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ARTICLE

Is bacterial vaginosis associated with a pro-inflammatory cytokine profile in endometrial secretions of women undergoing IVF?

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Abstract The objective of this prospective cohort study was to elucidate whether bacterial vaginosis (BV) is associated with a pro-inflammatory endometrial secretion cytokine profile and whether there is a relationship between BV and the concentrations of a number of key regulatory cytokines, chemokines and growth factors. A total of 198 women undergoing IVF treatment were included. Prior to embryo transfer, participants underwent screening for BV according to Nugent criteria by a Gram-stained cervical smear. The concentrations of 17 soluble mediators of human implantation were measured by multiplex immunoassay in endometrial secretions aspirated prior to embryo transfer. Seventeen (8.6%) women had BV (Nugent score >6). Multivariable logistic regression showed a significant positive association between interleukin-β and the presence of BV ($P = 0.011$; Nugent score >6 versus ≤6) and a significant negative association between eotaxin and BV ($P = 0.003$). No significant differences were found in the ratios of distinct pro- and anti-inflammatory cytokines in endometrial secretions from women with or without BV. In conclusion, BV is associated with higher concentrations of interleukin-β in endometrial secretions compared with women without BV. However, no distinct difference in pro- and anti-inflammatory profiles is present. An effect on endometrial receptivity is unlikely.

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KEYWORDS: bacterial vaginosis, endometrial secretion, IVF, pro-inflammatory cytokines

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Introduction

Bacterial vaginosis (BV) represents a common imbalance of the vaginal bacterial flora. An overgrowth of anaerobic organisms, such as *Gardnerella*, *Bacteroides* and *Mobinculus*, leads to replacement of lactobacilli and an increase in vaginal pH from less than pH 4.5 to as high as pH 7.0 (Morris et al., 2001). While BV may be associated with a malodorous vaginal discharge, up to 40–50% of women are asymptomatic (Klebanoff et al., 2004). BV is known to be a risk factor for preterm birth (Leitich and Kiss, 2007) and antibiotic treatment before 20 weeks of gestation has been shown to reduce this risk (McDonald et al., 2007).

There is evidence that the pathogenic effects of BV may not be confined to complications during pregnancy, but may also interfere with embryo implantation. BV has been reported to be associated with a significant increased risk of miscarriage in the first trimester in women undergoing IVF, independent of other risk factors (Ralph et al., 1999). Another study concluded that women with BV compared with women with a normal flora may have decreased conception rates (30% and 52%, respectively) (Eckert et al., 2003). However, these results were of borderline significance in a cohort of 91 patients and could not be confirmed by others (Gaudoin et al., 1999; Liversedge et al., 1999).

The mechanism by which BV may diminish IVF outcome is unclear. The term 'bacterial vaginosis' rather than 'vaginitis' is used, because of the absence of associated inflammation. However, there is evidence for an inflammatory response to BV. It has been shown that almost half of patients with symptomatic BV showed histopathological evidence of plasma cell endometritis (Korn et al., 1995). In addition, a number of studies have shown correlations between the concentrations of cytokines in both vaginal fluid and cervical mucus and the presence of BV (Cauci et al., 2003; Hedges et al., 2006; Mattsby-Baltzer et al., 1998; Spandorfer et al., 2001; Platz-Christensen et al., 1993; Sturm-Ramirez et al., 2000). The effects of BV on the endometrium may be brought about by endotoxins, such as lipopolysaccharides, a component of the outer membrane of Gram-negative bacteria. Significantly diminished pregnancy rates after IVF have been reported when endotoxin concentrations >200 pg/ml were found in menstrual effluent (Kamiyama et al., 2004). Microbial flora of the cervix could be identified on embryo transfer catheters in up to 50% of cases and have been reported to adversely affect IVF outcome (Egbase et al., 1996; Fanchin et al., 1998; Moore et al., 2000).

