

## Intrauterine Infection and Spontaneous Midgestation Abortion: Is the Spectrum of Microorganisms Similar to That in Preterm Labor?

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### ABSTRACT

**Objective:** To determine whether microorganisms associated with intrauterine infection and preterm labor play a contributing role in midgestation abortion.

**Methods:** A 4 year retrospective review of spontaneous midgestation abortions for which autopsy and microbiological cultures of placental and fetal tissue were performed was conducted for a tertiary obstetrics hospital, which included a regional referral service for perinatal and fetal pathology. One hundred twenty-nine spontaneously delivered, nonmacerated, midgestation fetuses or stillbirths (of between 16 and 26 weeks' gestation) and placentas were examined and cultured for aerobic and anaerobic bacteria, yeasts, and genital mycoplasmas.

**Results:** Microorganisms were recovered in 85 (66%) cases (57% placentas, 49% fetuses). Among the culture positive cases, 81% had histological chorioamnionitis, 28% fetal pneumonitis, 38% clinical signs of infection, and 62% ruptured membranes at the time of miscarriage. These differed significantly from culture-negative cases (44%, 5%, 13%, and 34%, respectively). Group B streptococcus (GBS) was the most significant pathogen, recovered in 21 cases, 13 as the sole isolate, 94% with chorioamnionitis, and 47% in women with intact membranes. *Escherichia coli* and *Ureaplasma urealyticum* (22 and 24 cases, respectively) occurred mostly as mixed infections, with ruptured membranes. GBS, *U. urealyticum*, and *Streptococcus anginosus* group were individually associated with chorioamnionitis, *Bacteroides/Prevotella* and *S. anginosus* with fetal pneumonitis. The spectrum of microorganisms was similar to that in preterm labor at later gestations; however, GBS appeared to be the most significant pathogen in midgestation miscarriage, especially with intact membranes.

**Conclusions:** Unsuspected intrauterine infection underlies many spontaneous midgestation abortions. GBS is a key pathogen in this setting. *Infect. Dis. Obstet. Gynecol.* 8:220–227, 2000.

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### KEY WORDS

group B streptococcus; placenta; chorioamnionitis; fetal pneumonitis; intact membranes

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The role of ascending genital infection, particularly subclinical amniotic fluid infection, in initiation of preterm labor is now well-recognized.<sup>1–6</sup> However, the association between intrauterine infection and spontaneous midgestation

abortion has been largely unrecognized. Several studies have shown that, the earlier the gestational age, the higher the rate of positive amniotic fluid cultures,<sup>7</sup> of histologic chorioamnionitis,<sup>8</sup> and of recovery of microorganisms from the chorioam-

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niotic space.<sup>3</sup> Several studies have shown a significantly higher prevalence of bacterial vaginosis microorganisms among women with preterm labor before 37 weeks of gestation.<sup>3,5,9,10</sup> Certain vaginal pathogens in labor (*Escherichia coli*, *Klebsiella* species, *Haemophilus* species) have also been associated with an increased risk of preterm labor.<sup>5,11</sup>

Although the association between intrauterine infection and midgestation abortion is now beginning to be recognised, attempts to define the role of infection in this group further have been limited by the absence of routine microbiological investigation of freshly delivered, morphologically normal fetuses with their placentas.<sup>12</sup> Frequently, these are submitted as formalin-fixed specimens for routine histopathological examination. We hypothesised that intrauterine infection might be an important aetiological factor in spontaneous midgestation abortion and that the microorganisms associated with spontaneous midgestation abortion may be the same as those associated with preterm labor between 26 and 36 weeks of gestation. Therefore, a 4 year retrospective review was undertaken to establish the extent to which intrauterine infection was an important contributing factor in spontaneous midgestation abortion and to determine the causative microorganisms.

## MATERIALS AND METHODS

### Subjects

Normally formed, midgestation fetuses and pre-viable newborns spontaneously delivered between 16 and 26 weeks of gestation, which were submitted fresh with their placentas to the regional specialist perinatal pathology service and on whom microbiological cultures had been performed by the pathology staff, were eligible for the study. Fetuses were excluded if they had been fixed in formalin before examination, if they showed established maceration, or if their gestational age was uncertain or size was inappropriate for gestational age. Any liveborn infant who survived long enough to be admitted to the neonatal intensive care unit was also excluded. Fetuses and placentas were transported and stored at 4°C.

