Risks associated with bacterial vaginosis in infertility patients: a systematic review and meta-analysis

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STUDY QUESTION: Is bacterial vaginosis (BV) associated with the cause of infertility and does BV impinge on conception rates and early pregnancy loss following IVF?

SUMMARY ANSWER: The incidence of BV is significantly higher among patients with tubal infertility when compared with patients with non-tubal infertility. BV does not impinge on conception rates but is significantly associated with preclinical pregnancy loss, though not with first trimester abortion.

WHAT IS KNOWN ALREADY: BV is prevalent in patients with infertility, as evident from studies across the world.

STUDY DESIGN, SIZE, DURATION: This study is a meta-analysis of data on the prevalence of BV in women with infertility, the association between BV and the cause of infertility, and the associations between BV and conception rates and early pregnancy loss following IVF. The meta-analyses of the various topics involved different numbers of studies: prevalence of BV with infertility—12 studies, association with tubal infertility—3 studies and associations with conception rates—6 studies, with early preclinical pregnancy loss—2 studies and with clinical pregnancy loss—4 studies.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Systematic literature searches of the electronic databases, PubMed, EMBASE, CINAHL, the Cochrane Library and ISI Web of Knowledge were performed up to September 2012. Studies were included if they reported on, at least, one of the following: prevalence of BV in infertility patients, association between BV and the cause of infertility, association between BV and conception rates with IVF or association between BV and early pregnancy loss. Studies were considered eligible if BV was diagnosed through standardized criteria like Nugent’s criteria or Hay-Ison’s criteria. In none of the studies, infertility as such was defined, but patients were described as unselected patients undergoing IVF.

MAIN RESULTS AND THE ROLE OF CHANCE: The estimated prevalence of BV (Nugent score > 6) in infertile women is 19% [95% confidence interval (CI): 14–25%]. Abnormal microflora including BV and intermediate microflora (Nugent scores 4–10) occurs in 39% of the infertile patients (95% CI: 26–52%). BV is significantly more prevalent in women with infertility compared with antenatal women in the same population [OR (odds ratio) 3.32, 95% CI 1.53–7.20].

BV is significantly more prevalent in women with tubal infertility compared with women with other causes of infertility (OR 2.77, 95% CI 1.62–4.75). BV is not associated with decreased conception rates (OR 1.03, 95% CI 0.79–1.33). Similarly, none of the studies found an association between abnormal vaginal flora and conception rates following IVF treatment.

BV is associated with a significantly elevated risk of preclinical pregnancy loss (OR 2.36, 95% CI: 1.24–4.51). BV is not associated with an increased risk of first trimester miscarriage (OR 1.20, 95% CI: 0.53–2.75).

LIMITATIONS, REASONS FOR CAUTION: All included studies were centre based. In addition, publication bias cannot be ruled out. Furthermore, all estimates are obtained using an absolute minimum of studies. The standard error on the estimates is so large that it does not allow for any formal statistical conclusions regarding heterogeneity between the effects reported in different studies.

WIDER IMPLICATIONS OF THE FINDINGS: It needs to be recognized that most inferences drawn in our study rely on a limited number of studies, potentially, endangering the generalizability of our findings. Moreover, all studies on cause of infertility in relation to BV included had a cross-sectional design and, therefore, do not allow for causal inferences. Still, there is strong circumstantial evidence that
Introduction

Under ideal circumstances, the vagina is populated by indigenous lactobacilli that constitute the normal vaginal microflora. Perturbation of the vaginal niche often occurs, however, most often leading to a dysbiosis called bacterial vaginosis (BV). BV is associated with a depletion of the normally predominant lactobacilli, while a variety of anaerobes display polymicrobial overgrowth as a biofilm covering the vaginal epithelium (Swidsinski et al., 2005). BV is worldwide the most common cause of vaginal discharge, but the condition remains asymptomatic in, at least, half of the cases. A vast body of evidence shows, however, that the pathogenic effects of BV are not confined to the lower genital tract. In particular, BV is strongly associated with reproductive failure, notably late fetal loss and preterm birth (Leitich et al., 2003; Leitich and Kiss, 2007). Several studies have shown that BV is particularly prevalent in patients with infertility (Ralph et al., 1999; Wilson et al., 2002), though it has not been firmly established what risks infertility patients with BV incur for pregnancy outcome.

