Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study

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Abstract

Objectives To assess whether bacterial vaginosis affects the rates of conception and miscarriage in the first trimester.

Design Cohort study.

Setting Assisted conception unit of a teaching hospital in Leeds.

Participants 867 consecutive women undergoing in vitro fertilisation.

Interventions Screening for bacterial vaginosis with a Gram stained vaginal smear before egg collection.

Main outcome measures The presence of bacterial vaginosis or normal vaginal flora, and the rate of conception and miscarriage in the first trimester.

Results 190 of 771 (24.6%) women had bacterial vaginosis. No difference in conception rate was found between those women with bacterial vaginosis and those with normal vaginal flora: 61 women (32.1%) and 146 of 493 women (29.6%) respectively (relative risk 1.08, 95% confidence interval 0.85 to 1.39; odds ratio 1.12, 0.77 to 1.64). However, 22 women (31.6%) with bacterial vaginosis who conceived had a significantly increased risk of miscarriage in the first trimester compared with 27 women (18.5%) with normal vaginal flora (crude relative risk 1.95, 1.11 to 3.42; crude odds ratio 2.49, 1.21 to 5.12). This increased risk remained significant after adjustment for factors known to increase the rate of miscarriage: increasing maternal age, smoking, history of three or more miscarriages, no previous live birth, and polycystic ovaries (adjusted relative risk 2.03, 1.09 to 3.78; adjusted odds ratio 2.67, 1.26 to 5.63).

Conclusions Bacterial vaginosis does not affect conception but is associated with an increased risk of miscarriage in the first trimester in women undergoing in vitro fertilisation, independent of other risk factors.

Introduction

Bacterial vaginosis is the most common cause of abnormal vaginal discharge among women of...
childbearing age. Prevalence rates of 13% to 31% have been reported in pregnant women.\textsuperscript{1–5}

Bacterial vaginosis develops when normal vaginal lactobacilli are replaced by an overgrowth of \textit{Gardnerella vaginalis}, anaerobes, and mycoplasmas. Factors responsible for this change are not fully understood. Bacterial vaginosis in pregnancy predisposes to an increased risk of late miscarriage,\textsuperscript{1–4} preterm labour,\textsuperscript{1–5} postpartum endometritis,\textsuperscript{6} and low birthweight infants.\textsuperscript{3} The reported odds ratios for preterm birth were between 6.9 and 9.1\textsuperscript{2,3} 15 the highest relative risks. Bacterial vaginosis was diagnosed between 16 and 20 weeks of gestation. A vaginal sample was cultured for \textit{Neisseria gonorrhoeae} (in-house culture medium), and endocervical \textit{G vaginalis} with a sterile cotton swab. The swab was smeared on to a glass slide then air dried and Gram stained. The women were simultaneously screened for sexually transmitted infections that can predispose to miscarriage. A vaginal sample was cultured for \textit{Trichomonas vaginalis} (in-house culture medium), and endocervical samples were tested for \textit{Neisseria gonorrhoeae} (GC Agar Base, Unipath, Basingstoke) and \textit{Chlamydia trachomatis} (enzyme immunoassay; Micro Trak, Behring Diagnostics, Milton Keynes).

The slides were read by two observers (SGR and JDW), blinded to each other's results and to pregnancy outcome. Modified Spiegel's criteria were used to diagnose bacterial vaginosis on Gram stain, as described by Hay et al.\textsuperscript{2} Thus, we graded flora as normal (predominantly lactobacilli), intermediate (reduced lactobacilli mixed with other morphotypes), and bacterial vaginosisis (few or absent lactobacilli with greatly increased numbers of \textit{G vaginalis}, other morphotypes, or both).

When the observers disagreed, the slide was reviewed until consensus was reached.

\textbf{Egg collection}

The vagina was cleansed with normal saline, and eggs were collected transvaginally under ultrasound guidance. Forty eight hours after egg collection, the vagina was washed with normal saline, and up to three embryos were placed into the uterus transcervically using a Wallace catheter (SIMS Portex, Hythe). The luteal phase was supported with 400 mg progesterone daily through vaginal pessaries.