### Methods

Full examinations of all fetuses and placentas were performed by experienced perinatal pathologists as soon as practicable after delivery. Tissue sampling

of placentas routinely included at least two full-thickness blocks from the placental disc, a roll of membranes, and a transverse section of umbilical cord. Major fetal organs including at least two blocks of lung were routinely sampled. Swabs were taken from fetal lung and other fetal sites as appropriate, using aseptic techniques. Placental swabs were aseptically taken from the subamniotic region as follows. With new sterile gloves and the heated flat of a blade, a small area of placental surface was seared. A shallow incision was made with a sterile scalpel to go just through the amnion. With sterilised forceps, the edge of the amniotic membrane was picked up and the membrane peeled back a bit. A swab was then moved around between the membranes, swabbing both chorion and amnion. If the amnion had already been disrupted, the swab was taken from the subchorionic intervillous fibrin layer. Swabs were transported to the microbiology laboratory for culture immediately after collection from placenta and autopsy.

Swabs were cultured for aerobic and anaerobic bacteria, yeasts and genital mycoplasmas, according to published methods.<sup>5</sup> For the recovery of aerobic bacteria and yeast, swabs were inoculated onto layered horse blood agar, chocolate agar, human blood agar, and cystine lysine-deficient (CLED) agar and incubated in 5% CO<sub>2</sub> at 37°C for 48 hr. A cooked meat broth was also inoculated, incubated overnight at 37°C, and subcultured to layered horse blood agar. For detection of genital mycoplasmas, swabs were cultured onto Ureaplasma Differential agar (Sheppard's A7) and incubated for 3 or 4 days in 5% carbon dioxide. For the recovery of anaerobic bacteria, the following media were used: prereduced brain heart infusion agar (Oxoid, Basingstoke, United Kingdom), with 5% sheep blood containing hemin and vitamin K each at 10 mg/liter (BHI), and BHI, with 75 µg of kanamycin/ml plus 7.5 µg of vancomycin/ml and 5% laked horse blood instead of normal sheep blood. Cooked meat medium with Schaedler's broth was also inoculated, incubated overnight at 37°C, and subcultured onto BHI. All anaerobic plates were incubated in an anaerobic chamber for up to 4 days at 37°C. A nonselective human blood agar was used for isolation of heavy growth of *Gardnerella vaginalis*. Group F and nongroupable microaerophilic streptococci were grouped together as the *S. anginosus* group, which incorporates *S. angini-*

TABLE 1. Demographic characteristics of women in the study

	Culture-positive <sup>a</sup> (n = 85)	Culture-negative (n = 44)	Total (n = 129)
Caucasian race (%)	66/77 (86)	24/27 (89)	90/104 (87)
Maternal age (years)	28.7 ± 6.0	27.5 ± 5.4	28.2 ± 5.8
Mean birth weight (g)	438.2 ± 211.7	418.5 ± 194.6	431.5 ± 205.5
Mean gestational age (weeks)	21.5 ± 2.6	21.1 ± 2.3	21.4 ± 2.5
Median interval (hr) between delivery and microbiological sampling (range)	46 (5-136)	48 (8-144)	47 (5-144)
Received from outside hospital (%)	33 (39)	26 (59)	59 (46)
Sex ratio (M:F)	1.66:1	1.10:1	1.43:1
Parity = 0 (%)	32/77 (42)	18/37 (49)	50/114 (44)
Married/stable partner (%)	63/76 (83)	29/33 (88)	92/109 (84)
Previous midgestation abortion (%)	12/77 (16)	6/36 (17)	18/113 (16)
Smoking (%)	30/64 (47)	10/25 (40)	40/89 (45)

<sup>a</sup>Placental and/or fetal.

*nosus*, *S. intermedius*, and *S. constellatus*. Anaerobes were grouped but not always speciated, and the term *Bacteroides* species includes those isolates now known as *Prevotella* and *Porphyromonas* species.