It is unclear whether cytokine patterns in the uterine cavity are altered in the presence of BV. Successful implantation appears to be associated with a predominance of anti-inflammatory cytokines (Wegmann et al., 1993). Any disturbance of the delicate immune balance in pro-and anti-inflammatory cytokines at the maternal–fetal interface may result in failure of implantation or early pregnancy loss (Chaouat et al., 1990). Next to a shift in pro- and anti-inflammatory cytokines, a dysregulation in the expression of other mediators involved in embryo implantation and endometrial maturation (Arcuri et al., 1999; Caballero-Campo et al., 2002; Dey et al., 2004; Ledee-Bataille et al., 2005; Lessey et al., 2002) may also reduce endometrial receptivity in women with BV. The objective of this study was to elucidate whether BV colonizing the cervix is associated with a more pro-inflammatory cytokine profile and to assess the correlation between the Nugent score and the concentrations of a number of key regulatory cytokines, chemokines and growth factors in endometrial secretions aspirated prior to embryo transfer.

Materials and methods

Study design

Ethical approval was received from the local institutional ethics committee and informed consent was obtained from all participants. Between December 2005 and October 2006, 198 patients attending the University Medical Centre Utrecht (The Netherlands) for IVF or intracytoplasmic sperm injection (ICSI) treatment, who had undergone no more than one prior embryo transfer and showed no signs of hydrosalpinges on the ultrasound, consented to participate in this prospective cohort study.

In-vitro fertilization

The IVF procedure has been described previously in detail (Boomsma et al., 2009). A maximum of two embryos were transferred 4 days after oocyte retrieval. Women with endometriosis or tubal pathology received an oral dose of ampicillin with clavulanic acid (875/125 mg) and doxycycline (200 mg), 2 h prior to oocyte retrieval. A urinary pregnancy test was performed 14 days after embryo transfer and if positive, an ultrasound was performed at 9 weeks of gestation.

Study procedure

Immediately prior to embryo transfer, participants underwent an endometrial secretion aspiration and screening for BV with a Gram-stained smear. BV was assessed from an (endo) cervical smear, since colonization of the cervix rather than the vagina may increase the risk of intrauterine disturbances. With the patient lying in lithotomy position and after insertion of the speculum without lubrication, a sterile cotton swab was used to obtain a sample from the cervix and endocervix for diagnosis of BV. The swab was rolled onto a glass slide, fixed with acetone and allowed to dry in air. Afterwards, the cervix was cleansed and endometrial secretion was aspirated using the technique described previously in detail (Boomsma et al., 2009; van der Gaast et al., 2003). Briefly, an embryo transfer catheter (Wallace: SIMS Portex Ltd., Hythe, Kent, UK) was introduced transcervically. Suction was gradually applied with a 2 ml syringe. Cervical mucus contamination was prevented by covering the cervix with the outer layer of the embryo transfer catheter, while removing the inner part of the catheter after aspiration. The embryo transfer procedure was carried out thereafter. The aspirate was snapfrozen in liquid nitrogen and stored at −80°C. All patients were questioned on the day of embryo transfer regarding symptoms associated with BV, namely an abnormal aspect or malodorous vaginal discharge or itching and burning symptoms.

Endometrial secretion analysis

The procedure employed to analyse the endometrial secretions has been previously described in detail (Boomsma et al., 2009). Briefly, the endometrial secretion aspirates were analysed using a multiplex immunoassay detecting 17 soluble cytokines, chemokines and growth factors. The factors included in the assay are known to be involved in the process of implantation and a description of these mediators is provided in Boomsma et al. (2009). Distinct pro-inflammatory cytokines are interleukin (IL)-1β, IL-12, IL-18, interferon (IFN)-γ, tumour necrosis factor (TNF)-α and macrophage migration inhibitory factor (MIF), whereas IL-5 and IL-10 have more anti-inflammatory properties. IL-6, IL-15 and IL-17 have both pro- and anti-inflammatory characteristics. Three chemokines involved in implantation were included in the assay, namely eotaxin, monocyte chemotactic protein (MCP)-1 and IFNγ-inducible 10 kD protein (IP-10). Vascular endothelial growth factor (VEGF) and heparin-binding epidermal growth factor (Hb-EGF) are growth factors. Lastly, Dickkopf homologue (Dkk)-1 inhibits Wnt signalling proteins, which comprise a family of molecules with pivotal roles in cell proliferation and differentiation. MIF was determined in 400× diluted samples using a commercially available enzyme linked immunosorbent assay (ELISA) (R and D systems, Abingdon, United Kingdom). To investigate whether BV was associated with a shift towards a pro- or anti-inflammatory profile, the ratios of TNFα or IL-1β divided by IL-10 or IL-5, two distinct pro- and anti-inflammatory cytokines respectively, were compared in women with or without BV. In the literature, ratios of pro-and anti-inflammatory cytokines are frequently used to demonstrate a bias of the milieu towards one or the other side (Cervera et al., 2007; Lira et al., 2009; Sahiratmadja et al., 2007; von Känel et al., 2008). Aspirate volumes could not be reliably measured, since the aspirates are highly viscous, but were between 1 and 4 μl. Therefore, total protein content of the aspirate was measured by NanoOrange Protein Quantitation Kit (Molecular probes Europe, Leiden, The Netherlands). The concentration of each mediator was expressed in pg/mg protein.

