Relationship between bacterial infections and recurrent abortion in pregnancy women

By

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Abstract:

Objective : The purpose of this study was to look at potential connections involving bacterial vaginosis detection in the first trimester and abortion at <20 weeks gestational. **Study design:** The microbiologic flora of the cervix was studied in 228 women (n = 228) who had a live singleton fetus at 14 weeks pregnant and received regular prenatal care in Al-Yarmouk Teaching Hospital , Baghdad ,Iraq. The three methods used to evaluate the infection were via microscopy (using clue cells), clinically (using the Amsel et al criteria), or by cultivating bacteria linked to bacterial vaginosis. Both unilateral (relative risk) and comprehensive analyses of the data were done. **Results:** A substantial correlation was found between the discovery of bacteria-related vaginosis at the initial prenatal visit and recurrent early pregnancy loss (relative risk: 5.4; 95% confidence interval: 2.5-11). Following a multivariate study, the microbes Salmonella hominis, Ureaplasma urealyticum, and bacteria that cause vaginosis were found to be related to a greater chance of premature delivery, but not other types of microbes. **conclusion :**Premature pregnancy termination and spontaneous abortion can both be caused by the parasites and vaginosis of bacteria.

Key words: Microorganisms, spontaneous abortion, early pregnancy loss, and infections caused by bacteria

Introduction :

The growing amount of research indicates a link between viral vaginosis and bacterial vaginal flora and gestational infection, limitation of intrauterine development, abrupt tear of the membrane, and early delivery. [1–7] This substance release, transmembrane declining, and climbing infection may all be prevented in part by defense from lactobacilli [2] that produces oxidase. Late miscarrying is linked to lower levels of lactic acid bacteria and bacterial vaginosis.[1, 7] However, a clear correlation between the infection and second-trimester miscarriage has not yet been shown, as the majority of these research on pregnancies have focused on monitoring initiatives in the following or late first period. In this study, we evaluated female vaginal flora in the early stages of gestation and examined the association between it and later unexplained pregnancy loss.

Patients and materials :

A total of 228 not chosen women who had a live solitary fetus and were examined for a normal prenatal checkup at less than 14 finished weeks during pregnancy agreed to have a typical vaginal scan between March 2023 and March 2024. On the same day of the week, all of the new patients were seen, and they were all less than 14 weeks pregnant. Because of the day-of-the-week specifications, Gilbert G.G. Donders, MD, who specializes in viral diseases and vaginal fluid microscopes, was able to examine the patient.

A hardwood Ayre spatula was used to remove vaginal fluid from the posterior vaginal vault and spread it over two different glass slides. Before the inspection, glass slides were preserved in 100% alcohol and stained with The Greek scientist for the 130 women who were seen throughout the study's first phase. Analyzing these data, which were initially part of another study on early gestation,8 showed that women who contracted a viral infection in the early stages of pregnancy had an unexpectedly high risk of termination. A hardwood Ayre blade was used to remove the urine from the anterior uterine vault and spread it over two different slides of glass. Before the inspection, glass slides were preserved in 100% alcohol and stained with The Greek scientist for the 130 women who were seen throughout the experiment's first phase. Analyzing these data, which were initially part of another study on early gestation,8 showed that women who contracted a viral infection in the early stages of childbirth had an unexpectedly high risk of termination.

During this additional study period, phase-contrast microscope inspection on fresh wet genital tract attaches was used for clue cell identification (n = 98) by the same police (Gilbert G.G. Donders, MD), who was acquainted with the two methods, for reasons of convenience and given that the proportion of clue cells proved to be similar in fresh fluid from the vagina.

From the bottom of the vault, a cotton-tipped swab was removed and placed right away in Amies' adapted Stuart solution. Following cleaning of the ectocervix, an endocervical swab was rotated three times in the endocervical canal, let to soak for twenty seconds, and then placed in a second section of Amies modified Stuart medium. Gardnerella vaginalis, Ureaplasma urealyticum, and Chlamydia hominis, three bacteria linked to bacterial vaginosis, were cultivated in vitro and cervical cultures on

certain readily accessible ampicillin-pretreated A3 medium for eighteen hours at 37°C. The ability to hydrolyze urea or L-arginine, respectively, allowed color interactions to be used to identify U urealyticum and M human DNA. Blood-chocolate agar was used to cultivate additional organisms, including yeasts, enterococci that Klebsiella species, varieties of Acinetobacter [enterobacteriaceae], Pseudomonas species, and microorganisms Only growth above 1+, or growth encompassing more than half of the incubator plate, was excluded to shield patients with typical mixed concomitant flora from inclusion. Herpes simplex virus and chlamydia trachomatis were isolated through culture on living McCoy or HeLa cell lines. The data (n = 10) were not included in Tables I, II, and III for additional analysis if the cultures were not taken correctly, if they were insufficient, or if the trip to the lab took longer than six hours.

