

BACTERIAL VAGINOSIS DURING PREGNANCY

REET MÄNDAR, HENN SAAG, PEETER PEIL, and MARIKA MIKELSAAR

SUMMARY

We investigated the vaginal microflora of 42 women repeatedly (4-7 times) during pregnancy (in the period between 6 and 38 weeks of gestation). Dilutions of the material were inoculated into different media and incubated aerobically and anaerobically. Subsequently the total count of microorganisms per swab and the relative percentage of lactobacilli in the total microbial count of the vagina were calculated. From the first dilution of the material also a Gram-stained smear was made which was evaluated using the scoring system of *Nugent et al.* (1991) for diagnosing bacterial vaginosis (BV). Also the presence of "clue cells" was registered.

We found BV in 31.3% of the samples. At least one episode of BV during pregnancy occurred in 20 women out of 42, seven of them had BV in all the samples. "Clue cells" were found in 68 out of 72 samples with BV and in two cases out of 114 intermediate microflora.

Lactobacilli were found in 39 out of 42 women and in 139 out of 229 samples. Their median relative amount in the microflora was similar in case of normal and intermediate vaginal flora whereas it was 0 in all the scores of BV. The incidence and relative amount of lactobacilli in the vaginal microflora increased during pregnancy, at the same time the incidence of BV by Gram-stain decreased. A dynamic survey of pregnant women can provide a model for studying the microbial ecology of BV.

INTRODUCTION

Bacterial vaginosis (BV) is found to be quite common among pregnant women (*Cristiano et al.*, 1989; *Kurki et al.*, 1992; *McGregor et al.*, 1990; *Thomason et al.*, 1991). It has been associated with several pregnancy complications (*Baron and Finegold*, 1990; *Hoyme*, 1989; *Kurki et al.*, 1992; *Mardh*, 1991; *McGregor et al.*,

1990; *Nugent et al.*, 1991) and the altered vaginal microflora may induce changes in the microflora formation of the new-born (*Lundequist et al.*, 1985).

In BV the absence or low numbers of lactobacilli is reported (*Spiegel*, 1991; *Thomason et al.*, 1991). However, it is not known whether the

Table 1: Characteristics of the pregnant women investigated

Study group	No.	Age (range and mean)	Number of previous pregnancies (range and mean)	Number of women with		Adverse pregnancy outcome ³ (%)
				anti-bacterial treatment	hormonal treatment	
Group I	23	19-29 (22.9)	0-2 (0.3)	9 ¹	0	11/23 (47.8)
Group II	19	20-35 (26.7)	0-5 (2.2)	8 ¹	19 ²	11/19 (57.9)

¹ Nitrofurans for urinary tract infections (4 women of Group I and 4 women of Group II), Metronidazole for trichomoniasis (1 and 1), Clotrimazole (3 and 0) or Nystatin (0 and 1) for vaginal candidiasis, Sulphonamides (2 and 3), Ampicillin (0 and 1) or Oxacillin (0 and 1) for respiratory tract infections.

² Turinal (Gedeon Richter, Hungary), contains 5 mg allyloestrenol. The hormonal therapy (5-15 mg daily) was stopped before the 20th week of gestation.

³ Threatened prematurity, preterm delivery and/or premature rupture of membranes.

changes in counts of lactobacilli are directly correlated with the incidence of BV. Several investigators have described the increase in vaginal lactobacilli before birthgiving (*Galask*, 1988; *Redondo-Lopez et al.*, 1990). At the same time there are very few dynamic studies of BV (*Platz-Christensen et al.*, 1993) and no studies comparing these two indicators dynamically during pregnancy. Also we

could not find any investigations comparing by cultivation the relative percentage of lactobacilli in the microflora and by Gram-stained slides in the incidence of BV.

The aim of the present survey was to study the occurrence and dynamics of BV during pregnancy and to compare it with the presence and amount of lactobacilli.

MATERIAL AND METHODS

Two groups of pregnant women were investigated during a prospective study at the Tartu Maternity Hospital (Table 1). The first group consisted of 23 consecutive pregnant women presenting before the 17th week of gestation who had never delivered before (Group I), and the second group of 19 consecutive women presenting with a threatened abortion before the 12th week of gestation (Group II). Each woman was investigated 4-7 times; altogether 229 investigations were performed. The periods of sampling were ≤ 10 weeks, 11th-16th,

17th-22nd, 24th-26th, 28th-30th, 32nd-34th and 36th-38th weeks of gestation, during ordinary visits to gynaecologist. Forty out of 42 women delivered live babies, one delivered a foetus mortus with multiple abnormalities and we lack information about the course of delivery of one person.