Histopathologic slides of placental disc, membranes, and umbilical cord and of major fetal organs were subsequently reviewed by one of us (H.M.C.). The presence of chorioamnionitis, defined as a dense polymorphonuclear leucocyte infiltration of the chorionamniotic component of the placenta and membranes, was recorded. Inflammatory reactions confined to either the decidua or the subchorial intervillous space of the placental disc, without infiltration of the chorion or amnion, were recorded as negative for chorioamnionitis. The presence and severity of fetal pneumonitis, defined as an acute or chronic inflammatory infiltrate within the fetal lung interstitium, with or without accompanying inflammatory cells in the air spaces, were recorded. The presence of inflammatory cells in the terminal air spaces only, although indicative of aspiration of amniotic fluid containing an inflammatory exudate, was not considered evidence per se of fetal lung infection.

Data recorded included gestation at delivery (determined by combination of last menstrual period and first- or second-trimester ultrasonography), duration of time between death and autopsy, birth weight, gender, and maternal demographic, obstetric, and behavioural characteristics. Preterm prelabor rupture of membranes (PPROM) was defined as rupture of membranes 12 hr or more before delivery. Clinical signs suggestive of infection included maternal pyrexia, maternal and fetal tachycardia, uterine tenderness, purulent vaginal dis-

charge, raised white cell count ( $>15 \times 10^9$ /liter) or raised C-reactive protein ( $>12$  mg/liter).

Statistical analysis was performed using the  $\chi^2$  test with Yates's or Mantel-Haenszel's correction or Fisher's exact test, as appropriate.  $P \leq 0.05$  was considered statistically significant.

## RESULTS

One hundred seventy-five cases fulfilled the study criteria, and one hundred twenty-nine of these had fetal and placental examinations with microbiology. The demographic characteristics of the study population and their relationship to recovery of microorganisms are summarised in Table 1. The mean birth weight was 432 g, and the median gestational age was 21 weeks. The male to female sex ratio was significantly different, 1.43:1. Microbiological cultures were performed in 122 of the 129 placentas received, and one or more types of microorganisms were recovered in 70 (57%). Fetal cultures were performed in 118 cases, the lung being the most frequently sampled site (115), and positive cultures were recovered from one or more sites in 58 (49%).

In 85 (66%) cases microorganisms were recovered from the placenta and/or fetus. There was no significant difference in mean birth weight or gestational age between the culture-positive and culture-negative groups. No significant difference was found in the mean interval between delivery and microbiological sampling in the culture-positive group and culture-negative groups, indicating that recovery of microorganisms was not attributable to postmortem overgrowth or postdelivery contamination. However, 46% were received from outside the

TABLE 2. Relationship between clinical and histological findings and infection

	Culture-positive <sup>a</sup> (%)	Culture-negative (%)	Total (%)	Odds ratio (95% CI)	P value
Clinical signs	27/72 (38)	4/31 (13)	31/103 (30)	4.1 (1.2–17.4)	<0.05
Ruptured membranes	45/73 (62)	12/35 (34)	57/108 (53)	3.1 (1.2–7.8)	<0.05
Histologic chorioamnionitis	68/84 (81)	19/43 (44)	87/127 (69)	5.4 (2.2–13.2)	<0.0001
Fetal pneumonitis	22/77 (28)	2/42 (5)	24/119 (20)	8.0 (1.8–73.1)	<0.005

<sup>a</sup>Placental and/or fetal.

hospital, and the recovery of microorganisms was less frequent in these cases than in hospital-born cases (55.9% vs. 74.3%).

Histological chorioamnionitis was present in 69% of cases, and, as expected, there was a significant correlation with recovery of microorganisms from the placenta (OR 5.4 95% CI 2.2–13.2; Table 2). Fetal pneumonitis also correlated with recovery of microorganisms (OR 8.0 95% CI 1.8–73.1; Table 2).

Clinical signs suggestive of infection were absent in 70% of women, yet microorganisms were found in 62% of these cases, and 61% had histological evidence of chorioamnionitis. As expected, recovery of microorganisms and clinical signs of infection were common in women with ruptured membranes. Surprisingly, among women with intact membranes, although only 15% had clinical signs, 55% were culture-positive and 51% had histological evidence of chorioamnionitis (Table 3).