Through a systematic literature review and meta-analysis, we aimed to assess the prevalence of BV in infertility patients, as well as to quantify the magnitude of the association between BV and the cause of infertility on the one hand, and conception rates and early pregnancy loss following IVF on the other hand.

Materials and Methods

Literature search methodology

We aimed to identify studies that reported on the prevalence of BV in infertility patients, as well as studies that assessed a putative association between BV and the cause of infertility, between BV and conception rates after IVF, and between BV and early pregnancy loss.

A systematic literature search using the MeSH terms, BV, Gardnerella vaginalis, vaginal flora and subfertility, infertility, subfertile, infertile and IVF was performed in MEDLINE (1966 through September 2012). No language restrictions were applied. Additional searches in EMBASE (1974 to September 2012), CINAHL (1981 to September 2012), the Cochrane Library (1970 to November September 2012) and ISI Web of Knowledge (1955 to September 2012) did not yield any additional relevant papers. Cross references were also checked for relevance by hand searching.

Study selection

Studies were included if they reported on, at least, one of the following: prevalence of BV in infertility patients, association between BV and cause of infertility, association between BV and conception rates with IVF or association between BV and early pregnancy loss. Studies were considered eligible if BV was diagnosed through standardized criteria like Nugent’s criteria (Nugent et al., 1991), Hay-Ison’s criteria (Ison and Hay, 2002) or Amsel’s criteria (Amsel et al., 1983). The study identification and selection process is presented as a PRISMA flowchart (Fig. 1).

Data extraction

The selected studies were assessed for methodological quality by applying the MOOSE guidelines for non-randomized studies (Stroup et al., 2000). Raw data were extracted to allow for the calculation of events per woman rather than per cycle.

Statistical analysis

The calculations were carried out using the metafor package (Viechtbauer, 2010) within the statistical programming environment R (R Core Team, 2012). Average proportions of combined prevalence and related confidence intervals (CIs) were calculated by fitting a random effects model using restricted maximum likelihood in order to obtain unbiased estimates of the average proportion and the variance thereof (Viechtbauer, 2005). In the prevalence study, the influence of each study on the total result is evaluated by using a leave-one-out approach and calculating the Cook’s distance for each study (Viechtbauer, 2010).

For each research question, a meta-analysis was carried out by fitting a random effects model using restricted maximum likelihood. In this approach, studies are weighted proportional to the inverse of the unconditional variance (Hedges, 1983). Heterogeneity is reported in the form of the between-study variance ($\tau^2$) and the standard error on this estimate. Also, the proportion of the between-study variance in the total variance ($I^2$) is reported. In case any of the cells in the $2 \times 2$ table for a particular study contained a zero count, 0.5 was added to all cells of that particular table in order to be able to calculate the odds ratio (OR).

Results

Prevalence of BV with infertility

We identified 12 studies, which reported on the prevalence of BV in patients with infertility, totalling 3229 patients (Table I). Five studies originated from the UK, two from the USA, two from Egypt, one from the Republic of Ireland, one from India and one from The Netherlands. In all studies, vaginal microflora status had been assessed through use of the Nugent’s criteria. One study specifically dealt with patients with idiopathic infertility (Aboul Enien et al., 2005). The remainder of studies described unselected patients recruited at IVF centres, regardless of cause of infertility, totalling 3189 patients. Based on these studies, the estimated prevalence of BV (Nugent score $\geq 5$) in fertile women is 19% [95% confidence interval (CI): 14–25%]. Abnormal microflora including BV and intermediate microflora (Nugent scores 4–10) occurs in 39% of the infertile patients (95% CI: 26–52%). Compared with the other studies, the prevalence of intermediate microflora (Nugent scores 4–6) is higher in the study of Selim et al. (2011). Influence analysis shows that the study of Selim et al. has a large influence on the calculation of the prevalence of all
abnormal flora in infertility patients (Cook’s distance = 0.88). If we leave the study of Selim et al. out, the prevalence of all abnormal microflora becomes 33% (95% CI: 26–39%).