Endocervical slides for diagnosis of BV

The slides were Gram-stained and examined microscopically under oil immersion at 1000× magnification. Specimens were scored according to a standardized scoring system providing a 0–10 point scale for the evaluation of vaginal flora (Nugent et al., 1991). The scale is based on a weighted sum of different bacteria with certain morphology, namely *Lactobacilli* (large Gram-positive rods), *Gardnerella vaginalis* and *Bacteroides* (small Gram-negative to -variable rods) and *Mobinculus* species (curved Gram-negative rods). Each morphotype was quantitated from 1 to 4+ with regard to the number of morphotypes per oil immersion field (Table 1). A Nugent score from 0–3 is considered normal, 4–6 intermediate and 7–10 is the criterion for BV. The slides were independently scored by two trained observers (NB and CB), who were blinded for clinical parameters or pregnancy outcome.

The Gram-stain technique employed in this study is considered to be the gold standard for the diagnosis of BV (Nugent et al., 1991). Its use is usually restricted to research settings since it is more cumbersome to use than the Amsel criteria (Amsel et al., 1983), which are more commonly applied in clinical practice. These criteria use surrogate markers, such as a homogeneous vaginal discharge, an amine odour when potassium hydroxide solution is added, the presence of clue cells on microscopy and a vaginal pH greater than pH 4.5. Compared with the Amsel criteria, the Nugent score allows for assessment of alteration in vaginal flora as a continuum rather than a dichotomy. Culture of vaginal discharge has no role in diagnosis of BV, since there are no bacteria that are specific for BV. DNA probes for *Gardnerella vaginalis* are expensive and cannot be used to diagnose bacterial vaginosis because it can be cultured from the vagina of more than 50% of normal women (Amsel et al., 1983). Nugent score has been favoured for diagnosing BV due to superior reproducibility and sensitivity (Krohn et al., 1989). The Papanicolaou smear has been shown to be poorly reliable for the diagnosis of BV (Greene et al., 2000).

Statistical analysis

On the basis of data previously derived and reported and the incidence of BV previously reported in literature, a power calculation was performed to assess the feasibility of the study. The value of the ratio of TNFα and IL-10 concentrations in endometrial secretions ranges from less than 0.12 to 32, with an observed standard deviation of 7.5. Highest values may be expected in women with BV. With an expected incidence of BV of 25% (Liversedge et al., 1999; Ralph et al., 1999; Wilson et al., 2002), 197 patients would allow us to identify a significant difference in the ratio of TNFα over IL-10 of 0.46 SD (Cohen's d) with 80% power. Therefore, the present study was considered to be sufficiently powered for this outcome. The ratio of TNFα and IL-10 was utilized because of the well-defined pro-inflammatory character of TNFα and because the other anti-inflammatory cytokine, IL-5 showed a higher number of values below the detection limit.

