

design and supervision of project. JH participated in development of study design, methods, and questionnaire for European study, management and screening of European population, and data coding. SP participated in screening South Asians, community liaison and recruitment to study, and data coding. NA contributed to development of South Asian questionnaire, translation, sampling, screening, recruitment, and data preparation. NU, MW, JY, LW, KGMM, JH, SP, and NA commented on the manuscript. CT participated in screening, development of questionnaire, recruitment, and community liaison. BW managed and participated in screening for European and South Asian samples and helped with entry and preparation of data for analysis. DK participated in coordination of recruitment and data collection for the South Asian study, data analysis on response rates, and drafting text. AK participated in screening, development of questionnaire, community liaison, and recruitment. ML advised on biochemical methods, supervised laboratories doing biochemical tests, and had responsibility for Lp(a) lipoprotein assays. AT participated in the Lp(a) lipoprotein study and provided data and advice on Lp(a) lipoprotein. RB, GA, NU, and MW are the study guarantors.

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## 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.<sup>1-5</sup>

Bacterial vaginosis develops when normal vaginal lactobacilli are replaced by an overgrowth of *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,<sup>3,4</sup> preterm labour,<sup>1-5</sup> postpartum endometritis,<sup>6</sup> and low birthweight infants.<sup>5</sup> The reported odds ratios for preterm births were between 1.4 and 6.9,<sup>1-5</sup> the highest rates being when bacterial vaginosis was identified before 16 weeks of gestation.<sup>1,3,4</sup> Riduan et al screened for bacterial vaginosis in pregnant women in both the mid and third trimester.<sup>2</sup> The authors found a significant risk of preterm birth when bacterial vaginosis was diagnosed between 16 and 20 weeks of gestation, but not if bacterial vaginosis developed just in the third trimester. The highest relative risk of preterm birth was reported at 6.9 by Kurki et al.<sup>1</sup> All the women were screened between 8 and 17 weeks of gestation, which is earlier than in other studies. Hay et al<sup>3</sup> and McGregor et al<sup>4</sup> found an increased relative risk of 3.9 and 3.1 for miscarriage in the mid trimester, when bacterial vaginosis was diagnosed before 16 weeks of gestation. The findings of these studies support a pathological role for bacterial vaginosis in the mid rather than third trimester, as was previously thought. Consequently, bacterial vaginosis may have a detrimental effect on the outcome of the first trimester, yet there are no published data on bacterial vaginosis in early pregnancy.

We aimed to assess whether bacterial vaginosis affects the rate of conception and miscarriage in the first trimester. We chose to study a cohort of women undergoing in vitro fertilisation, for two reasons: the exact date of conception was known, and vaginal samples could be taken at this time.

## Participants and methods

### Participants

We recruited 867 consecutive women undergoing in vitro fertilisation between April 1996 and June 1997 at the assisted conception unit of a teaching hospital in Leeds. All participants gave their written consent to participate, and ethical approval for our study was obtained from the local ethics committee. The women entered the study on the day of egg collection. We recorded baseline data for age, smoking habit, ethnic origin, previous obstetric history, and reason for infertility.

### Methods

#### *Vaginal samples*

The women were treated with a conventional superovulation regimen of pituitary downregulation followed by stimulation with gonadotropins.<sup>7</sup> Immediately before egg collection, a non-lubricated bivalve speculum was inserted into the vagina, and a sample was taken from the vaginal walls and posterior fornix 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 *Trichomonas vaginalis* (in-house culture medium), and endocervical samples were tested for *Neisseria gonorrhoeae* (GC Agar

Base, Unipath, Basingstoke) and *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.<sup>3</sup> Thus, we graded flora as normal (predominantly lactobacilli), intermediate (reduced lactobacilli mixed with other morphotypes), and bacterial vaginosis (few or absent lactobacilli with greatly increased numbers of *G vaginalis*, other morphotypes, or both). When the observers disagreed, the slide was reviewed until consensus was reached.

#### *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.

#### *Pregnancy confirmation*

Pregnancy was confirmed 14 days after egg collection by measuring serum concentrations of  $\beta$  human chorionic gonadotropin (Delfia, EG and G Wallac, 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.<sup>8</sup> Follow up ultrasound at 6 and 8 weeks of gestation confirmed clinical pregnancy. We followed all the women through to completion of the pregnancy.

#### *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).

### 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.

**Table 1** Previous obstetric history of 771 women undergoing in vitro fertilisation

Obstetric history	No (%) of patients
Never pregnant	439 (57.0)
At least one live birth	128 (16.6)
Miscarriage only	118* (15.3)
Termination only	45 (5.8)
Ectopic pregnancy only	41 (5.3)

\*Includes 16 women with history of  $\geq 3$  miscarriages.

