid samples were taken from each woman with a cytobrush before conducting the pelvic examination. \(\text{pH}\) was measured by putting a drop of the cervicovaginal fluid on a \(\text{pH}\) strip (ranging \(\text{pH} = 4-7\)). For Whiff test, the cervicovaginal fluid was smeared on slide, and one drop of 10% potassium hydroxide (KOH) was added. Smears having fishy odor were accepted as Whiff (+). After that, the cervicovaginal fluid was smeared on slide in one direction and fixed with 96% ethanol without air-drying. Smears were stained using Papanicolaou (PAP) method and examined by light microscope in detail.

In the cytological examination, the diagnosis of BV was established by detecting clue cells covered by adherent bacteria. The absence of \textit{Lactobacilli}, the lack of neutrophil leukocytes, and increase in the number of free cocci were accepted as the other identification criteria of light microscopic examination for BV. A homogeneous, thin, gray vaginal discharge; a fishy odor with Whiff test; and a vaginal \(\text{pH}\) of >5 were also considered.\[^{16}\]

Gram stain method
The cervicovaginal fluid was smeared on slides and these slides were air-dried. Gram staining differentiates bacteria by properties of their cell walls. Gram-positive bacteria that have thick cell wall stained purple, whereas Gram-negative bacteria that have thin cell wall stained pink. After staining with the Gram stain, some bacteria showed a mix of pink and purple stain. These bacteria were considered Gram variable. All slides were examined under an oil immersion objective. Gram-stained smears were evaluated according to Nugent et al. The Nugent score was calculated in the following methods.\[^{17}\]\ The decrease in large Gram positive rods; \textit{Lactobacillus} spp. were scored as 0-4. Gram variable small rods, \textit{G. vaginalis}, were scored as 0-4, whereas curved Gram variable rods, \textit{Mobilincus}, spp., scored as 0-2. Scores summed, and results graded as 0-3 (normal vaginal flora), 4-6 (intermediate flora), and 7-10 (BV).

Culture
The cervicovaginal fluid was obtained and transferred to the Microbiology Laboratory by Stuart Transport Media (Thermo Scientific, UK) and was then cultured on Blood agar (Neogen, US) and \textit{Gardnerella Selective} Agar with 5% human blood (Mast Diagnostics, UK). These plates were incubated at 37°C for 48 h in 5-10% \(\text{CO}_2\). After the incubation period, \textit{G. vaginalis} were identified for a positive beta-hemolysis and hippurate hydrolysis as well as negative catalase and oxidase reactions.

Statistical analysis
The aim of this study was to find out whether or not the presence of BV was associated with the history of abortion. For these comparisons, \(\chi^2\) or Fischer’s exact test was used, and \(P\) values less than 0.05 were considered as statistically significant.

Results
In this study, 200 fertile women were evaluated in view of the presence of BV and the history of abortion. Sixty-one (30.5%)
of 200 women had the history of a spontaneous abortion in the last 6 months and recurrent pregnancy losses (at least three times). These women were taken as a study group \((N = 61)\) and 139 (69.5\%) women not having the history of abortion were accepted as the control group. The percentage and the mean of ages of groups were shown in Table 1.

When we examined the study group with regard to the kind of abortion, 30 (49.2\%) of the 61 women were detected as having the history of a spontaneous abortion in the last 6 months, 31 (50.8\%) of 61 women had recurrent pregnancy losses. The women in the study group were also evaluated in view of the abortion whether it has happened in the first or second trimester of gestation. Forty-seven (77\%) of the 61 women had the first trimester miscarriage (≤12 weeks), and the remaining 14 (23\%) of 61 women had the second trimester miscarriage (>12 weeks).

According to the cytological examination, BV was diagnosed in 17 (27.9\%) women in the study group \((n = 61)\) and BV was positive in 19 (13.7\%) women in the control group [Figure 1a and b]. In BV (+) women, pH was higher than 5 in 14 (82.4\%) and 17 (89.5\%) women of the study and control groups, respectively. In the study group, Whiff test was positive in 5 of 17 (29.4\%) women with BV.

To obtain microbiological data, Gram staining and culture methods were employed. According to Gram staining results, Nugent score ranged 7-10 (BV) was observed in 7 (11.5\%) of 61 in the study group and 6 (4.3\%) of 139 in the control group [Figure 1c]. \(G.\) vaginalis was isolated by culture method in all women diagnosed as BV (+) by Gram stain in both study and control groups.