Specimens were taken from the lateral side of the internal part of the vagina. To maintain the viability of fastidious microorganisms blood-thioglycollate-agar-coated cottonwool swabs were used. The swabs were put

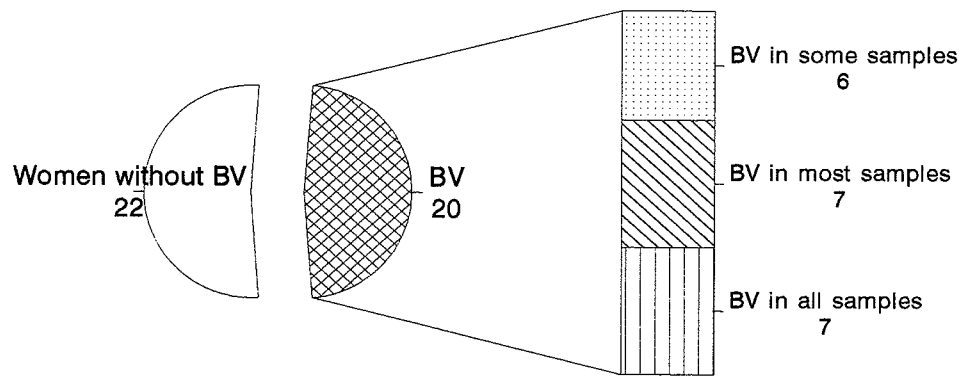


Figure 1: Occurrence of BV among pregnant women.

into tubes containing carbon dioxide sealed with rubber stoppers. The specimens were sent to the laboratory within 2 hours of collection.

Before inoculating the plates, the swabs were shaken in 2 ml of pre-reduced phosphate buffer under a gentle stream of oxygen-free CO₂. Dilutions 10⁻¹-10⁻⁵ (0.1 ml) of the bacterial suspension were then inoculated into pre-reduced blood-thioglycolate-agar- medium handled as modified roll-tubes for anaerobic microorganisms (Mikelsaar et al., 1984). The material was also inoculated onto freshly prepared blood-agar with 5% human blood, onto lactobacilli and streptococci selective MRS-4 agar (Lenzner et al., 1984), and onto Endo and Sabouraud media. The blood agar and the Endo and Sabouraud media were incubated aerobically at 37°C and examined 48-72 h later. The MRS-4 medium was incubated at 37°C in 10% CO₂ for 72 h and the roll tubes at 37°C for 72-120 h.

The lactobacilli were identified on the basis of colony and cellular morphology and negative catalase production (Lenzner et al., 1984). In case of each separate sample we calculated the total count of microorganisms per swab and the relative percentage (%) of lactobacilli in the total count of microbes of the vagina.

From the first dilution of the material also a Gram-stained smear was made for diagnosing BV. The slides were read using the scoring system of Nugent et al. (1991): large Gram-positive rods, small Gram-negative and -variable rods and curved Gram-variable rods have to be counted in 3 different oil-immersion fields and the criterion for BV is a score of 7 or higher; a score between 4 and 6 is considered intermediate, and a score between 0 and 3 normal.

Also the presence of "clue cells" was registered.

Data were analysed by regression analysis.

RESULTS

We found bacterial vaginosis in 72 (31.3%) out of 229 investigations. At least one episode of BV during preg-

nancy had occurred in 20 (47.6%) women out of 42 (Figure 1). Fourteen of them had BV in most of the sam-