A variety of microorganisms was recovered from placental and fetal cultures, as listed in Table 4, and in 51% of cases only one type of microorganism was found. The isolates can be considered as falling into four key groups: GBS; *G. vaginalis*, *S. anginosus*, *Bacteroides/Prevotella* (microorganisms found in high concentrations in bacterial vaginosis); *E. coli*, *Haemophilus*, *S. aureus*, [termed enteropharyngeal microorganisms because these microorganisms normally colonise either the nasopharyngeal region (*S. aureus*, *H. influenzae*) or the lower bowel (*E. coli*, *Klebsiella* species)]; and the genital mycoplasmas (*U. urealyticum* and *M. hominis*), plus a group of "other" microorganisms. GBS was the most significant pathogen, recovered from one-fourth of culture-positive cases and the only microorganism present in 62% (Table 4). GBS was also the most significant pathogen recovered in women with intact membranes, detected in eight of

twenty-eight culture-positive cases and the sole isolate in six (Table 4). Microorganisms present in bacterial vaginosis such as *Bacteroides/Prevotella*, *S. anginosus*, and *G. vaginalis* generally occurred as mixed infections, with ruptured membranes (Table 4).

The enteropharyngeal microorganisms (*E. coli*, *Haemophilus*, *S. aureus*), previously shown to be associated with preterm labor,<sup>5</sup> were also common in midgestation abortion (31 cases, 11 as sole pathogen). *E. coli* was the third most frequent isolate (22 cases), occurring as the sole pathogen in eight.

The genital mycoplasmas, *U. urealyticum* and *M. hominis*, were not included in the bacterial vaginosis group for analysis, although they do occur in increased concentrations in bacterial vaginosis, because in this study they were found largely in the placenta and in women with ruptured membranes, and their pathogenic role is unclear. *U. urealyticum* was the most common microorganism recovered (24 cases); *M. hominis* was much less common (four cases). However, both were mostly found as mixed infections.

Among 51 women with intact membranes, 28 were culture-positive. The most frequent isolates were GBS (n = 8), *E. coli* (n = 6), *Peptostreptococcus* (n = 5), and *U. urealyticum* (n = 4; Table 4). Most microorganisms, with the exception of GBS, *Peptostreptococcus*, and *G. vaginalis*, were more than twice as common in women with ruptured compared to intact membranes, and *U. urealyticum* was four times as common. On the other hand, GBS, *Peptostreptococcus*, and *G. vaginalis* were found in similar proportions in the intact and ruptured membrane groups.

As expected, microorganisms were more common when chorioamnionitis was present, and GBS, *S. anginosus*, and *U. urealyticum* placental isolates

TABLE 3. Relationship between chorioamnionitis, infection, and membrane status

	Intact membranes (%)	Ruptured membranes (%)	Unknown membrane status	Odds ratio (95% CI)	P value
Clinical signs	7/47 (15)	25/52 (48)	1/4	0.2 (0.1–0.5)	<0.001
Culture positive <sup>a</sup>	28/51 (55)	45/57 (79)	12/21	0.3 (0.1–0.8)	<0.05
Histologic					
chorioamnionitis	26/51 (51)	49/57 (86)	12/20	0.2 (0.1–0.5)	<0.0005
Fetal pneumonitis	8/50 (16)	11/57 (19)	5/21	0.8 (0.3–2.4)	ns

<sup>a</sup>Placental and/or fetal.