All women who were accepted as BV (+) by microbiological methods (Gram staining and culture) were also found positive by cytological examination. Therefore, PAP stain results were used for statistical comparison. As seen in Table 2, when the study and control groups were compared to each other, there was a significant correlation between the presence of BV and the history of abortion \((P < 0.05)\). In the study group, 12 of 17 (70.6\%) women with BV had a history of spontaneous abortion in the last 6 months and only 5 of 17 (29.4\%) women with BV had recurrent pregnancy losses [Table 3]. According to the statistical data, a significant association between BV and the history of spontaneous abortion \((P < 0.05)\) was determined; however, there was no association between BV and the history of recurrent pregnancy losses \((P > 0.05)\). The effect of BV on the gestation periods was also examined. The presence of BV had no effect on the first trimester miscarriage \((P > 0.05)\), but BV had strongly affected the second trimester pregnancy losses \((P < 0.05)\). These results were shown in Table 4.

The gynecological complaints of women were analyzed statistically, and these data were shown in Table 5. There was no association of the presence of BV with the gynecological complaints, such as vaginal discharge, itching, and pain \((P > 0.05)\).

### Table 1: Percentages and the mean of ages of the study and control groups

<table>
<thead>
<tr>
<th>Number of group</th>
<th>Percentages (%)</th>
<th>The mean of ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ((N = 200))</td>
<td>100</td>
<td>19-45, 32.1±5.56</td>
</tr>
<tr>
<td>Study group ((N = 61))</td>
<td>30.5</td>
<td>21-40, 30.55±4.13</td>
</tr>
<tr>
<td>Control group ((N = 139))</td>
<td>69.5</td>
<td>19-45, 32.71±4.76</td>
</tr>
</tbody>
</table>

### Table 2: Correlation of the study and control groups in view of BV

<table>
<thead>
<tr>
<th>Bacterial vaginosis (BV)</th>
<th>Study group ((N = 61, %))</th>
<th>Control group ((N = 139, %))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV (+)</td>
<td>17 (27.9)</td>
<td>19 (13.7)</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>BV (−)</td>
<td>44 (72.1)</td>
<td>120 (86.3)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Relationship among the presence of BV, history of spontaneous abortion, and recurrent pregnancy losses

<table>
<thead>
<tr>
<th>Study group</th>
<th>BV (+) ((N = 17, %))</th>
<th>BV (−) ((N = 44, %))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous abortion</td>
<td>12 (70.6)</td>
<td>18 (40.9)</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>Recurrent pregnancy losses</td>
<td>5 (29.4)</td>
<td>26 (59.1)</td>
<td>(P &gt; 0.05)</td>
</tr>
</tbody>
</table>

Figure 1: (a) Clue cell (arrow) and free cocci were seen around the squamous epithelial cells (PAP stain, ×400). (b) The cell borders were irregular (arrow) in clue cell (PAP stain, ×1000). (c) The cytoplasmic loss was observed (arrow) in clue cell (Gram stain, ×1000)
Discussion

BV is associated with pregnancy outcomes, including abortion, preterm labor, and premature rupture of membranes.\cite{4,18,19} According to the National Health and Nutrition Examination Survey, BV was positive in 29% of the fertile women aged 14-49 years.\cite{20} Jacobsson, Sware, and McGregor et al. studied pregnant women, and the prevalence of BV was found between 15.6% and 32.5% among their study subjects.\cite{7,8,21} The effects of BV on abortion were examined generally in pregnant women so far. For this reason, we aimed to understand the relationship between BV and the history of abortion using cytological and microbiological methods in fertile women.

In our study, BV was detected in 17 of 61 (27.9%) women by cytological methods (we accepted cytological results to make statistical analysis because only G. vaginalis was isolated microbiologically. The other microorganisms associated with BV, such as Bacterioides, Mobilincus spp., Ureaplasma urealyticum, M. hominis, and Prevotella, were not isolated). In the study group, there was a statistically significant association between the history of abortion and the presence of BV ($P < 0.05$). We obtained significant findings in light microscopic examination of cervicovaginal smears. The cell borders of clue cells were irregular, and cytoplasmic loss was observed [Figure 1b and c]. We thought that lytic enzymes produced by BV-associated microorganisms may have caused these changes in clue cells. According to studies which examined the reason of abortion, lytic enzymes, such as proteases, Phospholipase A2 and Phospholipase C produced by BV-associated microorganisms cause lysis of phospholipids of fetal membranes and cell membranes of clue cells. In other studies, after lysis of phospholipids, arachidonic acid is formed, and this acid causes induction of prostaglandins (PGs). PGs induce uterine muscle contraction, sulfated Glucoseaminoglycan (GAG) decreasing, reorganization of collagen fibrils, and decrease the cervical resistance.\cite{22,23} Some cytokines, such as interleukine-1 (IL-1), IL-6, IL-8, granulocytes stimulating factors, and tumor necrosis factor alpha (TNFα), have increasing level in amniotic fluid of women with BV.\cite{24,25} These cytokines also cause synthesis of PGs. In addition, PGs induce the release of inflammatory cytokines for stimulating the release of metalloproteinases (MMPs) from neutrophils. MMPs degrade connective tissue, such as chorioamniotic membranes, and it can be cause of abortion.\cite{26}