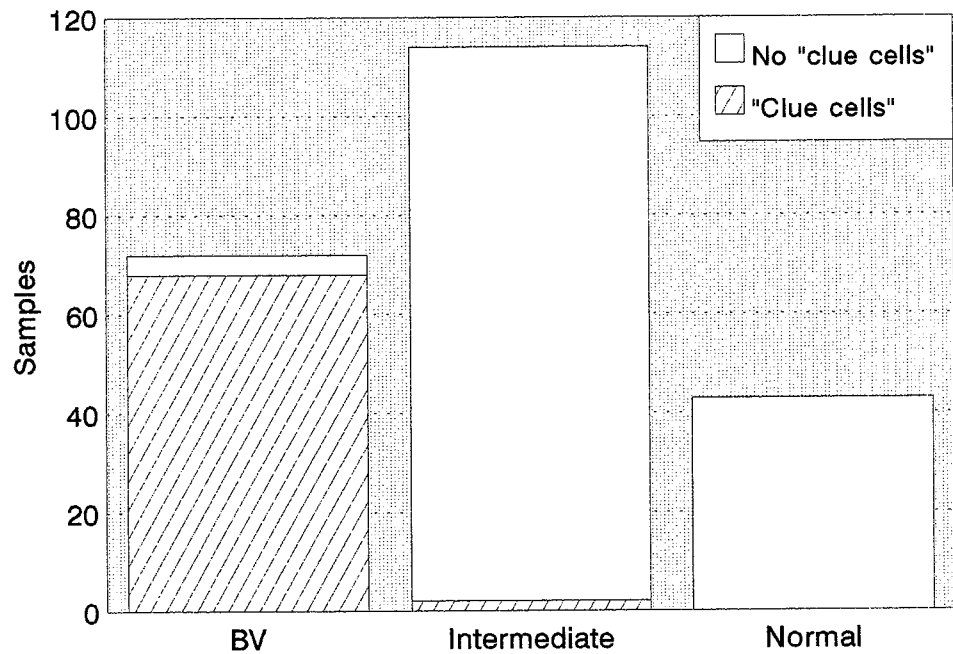


Figure 2: Occurrence of "clue cells" in different vaginal microfloras.

ples, in 7 of them it was found in all the samples. In 22 women BV was never revealed.

Nearly half (49.8%) of all the investigations showed the intermediate vaginal microflora, the normal microflora being found only in 18.8% of the samples. Not a single woman was found to harbour a normal microflora (score 0 - 3) during their complete pregnancy, everyone of those 22 without BV had at least one

episode of intermediate vaginal microflora.

"Clue cells" were found in 68 out of 72 samples with BV (Figure 2). They were also found in two cases out of 114 in the intermediate and never once in the normal microflora ($r = 0.94$).

We could find little difference between two groups studied; in women with threatening abortion BV could be detected less frequently (Table 2).

Table 2: Occurrence of BV in the different groups

Study group	Occurrence of BV (%)			
	Total % of samples	No. of women with BV in the first sample	No. of women with BV in the last sample	Stable BV ¹
I	36.2	8/23 (34.8)	8/23 (34.8)	7/23 (30.4)
II	26.5	8/19 (42.1)	2/19 (10.5)	5/19 (26.3)

¹ in more than half of samples.

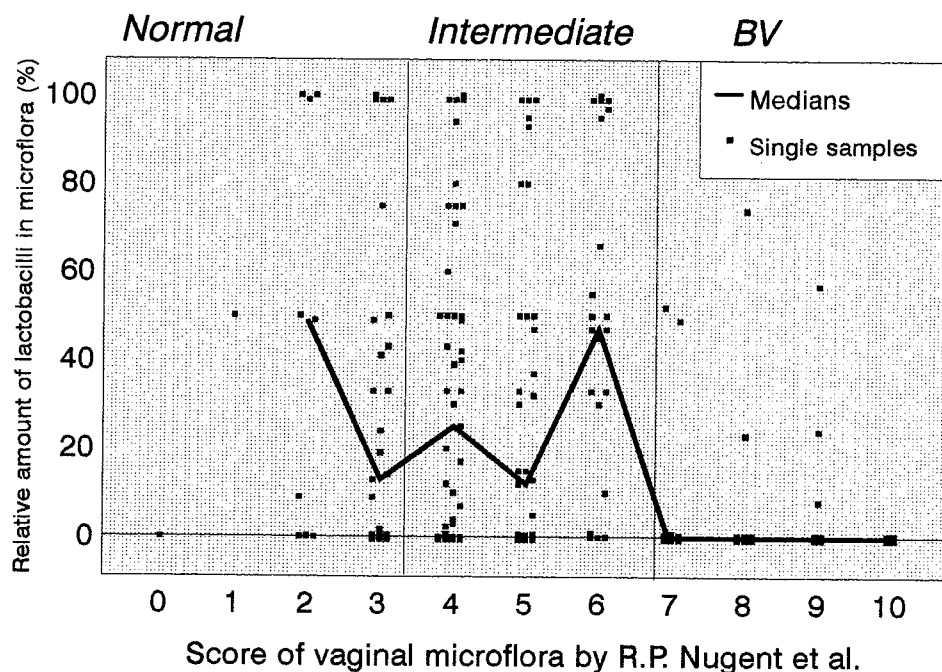


Figure 3: Relative amounts of lactobacilli in different vaginal microfloras.

Lactobacilli were found in 39 (92.8%) out of 42 women and in 139 (60.7%) out of 229 samples. Their median relative amount in the microflora did not differ in cases of the normal and intermediate vaginal flora, whereas it was 0 in all scores of BV (Figure 3).

