Carriage of *Gardnerella vaginalis* and anaerobes in semen

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SUMMARY *Gardnerella vaginalis* was isolated from 22 (38%) of 58 semen samples obtained from men attending an infertility clinic. Counts ranged from 1.2×10^3 to $>10^7$ colony forming units (cfu)/ml. There was no association between the isolation of *G vaginalis* and the sperm count. Twenty (34.4%) samples contained non-sporing anaerobes and nine (15.5%) both anaerobes and *G vaginalis*. The infective dose of *G vaginalis* is not known, but semen could act as a medium for its sexual transmission.

Introduction

Bacterial vaginosis (non-specific vaginitis) is a condition that has been associated with the isolation of *Gardnerella vaginalis*¹ and non-sporing anaerobes.^{2,3} It is not yet clear whether the condition is sexually transmitted or simply associated with sexual activity.

Evidence for sexual transmission has rested on the high prevalence of *G vaginalis* in the urine⁴ and urethra of sexual contacts of infected women.^{1,2,5,6}

If *G vaginalis* (or anaerobes) associated with bacterial vaginosis are sexually transmitted, the likely medium would be semen. We have examined semen for the presence of these organisms to assess its role in the causation and recurrence of bacterial vaginosis.

Patients and methods

PATIENT SELECTION

Semen samples are collected routinely from the male partner of all new couples attending the subfertility clinic at the Samaritan Hospital for Women, London. Samples were tested within four hours of collection for: pH (using narrow range pH paper, British Drug Houses, London, UK), total sperm count, and the presence of "clue" cells (wet and Gram stained preparations), *G vaginalis*, and anaerobes. The viscosity of semen and the presence of abnormal forms and motility of sperms were

investigated but not recorded for the purpose of this study.

ISOLATION AND IDENTIFICATION OF BACTERIA *G vaginalis* was isolated on bilayer human blood agar made selective by adding gentamicin, nalidixic acid, and amphotericin B.⁷ Numbers of *G vaginalis* were estimated by inoculating 10 µl of tenfold dilutions of semen on the same medium without added antibiotics. All media were incubated at 37°C for 48 hours in 5% carbon dioxide. All colonies that gave diffuse β haemolysis on human but not on horse blood agar, were Gram variable bacilli, and oxidase and catalase negative were identified as *G vaginalis*. The identity of some strains was confirmed further by the methods previously described by Piot *et al*,⁸ including the ability to hydrolyse hippurate to produce α and β glucosidase and acid from starch, maltose, and mannitol, and to be inhibited by metronidazole 50 µg, nitrofurantoin 150 µg, and bile 10%.

Obligate anaerobes were isolated on enriched blood agar, alone or with added kanamycin and vancomycin as previously described.⁹ After five days' incubation in an anaerobic cabinet (Don Whitley, West Yorkshire, UK) all strict anaerobes were identified by established criteria as described by Holdeman *et al*.¹⁰

Results

Semen samples were collected from 58 men. The pH of all samples was 7.0 to 8.0. In only 2/58 samples were "clue" cells seen. *G vaginalis* was isolated from 22 (37.9%) men, anaerobes from 20 (34.5%), and both groups of organisms from nine (15.5%). There

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was no association between the isolation rate of either *G vaginalis* or anaerobes and the total sperm count (table I).

TABLE I Relation of sperm count in 58 men to isolation of *Gardnerella vaginalis* and anaerobes

Sperm count ($\times 10^6/\text{ml}$)	No (%) patients colonised with:		
	<i>G vaginalis</i>	Anaerobes	<i>G vaginalis</i> and anaerobes
Nil (n = 6)	2 (33)	2 (33)	0
1-28 (n = 22)	7 (32)	7 (32)	3 (14)
>28 (n = 30)	13 (43)	11 (37)	6 (20)

All strains of *G vaginalis* were identified by differential haemolysis, Gram stain, and the absence of oxidase and catalase, and 13/22 were further identified as described above and were apparently consistent with published criteria for identifying *G vaginalis*. All anaerobes identified belonged to either the genus *Bacteroides* or *Fusobacterium*, *B bivuis* and *B disiens* being the predominant species (table II). The range and prevalence of anaerobes isolated were the same when found in association with *G vaginalis* (table II).