Two studies compared the prevalence of BV between women with infertility and pregnant women attending the affiliated antenatal clinic (Morgan et al., 1997; Mania-Pramanik et al., 2009). The between-study variance was non-significant (\( \tau^2 = 0.20, \ SE = 0.47, I^2 = 60\% \)). The estimated combined OR was 3.32 and significantly different from 1 (\( P < 0.01 \)). In Fig. 2, the individual ORs and the combined estimate are reported together with their 95% CIs. Aboul Enien et al. (2005) also found a significantly higher risk of BV in women with unexplained infertility compared with antenatal patients.

### Table I Prevalence of BV and abnormal vaginal microflora in women with infertility.

<table>
<thead>
<tr>
<th>Study name</th>
<th>Origin of study</th>
<th>BV prevalence</th>
<th>Prevalence of all abnormal microflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>McCaffrey et al. (1997)</td>
<td>Dublin, Ireland</td>
<td>10.0% (12/120)</td>
<td>25.0% (30/120)</td>
</tr>
<tr>
<td>Morgan et al. (1997)</td>
<td>London, UK</td>
<td>18.5% (37/199)</td>
<td>24.0% (48/199)</td>
</tr>
<tr>
<td>Ralph et al. (1999)</td>
<td>Leeds, UK</td>
<td>24.6% (190/771)</td>
<td>36.0% (278/771)</td>
</tr>
<tr>
<td>Livermore et al. (1999)</td>
<td>Bristol, UK</td>
<td>25.6% (77/301)</td>
<td>39.6% (119/301)</td>
</tr>
<tr>
<td>Gaudoin et al. (1999)</td>
<td>Glasgow, UK</td>
<td>16.3% (40/246)</td>
<td>—</td>
</tr>
<tr>
<td>Spandorfer et al. (2001)</td>
<td>New York, NY, USA</td>
<td>4.2% (14/331)</td>
<td>19.3% (64/331)</td>
</tr>
<tr>
<td>Wilson et al. (2002)</td>
<td>Leeds, UK</td>
<td>24.3% (182/749)</td>
<td>36.4% (273/749)</td>
</tr>
<tr>
<td>Eckert et al. (2003)</td>
<td>Seattle, WA, USA</td>
<td>11.0% (10/91)</td>
<td>45.0% (41/91)</td>
</tr>
<tr>
<td>Aboul Enien et al. (2005)</td>
<td>Alexandria, Egypt</td>
<td>25.0% (10/40)</td>
<td>—</td>
</tr>
<tr>
<td>Mania-Pramanik et al. (2009)</td>
<td>Mumbai, India</td>
<td>25.9% (29/112)</td>
<td>—</td>
</tr>
<tr>
<td>Boomsma et al. (2010)</td>
<td>Utrecht, The Netherlands</td>
<td>8.6% (17/198)</td>
<td>30.3% (60/198)</td>
</tr>
<tr>
<td>Selim et al. (2011)</td>
<td>Ismailia, Egypt</td>
<td>36.6% (26/71)</td>
<td>83.1% (59/71)</td>
</tr>
</tbody>
</table>
Association of BV with cause of infertility

Three studies compared the prevalence of BV in women with tubal infertility versus women with non-tubal infertility (Liversedge et al., 1999; Gaudoin et al., 1999; Wilson et al., 2002). Non-tubal infertility comprised all types infertility including male infertility in two studies (Gaudoin et al., 1999; Wilson et al., 2002), and only female causes in a third study (Liversedge et al., 1999). The between-study variance was non-significant ($\tau^2 = 0.14$, SE = 0.23, $I^2 = 63\%$). The estimated combined OR was 2.77 (1.62–4.75) and significantly different from 1 ($P < 0.001$). In Fig. 3, the individual ORs and the combined estimate are reported together with their 95% CIs. One study also found an elevated risk of BV in patients with anovulation (Wilson et al., 2002), and one study documented a significantly higher odds for unexplained infertility associated with BV compared with known causes of infertility (Spandorfer et al., 2001).