The incidence and relative amount

of lactobacilli in the vaginal microflora increased during pregnancy ($r = 0.90$, $p = 0.005$; Figure 4). At the same time the incidence of BV by Gram-stain decreased ($r = -0.62$, $p = 0.13$). Also the mean score of vaginal flora decreased during pregnancy (from 5.2 to 4.7).

DISCUSSION

We could detect BV quite frequently (in 31.3% of the samples) in the pregnant women that we examined. The incidence of BV corresponds to the data provided by other authors who have found BV in 10 to 26% of pregnant women (Cristiano et al., 1989; Kurki et al., 1992; McGregor et al., 1990; Platz-Christensen et al., 1993; Thomason et al., 1991). Our

study confirms the previous findings that the presence of the "clue cells" is really a good marker of BV which allows to diagnose BV rapidly without counting microbial morphotypes.

At the same time the total number of women harbouring BV in at least one sample during pregnancy was very high (20 out of 42 i.e. 47.6%). Our dynamical investigation showed

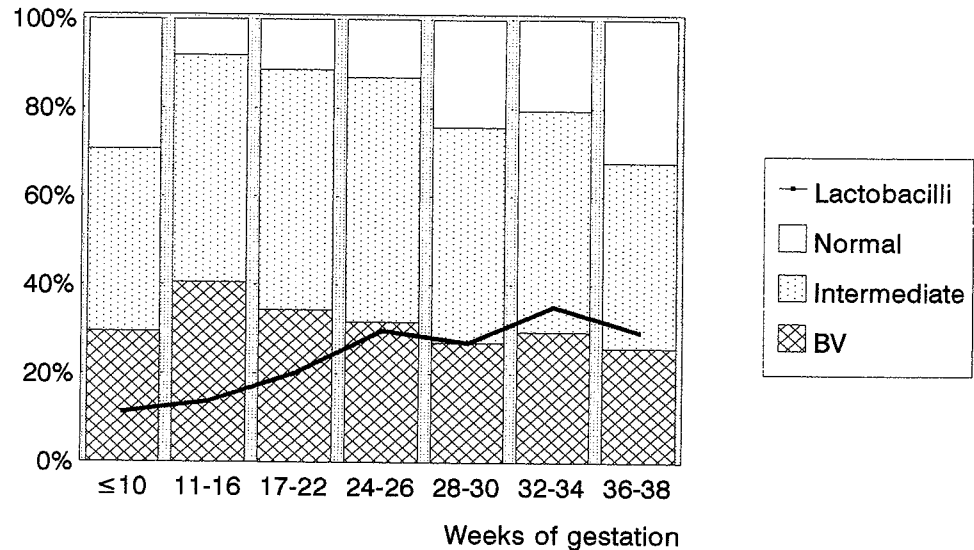


Figure 4: Dynamics of the relative amount of lactobacilli and occurrence of BV during pregnancy.

that in some women BV was unstable, whereas others had it throughout their pregnancy. The instability of BV has been previously described but mainly in nonpregnant women (*Bump et al., 1988; Platz-Cristensen et al., 1993; Livengood et al., 1990; Piot et al., 1983*). Consequently, the treatment of BV during pregnancy can be postponed till the diagnosis of BV is repeatedly confirmed.

In Group II the BV was somewhat less frequent, especially at the end of pregnancy. This may be connected with their hormonal therapy which has been shown to influence the microbial types and population levels in the vagina (*Redondo-Lopez, 1990*).

Lactobacilli were the frequent colonisers of pregnant women. We found that their relative amount in the microflora increased towards the end of pregnancy. This finding is in good correlation with the complementary decrease of incidence of BV in the same women. Seemingly the colonisation of the vagina by lactobacilli

really intervenes with the incidence of BV, apparently by improving the microbial ecology of the vagina.

We could not find differences between the relative amount of viable lactobacilli in case of the normal and the intermediate vaginal floras. The possible explanation may be that the "lactobacillus-morphotype" in Gram-stained slides partially includes also some other Gram-positive rods eubacteria, bifidobacteria, bacilli, and actinomyces. These microbes may help to perform the colonisation resistance of vaginal microflora. According to our survey it seems that the total amount of Gram-positive rods gradually decreases up to the point when in case of BV both lactobacilli and all the other Gram-positive rods disappear. So only by bacterioscopic studies it would not be possible to determine the real composition of Gram-positive flora and make conclusions about the interrelations between the persistence of lactic acid microflora and incidence of BV.