Analyses were performed using the Statistical Package for Social Sciences version 12.0 (SPSS, Chicago, IL, USA). A *P*-value less than 0.05 was considered to be statistically significant. Comparisons of clinical parameters in women with and without BV were performed using the independent *t*-test or Mann–Whitney *U*-test for continuous data and the chi-squared test for binary variables. Before analysis, all cytokine values were corrected for the total protein content in the sample. Thereafter, data were log transformed to obtain a normal distribution. Nine mediators (IL-1β, IL-6, IL-12, IL-18, TNFα, MIF, MCP-1, IP-10, VEGF) were detectable in 90–100% of the samples. Eotaxin was detectable in 88% of samples, IL-15 in 76% of samples, Dkk-1 68%, IL-10 56%, IL-17 55%, IL-5 34% and Hb-EGF was detectable in 23% of samples. IFNγ was not detectable in any of the samples and this was not due to technical problems (Boomsma et al., 2009). For statistical analysis of values below the detection limit of the assay, data was imputed by using the lower detection limit divided by $\sqrt{2}$, as recommended for log-transformed data by Schisterman et al. (2006). Blood

Table 1 Scoring system of the Nugent criteria for diagnosis of bacterial vaginosis.

Score	Lactobacillus	Gardnerella *and* Bacteroides	*Curved Gram-variable rods*
0	4+	0+	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	—
4	0+	4+	—

Average number of bacteria with a certain morphology (morphotypes)/field: 0+, no morphotype; 1+, <less than one morphotype; 2+, from one to four morphotypes; 3+, from five to 30 morphotypes; 4+, more than 30 morphotypes. Adapted from Nugent et al. (1991).

contamination of the endometrial secretion aspirate can affect the quantitation of a number of cytokines by the multiplex immunoassay method, as reported previously (Boomsma et al., 2009). The extent of blood contamination in the endometrial secretion samples was therefore visually graded and included as a covariate in the analyses.

The ratios of TNFα or IL-1β divided by IL-10 or IL-5 were calculated and subsequently log transformed to obtain a normal distribution. In order to investigate whether the ratios of distinct pro- and anti-inflammatory cytokines were different in women with or without BV, one-way analysis of covariance (ANCOVA) was performed with blood contamination (polynomial scale) and BV (Nugent score >6 versus ≤6) as fixed factors. In order to include all participants, women with normal and intermediate flora were combined. However, since the mediators included in the assay are known to be highly mutually correlated and molecular redundancy is present, multivariable analysis with backward elimination of mediators is the preferred method of analysis. The correlation between the concentration of mediators and the presence of BV (Nugent score >6 versus ≤6) was assessed by multivariable logistic regression analysis with a backward elimination procedure of the soluble mediators. A *P*-value >0.1 was used as a criterion for exclusion. Blood contamination was included as a covariate.

Results

From 198 women who underwent endometrial secretion aspiration and a cervical smear for diagnosis of BV, 197 were

Table 2 Clinical parameters of women with bacterial vaginosis (Nugent score ≥ 7) versus women with normal or intermediate flora (Nugent score <7).

Parameter	*Bacterial vaginosis (n = 17)*	*Normal flora and intermediate flora (n = 180)*	*P-value*
Age (years)	36.7 ± 3.9 (28—44)	34.8 ± 4.1 (26—44)	NS[a]
Duration of infertility (years)	2.8 ± 1.8	3.2 ± 1.7	NS[a]
Prinicipal type of infertility			
Andrological	65	48	NS[b]
Unknown	12	23	
Tubal pathology	6	19	
Endometriosis	6	2	
Other	12	8	
Body mass index (kg/m^2)	25.8 ± 5.3	24.3 ± 4.4	NS[a]
Smoking	0	16	NS[b]
Women treated with antibiotics prior to oocyte retrieval	18	17	NS[b]
IVF	60	58	NS[b]
ICSI	40	42	
Oocytes retrieved	5.7 ± 3.7	8.4 ± 4.8	0.015[c]
Embryos available	2.6 ± 1.3	3.8 ± 2.4	NS[c]
Embryos transferred	1.7 ± 0.5	1.5 ± 0.5	NS[b]
High-quality embryos	24	37	NS[b]
Pregnancy rate	47	33	NS[b]
Miscarriage/biochemical pregnancy rate per pregnancy	25	31	NS[b]
Women with subjective complaints on questionnaire			
Complaints absent	88	93	NS[b]
Complaints present	12	7	

Values are mean ± SD (range) or percentage.
[a]Independent *t*-test.
[b]Chi-squared test.
[c]Mann—Whitney *U*-test. ICSI = intracytoplasmic sperm injection; NS = not statistically significant.