\textbf{Pregnancy confirmation}

Pregnancy was confirmed 14 days after egg collection by measuring serum concentrations of \(\beta\) human chorionic gonadotropin (Delia, EG and G Wallace, Milton Keynes). This pregnancy test has a detection limit of 2 U/l of \(\beta\) human chorionic gonadotropin: we considered concentrations greater than 10 U/l as a positive result. When the concentration was less than 10 U/l we repeated the test 2 days later, and if the concentration had doubled, we considered this a positive result. These concentrations give a specificity of 100% for pregnancy.\textsuperscript{7} Follow up ultrasound at 6 and 8 weeks of gestation confirmed clinical pregnancy. We followed all the women through to completion of the pregnancy.

\textbf{Pregnancy loss}

We subdivided pregnancy loss before 13 weeks of gestation into (a) preclinical pregnancy (a positive pregnancy test followed by spontaneous abortion before 6 weeks of gestation, before confirmation of the pregnancy by ultrasonography), (b) anembryonic pregnancy (confirmation of a gestational sac by ultrasonography at 6 weeks of gestation but no fetus identified), (c) missed abortion (confirmation of a gestational sac and fetus by ultrasonography but no fetal heart visible), and (d) spontaneous abortion (confirmation of a viable fetus by ultrasonography, which aborts before 13 weeks of gestation).

\textbf{Statistical methods}

We used relative risk and odds ratio to compare the rates of conception and miscarriage in the first trimester among women with bacterial vaginosis and those with normal vaginal flora, and to assess the univariate risk of miscarriage due to other known factors. We investigated any association between bacterial vaginosis and these factors with Wilcoxon 2 sample, \(\chi^2\), or Fisher's exact tests. We included all the factors in a model to estimate by relative risk the probability of early miscarriage, and we used logistic regression to assess the significance of the factors by odds ratio, having adjusted for the other variables.
Results

Between April 1996 and June 1997 we recruited 867 consecutive women undergoing in vitro fertilisation. We excluded 96 women (11.1%); 75 (8.7%) failed to reach the embryo transfer stage, and in 20 cases (2.3%) slides were incorrectly labelled or broken. One woman had an ectopic pregnancy, which was surgically removed. No significant differences were found between the baseline data of those excluded and the study cohort.

Overall, 792 of the women (94.9%) were white, the median age was 33 years, and 139 (18.3%) were smokers. Table 1 outlines the previous obstetric history of the study cohort. As no sexually transmitted infections were detected in the first 200 women we recruited, we stopped screening.

The vaginal slides were graded as normal in 493 women (63.9%), intermediate in 88 (11.4%), and bacterial vaginosis in 190 (24.6%). Fifty five slides (7.1%) required review before a consensus was reached. Overall, 237 of the women (30.7%) conceived: 146 women (29.6%) with normal vaginal flora, 30 (34%) with intermediate vaginal flora, and 61 (32.1%) with bacterial vaginosis. No difference in conception rate was found between the women with bacterial vaginosis and those with normal vaginal flora (relative risk 1.08, 0.85 to 1.39; odds ratio 1.12, 0.77 to 1.64).

Discussion

Our study is the first to describe a definite association between bacterial vaginosis and miscarriage in the first trimester, and it suggests that the pathological processes of bacterial vaginosis may begin early in pregnancy. Previously published studies could not have reached this conclusion as these screened for bacterial vaginosis at 7-8 weeks of gestation at the earliest. In our study most of the miscarriages in the first trimester had already occurred by then.