The relationship between BV and the history of spontaneous abortion was investigated by the large meta-analysis, including 20,232 women, and BV was observed to be significantly associated with the spontaneous abortion.\cite{27} Recent studies showed women with BV during pregnancy increased two- to threefold spontaneous abortion risk compared to women without BV.\cite{13,14} In addition, Meningistie et al. and Goffinet et al. showed that BV was observed in pregnant women with the history of spontaneous abortion.\cite{28,29} In our study, BV was found in 12 of 30 (40%) women with a history of spontaneous abortion in the last 6 months. Consistent with previous reports, our data showed that BV is more frequent in fertile women with the history of spontaneous abortion in the last 6 months ($P < 0.05$) than the women with recurrent pregnancy losses ($P > 0.05$).

Study related to the recurrent pregnancy losses, Llahf-Camp et al. aimed to state whether or not BV was related to a history of recurrent pregnancy losses in 500 women. This report indicated that BV is more frequent in women with a history of late miscarriage (21%) than women with recurrent pregnancy losses (8%).\cite{15} Consistent with this study, only 5 of 17 (29.4%) women with BV had at least three recurrent pregnancy losses. As a result of these findings, we concluded that there was no association between BV and the history of recurrent pregnancy losses ($P > 0.05$).

In this study, the effects of BV on different periods of gestation were also evaluated. Some authors indicated that BV may cause the first trimester miscarriages even though the others stated that BV infection in the early periods of pregnancy may cause the second trimester miscarriage and preterm labor.\cite{11,13,14} In our study, we observed that percentages of BV (+) women with first trimester abortion ($N = 10, 58.8\%$) are less than that of the women without BV ($N = 37, 84.1\%$).

### Table 4: The effect of BV on the gestation periods (≤12 week, >12 week)

<table>
<thead>
<tr>
<th>Study group</th>
<th>BV (+) ($N = 17, %$)</th>
<th>BV (-) ($N = 44, %$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤12 week (first trimester)</td>
<td>10 (58.8)</td>
<td>37 (84.1)</td>
<td>$P &gt; 0.05$</td>
</tr>
<tr>
<td>&gt;12 week (second trimester)</td>
<td>7 (41.2)</td>
<td>7 (15.9)</td>
<td>$P &lt; 0.05$</td>
</tr>
</tbody>
</table>

### Table 5: Relationship between gynecological complaints and the presence of BV in the study and control groups

<table>
<thead>
<tr>
<th>Gynecological complaints</th>
<th>Study group ($N = 61$)</th>
<th>Control group ($N = 139$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV (+) ($N = 17, %$)</td>
<td>6 (35.3)</td>
<td>5 (26.3)</td>
<td>$P &gt; 0.05$</td>
</tr>
<tr>
<td>BV (-) ($N = 44, %$)</td>
<td>11 (25)</td>
<td>33 (27.5)</td>
<td>$P &gt; 0.05$</td>
</tr>
<tr>
<td>BV (+) ($N = 19, %$)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>$P &gt; 0.05$</td>
</tr>
<tr>
<td>BV (-) ($N = 120, %$)</td>
<td>1 (2.3)</td>
<td>3 (2.5)</td>
<td>$P &gt; 0.05$</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itching</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The frequencies of second trimester miscarriage in women with BV (N = 7, 41.2%) are higher than that in the women without BV (N = 7, 15.9%). In statistical analysis, the presence of BV is also significantly associated with second trimester miscarriages (P < 0.05). Rai et al. reported that untreated infections going on for a long time without any symptoms cause pregnancy losses.\cite{20} To our opinion, consistent with these results, untreated and asymptomatic BV infection in first trimester or before pregnancy may cause second trimester miscarriage.

In this study, the gynecological complaints of women were also correlated with BV [Table 5]. There were no significant relationship between the complaints, such as discharge, itching, and pain, and the presence of BV (P > 0.05). In previous studies, BV was asymptomatic in women with a prevalence of 50\%.\cite{21} Consistent with this previous report, BV was also found to be asymptomatic in our study.

**Conclusion**

In conclusion, women who had a spontaneous abortion in the last 6 months and recurrent pregnancy losses in the study group were evaluated in view of BV, and it was found that there was a significant correlation between the presence of BV and the history of abortion. BV was significantly higher in women with the history of spontaneous abortion than those with recurrent pregnancy losses and in the women with second trimester miscarriage than those with first trimester miscarriage. As a result, we suggest that the screening of BV in fertile women with the history of abortion is necessary to prevent from spontaneous abortion and second trimester miscarriage.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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