The lactobacillary characteristics are graded.

Schröder's initial classification scheme was applied to the wet mount.10–12 When it came to the presence of coccoid organisms, the normal grade I flora corresponded primarily to lactobacillus characteristics (although caution was exercised to prevent mistaking the cell fragments from lysed epithelial tissues [epitheliolysis] for coccoid bacteria). A decreased lactobacillary flora along with other bacteria was represented by the intermediate grade II flora. We divided the lactobacillary flora in this group into two categories: mildly affected but generally normal (IIa) and somewhat affected but somewhat aberrant (IIb). Lastly, there were no lactobacilli among the countless other bacteria that made up the egregiously aberrant grade III flora. The usefulness of this grading scheme in clinical settings throughout the latter stages of pregnancy led to its selection.4, 8 Red blood cells, Saccharomyces hyphae, the organism vaginalis, and epithelial epithelium cells' epitheliolysis were also observed.

Urinary erythrocyte classification.

A condition known as a grade of 1 (less than 5) was associated with less than 5 called leukocyte per high-power field (hpf; less than 400 magnification). If a value of 5 to 10 leuko-cytes / hpf was found, a score of 2 was given. When there were less than 10 leukocytes per epidermal cell, a sample with >10 leukocytes / hpf received a score of 3, and when there were more than 10 leukocytes per epithelial cell, the sample received a score of 4. According to our private assessments, we have observed that this score is very helpful in identifying early pathogenic risk during pregnancy brought on by vaginal infection.

The vaginosis bacterial prognosis.

Using the guidelines provided by Amsel et al.,9 vaginosis caused by bacteria was identified when three out of four criteria were : (1) a pH of less than 4.6 in the vagina, (2) a uniform outflow from the vagina, (3) a fishy smell (amine test), and (4) the existence of vaginal clue cells. We also examined the importance of finding clue cells on mounts with water or Greek scientific specimens, which can be done with ease by various people or at different times. Lastly, we looked into the possibility that the existence of bacteria linked to the infection or other microbes was a direct cause of miscarriages.

Table(1): Comparing the smallest fin dings of the vaginal tract among individuals that delivered babies after 20 weeks of conception vs those that lost their pregnancies early on their own.

Parameter	Early pregnancy loss No.(%)	Evolving pregnancy No.(%)	Statistical significance
Lactobacillary flora	21(10)	197(90)	NS
grade –I	4 (19)	58 (30)	* NS
grade –II-a	4 (19)	70 (36)	* NS
grade –II-b	5 (24)	31(16)	NS
grade –III	8 (38)	19(10)	*P* =.002
Mean ± SD (1-4)	2.81 ± 1.14	1.86 ± 1.10	*P* = .0002†
Clue cells	8 (38)	14 (8.7)	5.4 (2.5-11),† *P* = .0003‡
Candida hyphae	6 (29)	49(25)	NS
Red blood cells	2(10)	15 (7.6)	NS
Epithelial cytolysis	2 (10%)	27 (14)	NS
Vaginal leukocytosis <5 leukocytes / hpf	1 (4.8%)	29(15)	NS
<10 leukocytes / hpf (No.)	6 (29%)	67 (34)	NS
leukocytes / epithelial cell (No.) Mean ± SD (1-4)	2.0 ± 0.98	1.63 ± 0.99	NS
Totally (No.)	21	97	

Table(2): Comparing the clinical indications and signs observed in the group that had their pregnancies terminated at less than 20 weeks pregnant with the group that had their pregnancy evolve