Conception rates in infertility patients with BV

Conception rates in infertility patients undergoing IVF in association with disturbed vaginal microflora were reported in seven studies (McCaffrey et al., 1997; Gaudoin et al., 1999; Liversedge et al., 1999; Ralph et al., 1999; Spandorfer et al., 2001; Eckert et al., 2003; Selim et al., 2011). Six studies (Gaudoin et al., 1999; Liversedge et al., 1999; Ralph et al., 1999; McCaffrey et al., 1997; Eckert et al., 2003; Selim et al., 2011) specifically allowed the calculation of conception rates in association with BV as displayed in Fig. 4. None of the studies found a difference in conception rates following IVF in infertility patients with BV when compared with those with normal microflora. For all studies, conception rates have been calculated as a function of biochemical pregnancy, except in one study (Liversedge et al., 1999) where the conception rate was calculated as a function of clinical pregnancy. The between-study variance was virtually non-existent ($\tau^2 = 0$, SE = 0.07, $I^2 = 0\%$). The meta-analysis could not confirm an association between the conception rate and BV ($OR = 1.03$, 0.79–1.33). Similarly, none of the studies found an association between abnormal vaginal flora and conception rates following IVF treatment (data not shown).

Risk of early pregnancy loss in infertility patients with BV

The risk of early pregnancy loss (<13 weeks) after established pregnancy following IVF treatment in infertility patients with BV is reported in five studies (Liversedge et al., 1999; Ralph et al., 1999; Spandorfer et al., 2001; Eckert et al., 2003; Selim et al., 2011). One large study found a significantly elevated risk of early pregnancy loss, even after control for several confounders, though the risk was largely confined to preclinical pregnancy loss (Ralph et al., 1999). A much smaller study that also accounted for preclinical pregnancy loss found a similar trend (Eckert et al., 2003). The combined OR for both studies was estimated to be 2.36 (95% CI: 1.24–4.51), indicating a significant association between BV and early, preclinical pregnancy loss ($P < 0.01$; Fig. 5). Three smaller studies specifically reported on clinical pregnancy losses and failed to document an association between first trimester miscarriage and BV (Liversedge et al., 1999; Spandorfer et al., 2001; Selim et al., 2011). Meta-analysis on all data on the association...
infertility patients has BV and, at least, one in three has a disturbed natal patients from the reference population. An estimated one in five significantly more common in infertility patients compared with ante-

microbiota, in general, are commonly associated with infertility and in infertility patients associated with BV, we found, in the present sys-

Discussion

Albeit that very few studies have addressed reproductive outcome between first trimester abortion and BV yielded an OR of 1.20 (95% CI: 0.52–2.74, \( P = 0.65 \)). The study variance is non-significant (\( \tau^2 = 0 \), \( SE = 0.56, I^2 = 0% \)). Note that in the study of Spandorfer et al. none of the women with BV lost their child in the first trimester. Hence, all cells in the 2 \times 2 table were augmented with 0.5 in order to be able to calculate the OR (Fig. 6).

between cause of infertility and BV had a cross-sectional design, and hence do not allow for causal inferences, there is still strong evidence supporting a role for BV in the aetiology of tubal infertility.

It may be acknowledged that the risks reported will have a differential population impact depending on the BV prevalence in the background population. In particular, from population-based studies, it is evident that BV rates are highest in the USA (Koumans et al., 2007) and in Sub-Saharan Africa (Chico et al., 2012), with much lower rates being recorded in Europe. At the same time, it may be recognized that we found high rates of BV with infertility patients from a wide range of countries across the world. None of the studies actually defined infertility, but rather referred to patients attending infertility clinics. Clearly, this may have biased our results, as women attending infertility clinics may not be representative of the infertile population as such, and certainly represent only a subset of women in infertile couples. Similarly, bias may originate from between-centre variation in the determination of infertility cause.