included in the analysis. One patient was excluded, since insufficient material was available to measure the total protein content required for normalization purposes. Samples from 17 women (8.6%) met the criteria for diagnosing BV (Nugent score >6). The flora of 43 women (21.8%) was considered intermediate according to the Nugent score (>4) and the flora of 137 women (69.5%) was considered to be normal. None of the 197 women displayed symptoms of abnormal discharge at inspection during embryo transfer. On questioning, 7.6% of women complained of abnormal vaginal discharge or itching or burning symptoms. BV was diagnosed on Gram-staining in 13.3% of these cases, which did not significantly differ from the 8.2% prevalence of BV in women without symptoms on questioning. Univariate analyses of clinical parameters of women with BV versus women with normal or intermediate flora are presented in Table 2. No significant differences in demographic parameters between both groups were observed. With regard to treatment outcome, significantly less oocytes were retrieved ($P = 0.015$) and there was a non-significant trend towards development of fewer embryos in women with BV. With the number of oocytes retrieved included as a covariate, no significant difference in the number of embryos developed was found by ANCOVA analysis. No significant group differences were observed in the quality of the embryo transferred, pregnancy rates and early pregnancy loss rates in women with or without BV, but it should be noted that the study was not sufficiently powered to discern such a difference. The number of oocytes retrieved was significantly lower among women with BV. It was therefore included as a covariate in further analyses, since the ovarian response has been shown to significantly alter the intrauterine cytokine profile (Boomsma et al., 2010). The number of women who smoked did not significantly differ between women with or without BV. However, since the observed difference was sizeable (0% versus 17% in women with BV), smoking was also included as a covariate.

Intrauterine cytokine profile and presence of BV

To elucidate whether BV is associated with a pro-inflammatory cytokine profile, ratios of distinct pro- and anti-inflammatory cytokines were compared for women with and without BV. ANCOVA analysis, with blood contamination, smoking and BV (Nugent score >6 versus ≤6) as fixed factors and the number of oocytes retrieved as a covariate, did not show any significant differences (**Figure 1**). These results did not alter when women with BV (Nugent score >6) were compared with women without BV (Nugent score 0–3), neither when all values below the detection limit, which have been imputed, were excluded.

For women with or without BV, the concentrations of pro- and anti-inflammatory cytokines are presented in **Figure 2**, cytokines with both pro- and anti-inflammatory characteristics in **Figure 3** and other mediators in **Figure 4**. An independent *t*-test showed a significantly lower concentration of eotaxin ($P = 0.047$) in women with BV versus women without BV (Nugent score >6 versus ≤6). Multivariable logistic regression analysis with a backward elimination procedure of the soluble mediators and smoking, the number of oocytes retrieved and blood contamination included as covariates, showed a significantly positive association between IL-1β ($P = 0.011$) and the presence of BV (Nugent score >6 versus ≤6). A significantly negative association between eotaxin ($P = 0.003$) and the presence of BV was found. All results from multivariable analyses were confirmed when IL-5 and Hb-EGF with more than 50% imputed data were excluded from analyses. Results were also confirmed when smoking and the number of oocytes retrieved, which were unexpected potential confounders, were not included in the analysis.

Discussion

The hypothesis tested in this study was that BV, assessed from the cervix, may be associated with a pro-inflammatory cytokine profile in the uterus. No significant differences in the ratios of distinct pro- and anti-inflammatory cytokines were observed comparing endometrial secretions obtained from women with or without BV. Multivariable logistic regression analysis revealed a significantly positive correlation between the endometrial secretion concentration of IL-1β and the presence of BV and a significantly negative correlation between eotaxin and BV. IL-1β is a cytokine with pro-inflammatory characteristics. However, no further significant differences in pro- and anti-inflammatory cytokine profiles in endometrial secretions from women with or without BV were found.