We believe these results are genuine for several reasons. Firstly, the in vitro fertilisation procedures were identical in both groups of women. Secondly, we eliminated other infective causes of miscarriage. Thirdly, the association between bacterial vaginosis...
and miscarriage in the first trimester persisted after adjusting for other variables known to increase the risk of miscarriage—namely, smoking, older age, three or more previous miscarriages, polycystic ovary syndrome, and no previous live birth. Fourthly, an increasing miscarriage rate was seen with increasing abnormality of the vaginal flora: 18.5% in women with normal vaginal flora, 23.3% in women with intermediate vaginal flora, and 36.1% in women with bacterial vaginosis, as would be expected with a cause and effect relation. We did not include the intermediate group in the analysis because Hillier et al9 showed that intermediate vaginal flora can be a transitional phase between normal vaginal flora and bacterial vaginosis in pregnant women, with 32% of women developing bacterial vaginosis and 30% reverting to normal vaginal flora within 13 weeks. Fifthly, the overall miscarriage rate of 23.6% is in keeping with other studies of early pregnancy loss. Wilcox et al10 performed daily urinary assays for h human chorionic gonadotro- pin in couples trying to conceive. The total pregnancy loss was 31%: preclinical loss in 22% of women and clinically recognised miscarriages in 9%. Within our miscarriage rate of 23.6%, however, the proportion of women with bacterial vaginosis who miscarried was significantly higher than that of the women with normal vaginal flora, the increased risk being equivalent to one extra miscarriage for every six pregnant women with bacterial vaginosis.

The pathogenesis of miscarriages associated with bacterial vaginosis is not known. Most of the miscarriages in our study were preclinical, which represents a failure of implantation or early embryonic development. The increased risk of preclinical pregnancy was higher than the overall risk of a miscarriage in the first trimester (crude relative risk 2.69 and 1.95 respectively). Korn et al11 found plasma cell endometritis in 45.5% of women who had bacterial vaginosis but no other vaginal or cervical infections and no symptoms or signs of upper genital tract infection. If endometritis was present with bacterial vaginosis before in vitro fertilisation, this could impair implantation or early embryonic development.

An unavoidable component of in vitro fertilisation involves breaching the cervix with a catheter at the time of embryo transfer, thereby potentially assisting vertical spread of vaginal bacteria to the upper genital tract. Pelvic inflammatory disease after egg collection is, however, rare with a reported incidence of 0.2% to 0.5% per cycle12–14 and is mainly associated with accidental puncture of bowel or hydrosalphinx. Pelvic infection after embryo transfer is even rarer, with one reported case only.15 This therefore seems an unlikely cause of the miscarriages.

We specifically chose women undergoing in vitro fertilisation for two reasons: the exact time of conception was known, and a vaginal sample could be taken at this time. Such accurate information would be difficult to obtain in the naturally conceiving population. Whether these results apply to those conceiving naturally depends on the underlying cause of the miscarriages. If miscarriages were due to pre-existing endometritides these results would apply to all women trying to conceive either naturally or by assisted conception techniques. If instrumentation was responsible, an increase in miscarriage rate in the first trimester would be unlikely among the naturally conceiving population.

Key messages

- Bacterial vaginosis does not affect conception rate
- Bacterial vaginosis is associated with a two-fold risk of miscarriage in the first trimester
- The increased miscarriage rate is equivalent to one extra miscarriage for every six pregnant women with bacterial vaginosis
- The most likely cause of the miscarriages is due to pre-existing endometritides affecting implantation or early embryonic development, which could also affect naturally conceived pregnancies

Finally, infertility and in vitro fertilisation place a financial burden on the health service and a great personal cost on those affected. An intervention study is therefore required to assess whether treatment of bacterial vaginosis reduces the rate of associated miscarriage. As the diagnosis and treatment of bacterial vaginosis are simple and cheap, only a small improvement in pregnancy outcome would be required to be economically beneficial.

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Contributors: JDW conceived and designed the study, SGR coordinated the study and collected the baseline and outcome data. AJ Rutherford recruited and clinically cared for the participants, JDW and SGR interpreted the vaginal slides and analysed the data. SGR, AJR, and JDW wrote the paper. JDW will act as guarantor for the paper.

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