Parameter	Early pregnancy loss	Evolving pregnancy	Relative risk	95% Confidence Interval	Statistical significant
No.	21 (10%)	197 (90%)			
Genital symptoms					
Abnormal vaginal	10	56	1.7	1.02-2.8	*P*=.08

discharge					
Itching or irritation	3	13			NS
Other (smell, dyspareunia, bleeding)	2	11			NS
Unknown	1	26			NS
recent use of antimicrobial medication					
Vaginal cream or ovures	0	5	NS		
Oral antibiotics	1	14	NS		
Unknown	1	1			
Bleeding or congestion	11	52	1.8	1.1-2.9	*P* = .04
Endocervical pus	1	6			NS
Unknown	1	17			NS
Clinical diagnosis* of bacterial vaginosis	8	14	5.4	2.5-11	*P* = .0003

NS, Not important.

*A diagnosis based on Amsel's criteria: three tests (pH >4.6, homogenous her release, fishy odour, and clue cells) are present.

Table(3): Comparisons of vaginal microflora comparing the group that had an abortion at <20 weeks pregnant and the group that had a delivery at >20 weeks maternity during the first visit, at <14 weeks pregnant

Subjects (No.)	Early pregnancy loss	Evolving pregnancy	Relative risk	95% Confidence interval	Statistical significant
Bacterial vaginosis group					
G vaginalis	5	8	5.8	2.1-16	*P* =.004
U urealyticum	5	8	5.8	2.1-16	*P* =.004
M hominis	4	3	12.5	3.0-52	*P* =.002
Total	14	19	5.5	2.9-10	*P* <.0001
Yeast infection	4	14			N S
T vaginalis	2	0			N S
Herpes simplex virus	0	1			N S
Neisseria gonorrhoeae	0	0			N S
C trachomatis	1	6			
Enteric bacteria					
Enterobacteriaceae	1	8			N S

Enterococci	3	9			N S
Group B streptococci	3	10			N S
Any pathogen present except					
Candida species	12(63%)	43(25%)	2.6	1.6 -4.1	P=.001
No pathogen present	7(3%)	144(73%0	0.46	0.25-0.84	.0007

NS, * *E. Coli, Klebsiella species*, The bacterium A organism.

Creatures from a cervical cancer screening. Lastly, we looked at the possibility that the existence of pathogenic bacteria that cause avitaminosis or other microbes was a direct cause of abortion. posterior friability, or easy flowing when brushed by a cotton swab, was noted as cervical crowding and joined with it for analysis. On the initial appointment, every person after specimens were obtained for culturing and microscope.

Clinical indicators and pregnancy age validation.

Questions concerning genital infection complaints and recent (past two months) antibiotic use were asked of each woman. Vaginal crowding was identified as the presence of redness and edema in the cervix. Cervical softness, or easy weeping when contacted with a cotton swabbing instrument, was also noted throughout during the study. On the initial appointment, every single person after the samples were obtained for culturing and histology.

The fetal fingerprints profile at 6 to 14 weeks of the pregnancy differed by >7 days from the predicted pregnancy age on 2 separate ultrasonographic assessments made <1 week apart, and only then was the gestational age calculated based on the date of the last period of menstruation corrected, provided that positive fetal heart activity was verified.

Evaluation based on statistics.

The primary goal was to determine if pregnancy loss before the twentieth trimester week was associated with bacterial vaginosis and other indicators of vaginal microbiota disruption. Furthermore, we examined the average gestational age at delivery and the neonatal weight of women with and without the infection. In order to express any substantial variation in the result of women colonized by bacteria that cause vaginosis, the relative risk with a 95% confidence interval was determined using the Fisher exact test on 2 tables. Age, history of prior abortion, recent antibiotic usage, culture results, lactobacillus grades, indications of cervical inflammatory processes, and clinical evidence of infection with bacteria vaginosis were all considered essential considerations because interference from these factors was not implausible.

Table (4): Women with vaginosis due to bacteria (three or more of Amsel's screening criteria[9]), temporary flora (inappropriate vaginal microbes but two or fewer of Amsel's evaluation criteria), or normal flora are associated with premature pregnancy loss (less than 20 weeks during pregnancy), there was premature termination of an evolving conception (20–37 weeks

gestation) and term birth.