Previously, it has been shown that almost half of patients with symptomatic BV showed histopathological evidence of plasma cell endometritis (Korn et al., 1995). A marked difference in absolute numbers of cytokine concentrations in lochia of symptomatic women with endometritis has been reported previously (Sukhikh et al., 2005). The concentration of IL-1β and TNFα was increased 1.4-fold and 2.7-fold, respectively, in women with endometritis versus controls. In the present

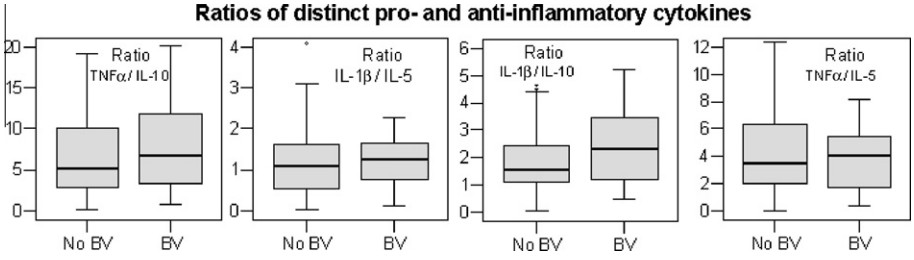


Figure 1 Ratios of distinct pro- and anti-inflammatory cytokines in women with bacterial vaginosis (BV; Nugent score ≥7, $n = 17$) or without (no BV; normal or intermediate flora, Nugent store <7, $n = 180$). IL, interleukin; TNF, tumour necrosis factor.

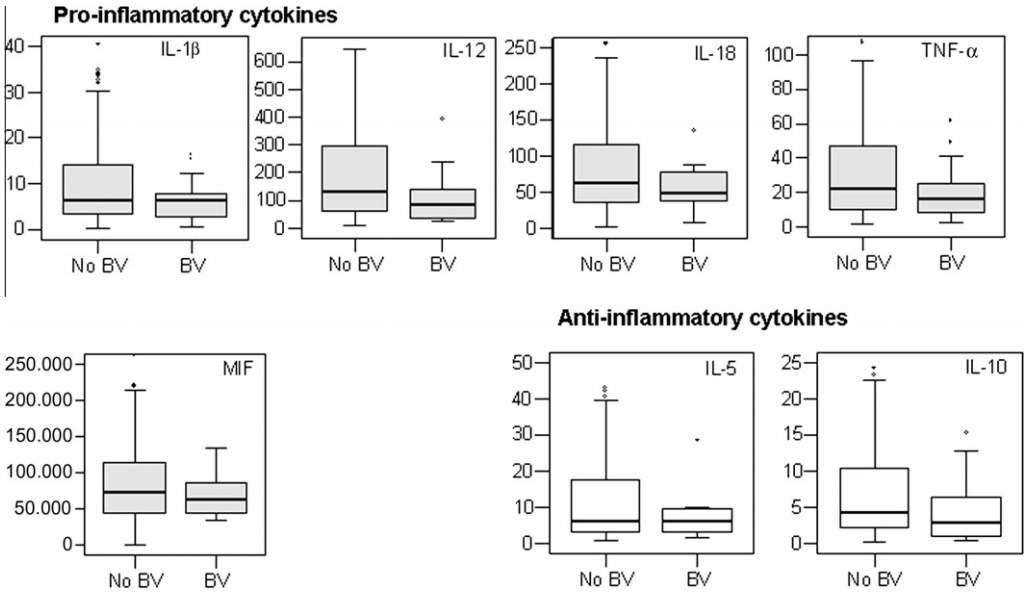


Figure 2 Concentrations of pro- and anti-inflammatory cytokines in women with bacterial vaginosis (BV; Nugent score $\geq$7, n = 17) or without (no BV; normal or intermediate flora, Nugent store $<$7, n = 180). Mediator concentration expressed in pg/mg protein. None of the *P*-values for independent *t*-test of log-transformed concentrations of mediators in women with or without bacterial vaginosis were statistically significant. IL, interleukin; MIF, macrophage migration inhibitory factor; TNF, tumour necrosis factor.