Consequently, pregnancy serves as a useful natural model for revealing dynamical ecological changes in vaginal microflora.

Reet Mändar, Marika Mikelsaar, Institute of Microbiology, University of Tartu, 46 Vanemuise Str, EE2400 Tartu, Estonia.
Henn Saag, Peeter Peil, Tartu Maternity Hospital, 6 Lossi Str, EE2400 Tartu, Estonia.

Correspondence: Reet Mändar, 46 Vanemuise Str, Institute of Microbiology, University of Tartu, EE2400 Tartu, Estonia; Phone: 372-34-28802/30617; Fax: 372-34-30365/35440; E-mail: hugo@physic.ut.ee

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LITERATURE

- Baron, E.J., and Finegold, S.M.: Diagnostic microbiology. Bailey & Scott, St.Louis (1990).
- Bump, R.C., and Buesching, W.J.: Bacterial vaginosis in virginal and sexually active adolescent females: Evidence against exclusive sexual transmission. *Am. J. Obstet. Gynecol.* 158, 935-939 (1988).
- Cristiano, L., Coffetti, N., Dalvai, G., Lorusso, L., and Lorenzi, M.: Bacterial vaginosis: Prevalence in outpatients, associations with some microorganisms and laboratory indices. *Genitourin. Med.* 65, 382-387 (1989).
- Galask, R.P.: Vaginal colonization by bacteria and yeast. *Am. J. Obstet. Gynecol.* 158, 993-995 (1988).
- Hoyme, U.B.: Bacterial vaginosis. *Zbl. Gynecol.* 111, 1589-1598 (1989).
- Kurki, T., Sivonen, A., Renkonen, O.V., Savia, E., and Ylikorkala, O.: Bacterial vaginosis in early pregnancy and pregnancy outcome. *Obstet. Gynecol.* 30, 173-177 (1992).
- Lenzner, A., Lenzner, H., Mikelsaar, M., Ttri, M., Vdljaots, M., Shilov, V., Lizko, N., Legenkov, V., and Reznikov, I.: Die quantitative Zusammensetzung der Lactoflora des Verdauungstraktes vor und nach kosmischen Flügen unterschiedlicher Dauer. *Die Nahrung* 28, 607-613 (1984).
- Livengood, C.H., Thomason, J.L., and Hill, G.B.: Bacterial vaginosis: treatment with topical intravaginal clindamycin phosphate. *Obstet. Gynecol.* 76, 118-123 (1990).
- Lundequist, B., Nord, C.B., and Winberg, J.: The composition of the fecal microflora in breastfed and bottle-fed infants from birth to eight weeks. *Acta Paediatr. Scand.* 74, 45-50 (1985).
- Mardh, P.-A.: The vaginal ecosystem. *Am. J. Obstet. Gynecol.* 165, 1163-1168 (1991).
- McGregor, J.A., French, J.I., Richter, R., Franco-Buff, A., Johnson, A., Hillier, S., Judson, F.N., and Todd, J.K.: Antenatal microbiologic and maternal risk factors associated with prematurity. *Am. J. Obstet. Gynecol.* 163, 1465-1473 (1990).
- Mikelsaar, M.E., Türi, M.E., Väljaots, M.E., and Lenzner, A.A.: Anaerobe Inhalts und Wandmikroflora des Magen-DarmKanals. *Die Nahrung* 28, 727-733 (1984).
- Nugent, R.P., Krohn, L.A., and Hillier, S.L.: Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J.*

- Clin. Microbiol. 29, 297-301 (1991).
- Piot, P., van Dyck, E., Godts, P., and Vanderheyden, J.: A placebo-controlled double-blind comparison of tinidazole and triple sulfonamide cream for the treatment of nonspecific vaginitis. *Am. J. Obstet. Gynecol.* 147, 85-89 (1983).
- Platz-Christensen, J.J., Pernevi, P., Hagmar, B., Andersson, E., Brandberg, A., and Wiquist, N.: A longitudinal follow-up of bacterial vaginosis during pregnancy. *Acta Obstet. Gynec. Scand.* 72, 99-102 (1993).
- Redondo-Lopez, V., and Cook, R.L.: Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Rev. Infect. Dis.* 12, 856-872 (1990).
- Spiegel, C.A.: Bacterial vaginosis. *Clin. Microbiol. Rev.* 4, 485-502 (1991).
- Thomason, J.L., Gelbart, S.M., and Scaglione, N.J.: Bacterial vaginosis: Current review with indications for asymptomatic therapy. *Am. J. Obstet. Gynecol.* 165, 1210-1217 (1991).