Although all studies included for systematic review of the relation between cause of infertility and BV had a cross-sectional design, and hence do not allow for causal inferences, there is still strong evidence supporting a role for BV in the aetiology of tubal infertility. In a recent prospective cohort study, Wiesenfeld et al. (2012) actually documented that a proportion of female infertility is attributable to subclinical pelvic inflammatory disease (PID), since subclinical PID decreases subsequent fertility even after provision of treatment for sexually transmitted diseases (STDs) and BV. Though the studies in this systematic review all measured incident BV, it is well established that BV has a high tendency to recur, and, therefore, incident BV may serve as a marker of past BV, which is also a risk factor for STD acquisition (Brotman, 2011). Of note, is that in the largest study on the subject, the risk of tubal infertility with BV persisted after controlling for several potential confounding variables, including age and smoking (Wilson et al., 2002), further giving support to a causal relationship between BV and tubal infertility. Unexpectedly, this study also found an elevated risk of BV in patients with anovulation (Wilson et al., 2002), and one study documented a significantly higher odds for unexplained infertility associated with BV compared with known causes of infertility (Spandorfer et al., 2001).

It is certainly reassuring that studies consistently found that BV is not associated with decreased conception rates, with virtually no heterogeneity across studies. BV, thus, does not seem to interfere with the vaginal microbiota. It could further be documented that the risk of BV is significantly higher among patients with tubal infertility when compared with patients with non-tubal infertility. Studies consistently show that BV does not impinge on conception rates, as confirmed by meta-analysis. Finally, BV is significantly associated with preclinical pregnancy loss, though not with first trimester miscarriage. Hence, the current findings suggest that BV may have a role in both the aetiology and pregnancy outcomes of infertility patients.

All included studies were centre based but of good quality, though we do acknowledge that we had limited selection criteria. In addition, publication bias cannot be ruled out. Furthermore, all estimates are obtained using an absolute minimum of studies. Although the REML estimator of the between-study variance \( \tau^2 \) is unbiased (Viechtbauer, 2005), the standard error on the estimates is so large that it does not allow for any formal statistical conclusions regarding heterogeneity between the effects reported in different studies. Furthermore, it needs to be recognized that most inferences drawn in our study rely on a limited number of studies, potentially endangering the generalizability of our findings.

It may be acknowledged that the risks reported will have a differential population impact depending on the BV prevalence in the background population. In particular, from population-based studies, it is evident that BV rates are highest in the USA (Koumans et al., 2007) and in Sub-Saharan Africa (Chico et al., 2012), with much lower rates being recorded in Europe. At the same time, it may be recognized that we found high rates of BV with infertility patients from a wide range of countries across the world. None of the studies actually defined infertility, but rather referred to patients attending infertility clinics. Clearly, this may have biased our results, as women attending infertility clinics may not be representative of the infertile population as such, and certainly represent only a subset of women in infertile couples. Similarly, bias may originate from between-centre variation in the determination of infertility cause.
Implantation process. In contrast, we did find that BV predisposes to early preclinical pregnancy loss following IVF. The latter association was first documented in a large and very well-designed study (Ralph et al., 1999). The authors of the former study point to several arguments supporting a causal relationship between BV and preclinical early pregnancy loss, including the control for a number of potential confounding variables.

Unfortunately, no study looked beyond first trimester fetal loss, although it is plausible that the high preterm birth rates observed after IVF are, at least, in part attributable to BV (Wilson et al., 1999).

Taken together the findings obtained from this systematic review further highlight the tremendous burden of disease, that is, associated with bacterial vaginosis and justify intensified research efforts to develop novel and long-term effective treatment strategies. Further research into the association between the vaginal microbiome and IVF outcome is also warranted, as it was recently shown that the vaginal microbiome on the day of embryo transfer affects pregnancy outcome (Hyman et al., 2012).

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Authors’ roles
H.V. conceived of the study; N.V.O. and H.V. performed the literature search and the data extraction; J.M. performed all statistical analyses; N.V.O., H.V., J.M. and P.D.S. drafted the manuscript.

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Conflict of interest
None declared.

References