TABLE 4. Microorganisms from placental and fetal cultures and membrane status

Organism group	Organism	Cases (sole isolate) (n = 129)	Placenta (n = 122)	Fetus (n = 118)	Intact membranes (n = 51)	Ruptured membranes (n = 57)	Unknown status (n = 21)
Group B streptococcus	Group B streptococcus	21 (13)	18	15	8	9	4
Bacterial vaginosis	<i>Bacteroides</i>	15 (93)	12	8	2	7	4
	<i>G. vaginalis</i>	4 (1)	2	3	2	2	0
	<i>S. anginosus</i>	9 (2)	7	7	3	5	1
Enteropharyngeal	<i>E. coli</i>	22 (8)	13	14	6	13	3
	<i>P. mirabilis</i>	4 (2)	3	2	1	2	1
	<i>Haemophilus</i>	5 (1)	3	3	0	3	2
	<i>Enterobacter</i>	2 (0)	2	0	0	1	1
	<i>S. aureus</i>	4 (2)	4	2	0	4	0
Genital mycoplasmas	<i>U. urealyticum</i>	24 (9)	21	10	4	17*	2
	<i>M. hominis</i>	4 (2)	3	2	1	3	0
Other anaerobes	<i>Peptostreptococcus</i>	9 (0)	7	2	5	3	1
	<i>C. perfringens</i>	2 (0)	1	2	1	1	0
	<i>Fusobacterium</i>	2 (0)	2	2	0	1	1
Other	<i>Enterococcus</i>	10 (0)	8	9	3	6	1
	Yeast	2 (0)	2	2	0	1	0
Any microorganism		85 (43)	70	58	28 (55%)	45 (79%)**	12

\*Ruptured vs. intact membranes,  $P = 0.008$ , OR 5.0 (95% CI 1.5–21.8).

\*\*Ruptured vs. intact membranes,  $P = 0.014$ , OR 3.1 (95% CI 1.2–7.8).

were significantly associated with chorioamnionitis (Table 5). Rates of recovery of *Peptostreptococcus* and *Enterococcus* were similar in women with and without chorioamnionitis, perhaps indicating a nonpathogenic role for these microorganisms. Fetal pneumonitis was demonstrated in 24 (20%) of 119 cases recorded, and 22 (92%) of these were culture-positive (Table 5). Certain microorganisms were strongly associated with fetal pneumonitis, *Bacteroides/Prevotella* and, in particular, *S. anginosus* (fetal pneumonitis was present in seven of eight cases; Table 5).

Among 37 women given antibiotics in labor, lower recovery rates of GBS (19% vs. 29%), *Bacte-*

*roides/Prevotella* (8% vs. 21%), *Peptostreptococcus* (5% vs. 18%), and *S. anginosus* (3% vs. 15%), but not *U. urealyticum*, *E. coli*, *S. aureus*, *G. vaginalis*, or *M. hominis*, were found compared to women not given antibiotics. In contrast, *S. aureus* and *M. hominis* were recovered only from women given antibiotics during labor.

GBS was found in both placental and fetal cultures in 60% of GBS-positive cases (excluding one case in which the fetus was not cultured). This is in contrast to *U. urealyticum* (32%) and *E. coli* (25%). Although culture methods were adequate to detect *Listeria monocytogenes*, this organism was not recovered.

TABLE 5. Chorioamnionitis and incidence of placental isolates

Organism group	Organism	Chorio amnionitis (n = 87) n (% with org)	No chorio amnionitis (n = 40) n (% with org)	Fetal pneumonitis (n = 24) n (% with org)	No fetal pneumonitis (n = 94) n (% with org)
Group B streptococcus	Group B streptococcus	17 (20) <sup>a</sup>	1 (3)	2 (8)	16 (7)
Bacterial vaginosis	<i>Bacteroides</i>	12 (14)	3 (7.5)	6 (25) <sup>****</sup>	8 (9)
	<i>G. vaginalis</i>	4 (5)	0	1 (4)	3 (3)
	<i>S. anginosus</i>	9 (10) <sup>*</sup>	0	7 (29) <sup>*****</sup>	1 (1)
Enteropharyngeal	<i>E. coli</i>	16 (18)	5 (12.5)	6 (25)	15 (16)
	<i>Haemophilus</i>	5 (6)	0	3 (13)	2 (2)
	<i>S. aureus</i>	4 (5)	0	0	3 (3)
Genital mycoplasmas	<i>U. urealyticum</i>	21 (24) <sup>**</sup>	3 (7.5)	3 (13)	14 (15)
	<i>M. hominis</i>	4 (5)	0	1 (4)	4 (4)
Other anaerobes	<i>Peptostreptococcus</i>	6 (7)	3 (7.5)	1 (4)	9 (10)
	<i>Fusobacterium</i>	2 (2)	0	1 (4)	2 (9)
Other	<i>Enterococcus</i>	7 (8)	3 (7.5)	4 (17)	6 (7)
Any microorganism		68 (78) <sup>***</sup>	16 (40)	22 (92) <sup>*****</sup>	54 (57)

<sup>a</sup>OR 9.5 (95% CI 1.4–405.8)  $P = 0.02$ .