Normal vaginal flora	12	20	160	11
Intermediate flora	1	5	5	50
Bacterial vaginosis	8	3	13	18
Total	21	28	178	13.6

Compared to women with normal flora or viral vaginosis, those with transitional flora had a 7.5 (range, 2-28) times higher risk of premature delivery (*P* <.005). One woman had a developing gestation; the date of her due date was undetermined.

About Twenty-one (10%) of the 218 women who had a viable fetus in the initial trimester and full culture results at their initial prenatal visit went on to have unplanned abortions. The mean (± SD) gestational of the women in this group at the initial trimester visit was 7.5 ± 1.9 weeks, while the women with progressing conceptions had a gestational of 9.3 ± 2.5 weeks (P =.001). At a mean of 11.3 ± 2.9 weeks during pregnancy (range, 5–13 gestational weeks), a miscarriage happened, and it happened at a mean of 25.7 ± 29.6 days (range, 1-105 days) following collection sampling. Except for one woman, whose premature birth occurred at 19 weeks of development, all women who had early pregnancy loss did so before the 14th embryonic week. For three women, the precise gestational age was unknown, but it was unquestionably less than twenty weeks. In terms of age, smoking, marital status, and reproductive history, women who experienced unplanned abortions at less than 20 weeks of gestational did not vary from those who had evolving conceptions (data not shown). However, they did exhibit higher gravidity.

A greater likelihood of miscarrying by 20 weeks of pregnancy was linked to the presence of clue cells (relative risk, 5.4; 95% confidence interval, 2.5-11) and disorganized (grade III) lactobacilli flora (relative risk, 4.0; 95% assurance interval, 2.0-7.8) in the vagina. There was no correlation seen between increased vaginal leukocytosis, Candida hyphae, hemoglobin, and epidermal cell lysis and this unfavorable result.

Remarkably, when clinical vaginosis from bacteria was diagnosed early on, the likelihood of recurrent premature births increased fivefold (relative risk, 5.4; 95% confidence range, 2.5-11). Remarkably, when clinical vaginosis with bacteria was diagnosed early on, the likelihood of recurrent premature births increased fivefold (relative risk, 5.4; 95% confidence range, 2.5-11).

When women with inter-mediate flora (unusual flora but only two or fewer of the Amsel et al standards) were juxtaposed with those with normal vaginal flora, infections (a minimum of 3 of the Amsel et al requirements), or neither, they showed an increased risk of preterm birth once pregnancy had progressed past the 20th during pregnancy week (relative risk, 7.5; 95% confidence range, 2-28; Table IV). However, in contrast to normal flora (11%), full-blown infections caused by bacteria did not seem to be a major risk factor for premature delivery (18%).

We also looked at the culture data (Table III) to rule out the impact of mixing with microbes other than those linked to bacterium vaginal infection. During the first conception visit, women who experienced early pregnancy loss had a higher likelihood of having heavy colonization of any type of organism (relative risk: 2.6; 95% confidence interval: 1.6-4.1). However, there was no apparent correlation found between the amount of digestive or sex-wise transferable bacteria and the risk of miscarriage. On the other hand, the risk of premature delivery was significantly elevated in an outbreak of bacteria linked to vaginosis by bacteria (relative risk, 5.5; (95% CI, 2.9–10). Among these bacteria, the group that was pregnant that resulted in the fetus expelling spontaneously had significantly higher prevalences of G vaginal tissue (relative risk: 5.8), U fictitious (relative risk: 5.8), and M hominis (relative risk: 12.5).

Step-wise multivariate statistical analysis was used to examine the variables' involvement (Table V). A recurrent pregnancy was linked to a history of spontaneous abortion as well as the presence of G vaginalis, M hominis, and U urealyticum. There was no evidence of any correlation between premature birth loss and coloration with bacteria (apart from those already mentioned) or not specific symptoms of infection like lactobacillus grades, cervical swelling, or clinical bacterial vaginosis. The correlation between current nicotine use and early loss was borderline.