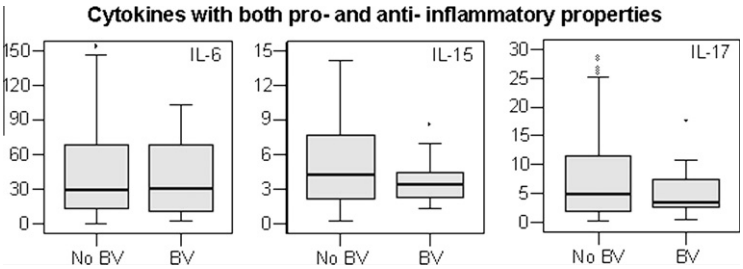


Figure 3 Concentrations of cytokines with both pro- and anti-inflammatory characteristics in women with bacterial vaginosis (BV; Nugent score $\geq$7, n = 17) or without (no BV; normal or intermediate flora, Nugent store $<$7, n = 180). Mediator concentration expressed in pg/mg protein. None of the *P*-values for independent *t*-test of log-transformed concentrations of mediators in women with or without BV were statistically significant. IL, interleukin.

study of women with mainly asymptomatic BV, cytokine concentrations were observed within the normal range compared with controls with a tendency to a more pro-inflammatory profile in women with a higher Nugent score.

Cervical smears from just 17 women (8.6%) met the criteria for BV, namely a Nugent score between 7 and 10. Complaint of abnormal vaginal discharge or itching or burning symptoms by the participants of this study, was not associated with a higher or lower prevalence of BV. This observation may be explained by the fact that BV was screened from the cervix rather than the vagina. This percentage of women with BV was lower than the prevalence of 25% in subfertile women previously reported (Liversedge et al., 1999; Ralph et al., 1999; Wilson et al., 2002), although other studies also reported lower incidences of BV, namely 16% (Eckert et al., 2003) or 4.2% (Spandorfer et al., 2001). However, in the latter study all patients received tetracycline prophylaxis at the time of oocyte retrieval. In the present study, 17% of women with either endometriosis or tubal pathology diagnosed on laparoscopic inspection received a prophylactic dose of ampicillin with clavulanic acid and doxycycline prior to oocyte retrieval. The use of prophylactic antibiotics in this group may explain the lower prevalence of BV observed. However, the same percentage of women received antibiotics in women with or without BV and the percentage of women with BV was similar among women who did receive antibiotics versus women who did not (8.8% and 8.6%, respectively). Moreover, doxycycline treatment at oocyte retrieval has been reported to have no substantial impact on the presence of BV (Moore et al., 2000). Although ampicillin is significantly less effective than the recommended therapy for BV by metronidazole or clindamycin (Joesoef et al., 1999; Piot, 1984), ampicillin with clavulanic acid has been shown to be effective against bacterial colonization of the cervix in women undergoing IVF (Salim et al., 2002). The difference in the prevalence of BV between the present study and the prevalence reported in literature may also be related to the method of diagnosis.

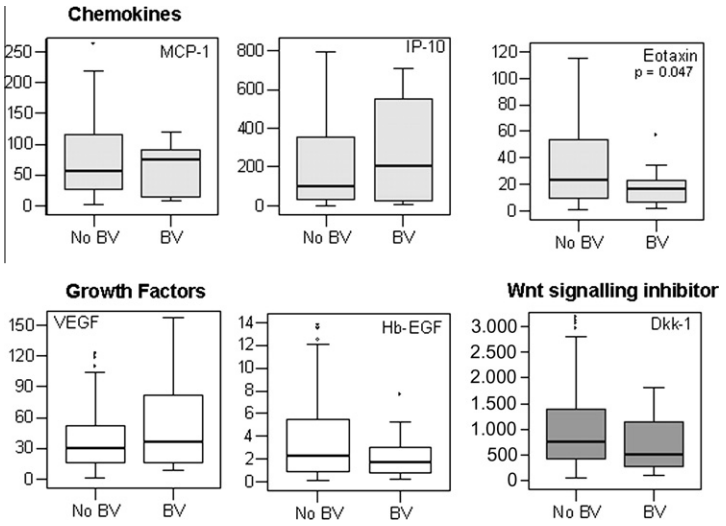


Figure 4 Concentrations of other mediators in women with bacterial vaginosis (BV; Nugent score ≥7, $n = 17$) or without (no BV; normal or intermediate flora, Nugent store <7, $n = 180$). Mediator concentration expressed in pg/mg protein. *P*-value for independent *t*-test of log-transformed concentrations of mediators in women with or without BV was only significant for eotaxin ($P = 0.047$). Dkk, Dickkopf homologue; Hb-EGF, heparin-binding epidermal growth factor; IP-10, interferon-γ-inducible 10 kD protein; MCP, monocyte chemotactic protein; VEGF, vascular endothelial growth factor.