TABLE II Identification of anaerobes alone or with *Gardnerella vaginalis* in semen from 58 men

	No of isolates found:	
	Total tested (n = 20)	In association with <i>G vaginalis</i> (n = 9)
<i>Bacteroides bivius</i>	5	4
<i>Bacteroides disiens</i>	5	2
<i>Bacteroides capillosus</i>	4	2
<i>Bacteroides oralis</i>	1	0
<i>Bacteroides ruminicola</i>	2	1
Other <i>Bacteroides</i> spp	4	3
<i>Fusobacterium</i> spp	3	2
Unidentified	9	5

In 9/22 patients from whom *G vaginalis* was isolated quantitative counts were available. These ranged from 1.2×10^3 to $>10^7$ cfu/ml. Only two patients had counts higher than 10^6 cfu/ml. The geometric mean was 9×10^4 cfu/ml.

Discussion

Ideally, a study of the role of semen in bacterial vaginosis would include taking vaginal swabs from the women, and urethral and semen samples from their male partners. This type of study, however, is difficult to organise. Our series of semen samples,

although they may not be truly representative, allow preliminary data to be collected on a readily available and sexually active group. Although 48% of patients had a low sperm count ($<28 \times 10^6/\text{ml}$), only 6/58 had azoospermia. Whereas there is no absolute correlation between a low sperm count and infertility, the count is generally lower in infertile men. The prevalence of *G vaginalis* and anaerobes was similar in men with varying sperm counts in this study, and there therefore appeared to be no indication that these organisms relate to infertility in men.

G vaginalis has been implicated as the aetiological agent in bacterial vaginosis.¹ If this condition is to be classed as sexually transmitted it would need to be present in semen in sufficient numbers to provide an infective dose.

Work with human volunteers suggests that the infective dose of *G vaginalis* may be high. Criswell *et al* did not succeed in infecting all their subjects using an inoculum of 10^9 cfu.¹¹ In contrast, an inoculum of only 10^5 cfu was sufficient to establish colonisation with *G vaginalis* in pigtail macaque monkeys in which the vaginal pH was high and large numbers of anaerobes were present.¹² The true infective dose of *G vaginalis* is not known.

If the figure of Criswell *et al* is taken, then the counts of *G vaginalis* that we found in semen, if representative, would be far too low to be infective. There are, however, two factors that Criswell *et al* did not take into account: firstly, any effects of semen itself in helping to establish infection with *Gardnerella*, possibly by promoting bacterial growth or adherence or by raising vaginal pH; and secondly the presence of non-sporing anaerobes.

Anaerobes,^{2,3} either alone or in combination with *G vaginalis*,¹³ have been suggested as causative agents of bacterial vaginosis, but there are no corresponding data to suggest a possible infective dose or indeed sexual transmission. There is also little information on the carriage of anaerobes in men, but our own work (unpublished data) suggests that their isolation from the urethra is similar to their prevalence in semen. Gardner and Dukes¹ showed that vaginosis was established in more volunteers using vaginal material than using pure cultures of *G vaginalis*, which suggested that other factors such as the presence of anaerobes might be involved.

The isolation of *G vaginalis* from semen raises again the question of whether this organism colonises men and provides a reservoir for reinfection or is transient, being merely the result of continued passive acquisition from the female. Urethral carriage of *G vaginalis* in unselected men is between 7% and 11%,^{14,15} four times less than we found in semen (37.9%). This difference is unexplained as we

were unable to obtain urethral samples from the patients in the study. It emphasises the need for taking both urethral and semen samples, together with vaginal material from the female partner, to clarify the role of semen as a means of sexual transmission in bacterial vaginosis.

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