Table(5): An investigation of factors increasing the risk of giving birth at less than 20 weeks gestation using modified steps in regression

Previous miscarriage	0.43	0.179	$P < .0001$
Bacterial vaginosis	0.21	0.041	$P = .0016$
M hominis	0.14	0.018	$P = .04$
U urealyticum	0.13	0.015	$P = .05$
Smoking		0.014	$P = .06$
Age		0.011	$P = .1$
Vaginal leukocytosis		0.006	$P = .2$
Any positive vaginal culture result		0.004	$P = .3$
Cervical inflammation		0.002	$P = .5$
Intake of antibiotics		0	$P = .9$
Lactobacillary grade		0	$P = .9$

Only women with live children took part in the study to rule out the potential that changed testosterone levels within women who eventually experienced pregnancy loss might favour the spread of bacterial vaginosis. Furthermore, there were no variances in the happenings of bacteria that cause vaginitis (3/7 vs 5/10), clue cells (3/7 vs 4/10), and bacterial virginities-associated organisms (4/7 vs 5/10) between the 7 women who experienced losing a pregnancy at or less than 7 days after the first visit and the 10 women who experienced conception loss >7 days after the first visit to the doctor.

About 14 women with bacterial vaginosis, 21 who had elevated development of gastrointestinal bacteria (such as E coli, group B staphylococcus, or microbes such as), 32 with either or both of these

disorders and 165 with typical vaginal micro biota were among the 197 women with progressing pregnancy who had complete culture results. The women with the condition had mean pregnancy ages at birth of 38.9 ± 1.5 weeks and birth weights of 3246 ± 424 g; those with enteric bacteria had mean pregnancy ages at birth of 39.1 ± 1.3 weeks and birth weights of 3276 ± 399 g; and those with normal gut bacteria had mean gestational diabetes ages at birth of 39.1 ± 1.5 several months and birth heavy objects of 3386 ± 498 g. These distinctions were not very substantial.

Discussion :

It is unclear what causes unexpected miscarriages. Women who experience miscarriages are traditionally taught that complicated polygenetic or autoimmune factors may have contributed to the outcome and that gradual selection has taken place. There is a shortage of information regarding the potential contribution of cervicovaginal infection to early pregnancy loss. Via an overwhelming maternal immune-stimulating response to its hot shock protein 60 antigen, *C.trachomatis* may cause abortion. Additionally, some authors have discovered a correlation between spontaneous abortion and an abundance of vaginal *U* urealyticum, group B microorganisms gonorrhea, and *U* urealyticum. [15]

In their planned study of aberrant bacterial colonization of the vagina, Hay et al. (7) discovered that the group with intermediary microbiology had a 5.5-fold higher chance of miscarrying between 16 and 24 weeks of gestation. Even while the group with grade III (completely aberrant) microbiota was not linked to the delivery of an immature fetus, the increase was substantial when combined with them. Since a variety of circumstances other than a viral infection may cause the lactobacillus morphological types to disappear, it is unclear whether equating temporary flora with partial bacterial infection is accurate, even though this relationship is usually taken as an upward correlation of premature birth with the infection.[4, 16, 17]

The relationship between pregnancy outcomes and infections caused by bacteria has been the subject of two further studies. Before the eighteenth week of pregnancy, Kurki et al. (18) examined for infections caused by bacteria and discovered that the presence of the infection at the initial pregnancy visit was prognostic of early rupture of the mucus membranes, preterm labor, and preterm birth. However, the study only covered baby results from 20 weeks of gestation onward, thus the matter of whether bacteria-related vaginosis causes mortality in the first half of pregnancy remains unsolved. When McGregor et al. (19) examined the vagi- nal flora of 1260 pregnant women on their first prenatal visit, they discovered that infection with bacteria was linked to a three-fold higher risk of miscarriage at less than 22 weeks of gestation. However, the number of abortions climbed from 1.8% to

2.9% in the course of the study arm (two 300-mg doses of amoxicillin), suggesting that the treatment for the infections had not been effective in averting premature baby losses.

This series covered a community with the smallest amount of infections imaginable, yet many medical scientists interested in transmissible illnesses may operate in places with a significant number of infections or their consequences. Even bacterial vaginosis was less common than in most other series (11%), with gonorrhea, the protozoan, syphilis, herpes, the human immunodeficiency virus infection, and Chlamydia occurring very infrequently (<0.5%). [1,2,3,6, 7,18&19.] It is less likely that confections and associated risk factors will have an impact on the connections discovered in this group of individuals, which is why this low-risk profile was purposefully chosen.