The above-mentioned studies used a vaginal smear, whereas this study assessed a cervical smear since colonization of the cervix rather than the vagina was considered more likely to increase the risk of intrauterine disturbances. The power calculation used in this study assumed a higher incidence of BV than observed. With an incidence of 8.6% BV, 197 patients would still allow us to identify a significant difference in the ratio of TNFα and IL-10 of 0.7 SD (Cohen's d).

Studies have shown associations between BV and cytokine concentrations in vaginal fluid or cervical mucus. As yet, the influence on the intrauterine milieu has not been assessed. In women with BV, higher concentrations of endotoxins in both vaginal fluid and cervical mucus have been observed (Larsson et al., 1991), which can induce the production of pro-inflammatory cytokines, such as TNFα and IL-1β (Romero et al., 1989, 1991), and also anti-inflammatory cytokines, such as IL-5 and IL-10 (Masuda et al., 2002). Cervical mucus from women with BV containing endooxins was shown to induce IL-6 expression in monocytic cells *in vitro* (Sturm-Ramirez et al., 2000). *In vivo*, the concentrations of IL-1β (Cauci et al., 2003; Hedges et al., 2006; Larsson et al., 1991) and IL-1α (Platz-Christensen et al., 1993) are reported to be significantly higher in vaginal fluid from women with BV, whereas no differences in IL-8 (Cauci et al., 2003; Hedges et al., 2006), TNFα and IL-6 (Hedges et al., 2006) were observed. In cervical mucus rather than vaginal fluid, BV has also been shown to be correlated to a higher concentration of IL-1β (Mattsby-Baltzer et al., 1998; Spandorfer et al., 2001; Sturm-Ramirez et al., 2000), IL-1α (Platz-Christensen et al., 1993), but also to IL-8 (Spandorfer et al., 2001) and TNFα (Sturm-Ramirez et al., 2000).

The previously reported positive association between BV and the concentration of IL-1β in cervical mucus as well as vaginal fluid (Cauci et al., 2002, 2003; Hedges et al., 2006; Mattsby-Baltzer et al., 1998; Romero et al., 1989; Spandorfer et al., 2001; Sturm-Ramirez et al., 2000) is consistent with the present findings of an increased concentration of IL-1β in endometrial secretions from women with BV. A significantly negative association was also observed between eotaxin and the presence of BV. Eotaxin was initially identified as a specific chemoattractant protein for eosinophils. Eosinophils are considered the main effector cells in allergic responses and asthma. These eosinophils have been shown to accumulate in the endometrium just prior to and during menstruation (Jeziorska et al., 1995) and may therefore also have a role in endometrial tissue remodelling.

Whether or not BV coincides with an intrauterine pro-inflammatory cytokine profile is of interest since this may be detrimental to endometrial receptivity. However, other mechanisms by which BV may impair pregnancy rates, such as introduction of bacteria into the uterus at embryo transfer or diminished oocyte fertilization rates due to contaminated follicular fluid (Deb et al., 2004; Nagata et al., 1993), cannot be excluded. We have determined the concentration of a series of prominent pro- and anti-inflammatory cytokines and are confident that this gives us a reliable idea of the pro- and anti-inflammatory milieu. However, it is possible that other cytokines that were not included in the test panel contribute to the inflammatory environment in the endometrium. For example, a number of putative mediators of implantation which may be modulated by inflammation were not included either because appropriate antibodies were not available or because of cross-interference between specific antibodies, such as IL-11 and LIF. However, this study has been able to show that BV does not significantly affect the intrauterine cytokine profile, since only marginal differences towards a pro-inflammatory milieu were observed. Therefore, it can be concluded that the impact of BV on endometrial receptivity is likely to be minor. Screening for BV in women with implantation failure is not recommended on the basis of these results.

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