**Table 2** Types of miscarriage occurring in first trimester in two groups of women undergoing in vitro fertilisation

Miscarriage	Bacterial vaginosis (n=22)	Normal vaginal flora (n=27)
Preclinical	18	16
Anembryonic	0	4
Missed abortion	2	4
Spontaneous abortion	2	3

## 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, 732 of the women (94.9%) were white, the median age was 33 years, and 139 (18%) 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).

**Table 3** Risk factors for miscarriage in 771 women undergoing in vitro fertilisation

Risk factor	Odds ratio (95% CI)	Relative risk (95% CI)
Increasing age	1.03* (0.95 to 1.12)	1.01* (0.94 to 1.08)
Smoking	1.05 (0.47 to 2.33)	1.04 (0.52 to 2.08)
Polycystic ovary syndrome	1.68 (0.55 to 5.18)	1.46 (0.58 to 3.67)
$\geq 3$ miscarriages	1.63 (0.14 to 18.31)	1.42 (0.20 to 10.26)
No previous live birth	1.54 (0.70 to 3.41)	1.38 (0.70 to 2.69)

\*For each additional year.

**Table 4** Adjusted relative risks and odds ratios in 771 women undergoing in vitro fertilisation

Risk factor	Relative risk (95% CI)	Odds ratio (95% CI)
Bacterial vaginosis	2.03 (1.09 to 3.78)	2.67 (1.26 to 5.63)
Increasing age	1.02 (0.94 to 1.10)	1.03 (0.94 to 1.12)
Smoking	0.83 (0.38 to 1.78)	0.75 (0.30 to 1.87)
Polycystic ovary syndrome	1.29 (0.47 to 3.52)	1.46 (0.43 to 5.03)
$\geq 3$ miscarriages	1.26 (0.14 to 11.13)	1.45 (0.09 to 22.20)
Previous live birth	1.11 (0.53 to 2.31)	1.17 (0.49 to 2.79)

Of the 237 women who conceived, 56 (23.6%) miscarried during the first 13 weeks of gestation: 27 of 146 women (18.5%) with normal vaginal flora, 7 of 30 women (23.3%) with intermediate vaginal flora, and 22 of 61 women (36.1%) with bacterial vaginosis. A significantly increased risk of miscarriage was found in women with bacterial vaginosis compared with women with normal vaginal flora (1.95, 1.11 to 3.42; 2.49, 1.21 to 5.12).

Table 2 classifies the miscarriages that occurred in the first trimester. No difference was found between the two groups in distribution of timing of the miscarriages: preclinical miscarriages occurred in 18 of 22 women with bacterial vaginosis and 16 of 27 women with normal flora ( $P=0.09$ ,  $\chi^2$ ). A significant difference was found, however, in the proportions of conceptions resulting in preclinical pregnancies: 18 of 61 pregnancies (29.5%) in women with bacterial vaginosis compared with 16 of 146 pregnancies (11.0%) in those with normal vaginal flora (2.69, 1.47 to 4.92; 3.4, 1.5 to 7.8). This risk is greater than that of overall miscarriage in the first trimester in women with bacterial vaginosis.

Increasing maternal age, smoking, polycystic ovary syndrome, history of three or more miscarriages, and no previous live birth all increase the risk of miscarriage in the first trimester. We performed a statistical analysis to assess whether there was any association between these factors and miscarriage. We found no significant associations, although our study was not powered to assess these as risk factors (table 3).

To eliminate an uneven distribution of these factors between the two groups of women, we sought any associations between the factors and bacterial vaginosis. The proportions of women with polycystic ovary syndrome ( $P=0.147$ , Fisher's exact) and three or more previous miscarriages ( $P=1.000$ , Fisher's exact) did not differ between the two groups, but the numbers were small in each category. Distributions of age, smoking habit, and previous live birth, however, were significantly different. Women with bacterial vaginosis were more likely to smoke ( $P=0.001$ ,  $\chi^2$ ) and less likely to have previously given birth ( $P=0.003$ ,  $\chi^2$ ). Women with normal vaginal flora were significantly older than women with bacterial vaginosis ( $P=0.008$ , Wilcoxon 2 sample).

After adjustment for these variables, we found no other factor to be significant (table 4). The increased risk of miscarriage with bacterial vaginosis remained (adjusted relative risk 2.03, 1.09 to 3.78; adjusted odds ratio 2.67, 1.26 to 5.63).

## 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 al<sup>9</sup> 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 al<sup>10</sup> performed daily urinary assays for  $\beta$  human chorionic gonadotropin 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 al<sup>11</sup> 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 cycle<sup>12-14</sup> and is mainly associated with accidental puncture of bowel or hydrosalpinx. Pelvic infection after embryo transfer is even rarer, with one reported case only.<sup>15</sup> 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 endometritis 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 endometritis 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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