Compared to women with progressing conceptions, those who later experienced an abortion received their first prenatal visit 10 days earlier on average. Could the women with changing conceptions' later registrations alone account for the differing pregnancy outcomes? One could argue that a woman's chances of experiencing a miscarriage decrease with the further along in her pregnancy she seeks therapy. There is still no explanation for why bacterial vaginosis was seven times more common in the group that experienced abortions than in the group that experienced evolving conceptions, even when the declining frequency of the disease from 15% to 10% with being pregnant advancement is taken into account. The probability of bacterial vaginosis being linked to spontaneous abortion is higher than the probability of the evolving gestation being linked to a 10-day postponement of the initial prenatal appointment.

At the initial visit, thirty-eight percent of the women who had miscarried showed signs of bacterial vaginosis, while the percentage for women who had normal pregnancies was 5.3% (relative risk, 7; P=.0003). At the initial visit, 48% of the women who experienced early pregnancy loss had their G vaginalis, U urea-lytic, or M hominis bacteria cultured, while only 8.2% of the control group had the same treatment (relative risk, 6; *P* <.0001). Therefore, premature births in 36% of these women would be predicted by bacterial vaginosis before Fourteen weeks (positive predictive value); in contrast, the contrary would be predicted in 93% of instances if bacterial vaginosis was absent. Since 82% of cases of bacterial vaginosis are attributable to the illness, eliminating antibacterial vaginosis in affected women should logically reduce their chance of spontaneous abortion by 82%. The population- attributable population risk (PAR) would be reduced by 30% if bacterial vaginosis was completely eradicated from the pregnant population. In a subgroup of women analyzed before 16 weeks' gestation, Hay et al.7 showed a similar relationship risk of

second-trimester abortion to the early pregnancy loss risk (relative risk, 5.4) among women diagnosed with vaginosis due to bacteria before 14 weeks' pregnancy.

Within a week of the first selection, the group that experienced pregnancy loss saw the same rates of bacterial vaginosis and colonization with species linked to bacterial vaginosis as the group that experienced pregnancy loss later on. This rules out the chance that the bacterial vaginal flora could be affected by the hormone levels that are dropping, which is indicative of an impending and inevitable expulsion. Moreover, at the first inclusion visit, an ultrasound revealed good cardiac activity in every fetus. Compared to the group of women with progressing conceptions, there were more women in the pregnancy loss group who had previously experienced prenatal loss. This begs the fascinating question of whether any members of the former group might have had recurring, chronic microbial varicella, which was implicated in both the index event and the prior accidental miscarriages. To determine whether or not bacterial vaginosis is one of the numerous pieces missing from the puzzle of recurring spontaneous abortion, more research is required.

There is indirect evidence suggesting that the pathway causing intrauterine infection in the latter stages of development could not be the same as the one causing abortion. Candida vaginosis was not associated with preterm delivery in this study, however it was with miscarriage. On the other hand, preterm birth was linked to intermediate flora. It is possible that bacterial vaginosis, based on most criteria, actually comprises a variety of illnesses comprised of both anaerobic vaginosis and other explanations for irritated flora, so the distinction is not always made in some early gestation studies, even though there may be a differential procedure according to gestational age. [17]

In these and earlier research, the majority of aborted fetuses did not exhibit histologic evidence of infection or infection itself. As a result, amnionitis may not always be caused via the ascent channel. Maternal and fetal infection was detected in 25–33% of instances of a condition known as caused by E. Coli and group B staphylococcus however, it was only observed in 1% of cases when G. vaginalis was discovered in the amniotic fluid. [20] Moreover, colonization with *Ecoli* and group B microorganisms elicits a strong immune response from mothers, whereas colonization with *G.vaginalis* does not elicit the same response.[21]

This could suggest that while infectious microorganisms may travel further along the ascending decidual pathway, causing deciduous and the resulting discharge of endotoxins, also cytokines, and asubstance called pros enteric bacteria may more easily enter the

mother's uterine bloodstream. Additional researchers have identified G vaginal tissue and anaerobes linked to the infection in the amniotic cavity and from the vagina up to the amniotic and chorionic membranes.[22,23,24] Therefore, an allergic reaction following escalating infection could be the workings by which spontaneous abortion results from bacterial vaginosis. This would also explain why the presence of an implantable contraceptive device, for example, facilitates the ascent and raises the risk of complications from vaginosis from bacteria[25,26]

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