\*OR undefined, RR 1.5 (95% CI 1.3–1.7) Fisher's one-tail test  $P = 0.03$ , Fisher's two-tail  $P = 0.056$ .

\*\*OR 3.9 (95% CI 1.05–21.7)  $P < 0.05$ .

\*\*\*OR 5.4 (95% CI 2.2–13.2)  $P \leq 0.0001$ .

\*\*\*\*OR 3.6 (95% CI 0.9–13.3)  $P = 0.04$ .

\*\*\*\*\*OR 38.3 (95% CI 4.3–1743)  $P \leq 0.0001$ .

\*\*\*\*\*OR 8.2 (95% CI 1.8–74.5)  $P = 0.004$ .

## DISCUSSION

This study confirms the frequency of intrauterine infection in midgestation abortion, particularly with intact membranes, suggesting that such infection may be an important factor in initiating this process. Notably, clinical signs of infection in the mother were uncommon, despite histological chorioamnionitis being present in more than two-thirds of the women and in 51% with intact membranes. This study reaffirms the now well-established association between recovery of microorganisms from the chorioamniotic space and histological chorioamnionitis<sup>6</sup> and supports previous findings that, the earlier the gestational age, the higher the rate of histological chorioamnionitis and amniotic fluid infection.<sup>7,13</sup> In a study of women with preterm labor, chorioamnionitis was more than twice as prevalent in the second trimester as it was between 27 and 34 weeks of gestation.<sup>14</sup> However, other researchers in the same year concluded that intraamniotic infection was not a common cause of preterm labor between 20 and 36 weeks of gestation in asymptomatic women with intact membranes.<sup>15</sup> Prior rupture of membranes is not necessary for development of ascending amniotic infection.<sup>13</sup>

The finding of established fetal pneumonitis in 28% of culture-positive cases, not reported in previous studies, is of considerable interest in that it reflects a well-established reaction to fetal infection prior to death. Our findings moreover suggest that there are likely to be two mechanisms of fetal loss associated with infection in the midgestation and that both are similar to those operating later in pregnancy. First, there may be bacterial invasion of the amniotic cavity or fetoplacental membranes, stimulating labor and delivery of a previable fetus, and, second, there may be intrauterine infection of the fetus presumably from swallowed and inhaled infected amniotic fluid, leading to fetal pneumonitis and/or septicaemia.

In this study, GBS was the most significant pathogen in spontaneous midgestation abortions, was often the sole pathogen recovered, and was found equally in women with intact and ruptured membranes. (The midgestation prevalence of GBS is approximately 13% in this population.) Heavy vaginal GBS colonization has been associated with preterm labor at later gestations.<sup>16</sup> Our study of midgestation abortions showed that GBS was significantly related to histological chorioamnionitis but not to fetal pneumonitis. This supports the

findings of Hillier et al.<sup>6</sup> that GBS in the placenta was significantly related to both prematurity and histological chorioamnionitis. However, only one-third of chorioamniotons with inflammation at  $\leq 34$  weeks yielded microorganisms in that study, and only 58% of culture-positive chorioamniotons had inflammation. Our results for preterm labor at earlier gestations demonstrated inflammation in 53% of chorioamniotons and 69% of chorioamniotons in which microorganisms were recovered. With the inclusion of several cases in which fetal culture indicated amniotic fluid infection although placental culture either was not performed or from which no microorganisms were recovered, the latter figure increased to 81%.

In a previous study, we demonstrated a significant relationship between the recovery of bacterial vaginosis microorganisms (*G. vaginalis*, *Bacteroides/Prevotella*) in the vagina in labor and an increased risk of preterm birth.<sup>5</sup> In this study *G. vaginalis* and/or *Bacteroides/Prevotella* were found in 11 cases and were the sole isolates in one and three cases, respectively. The low recovery of *G. vaginalis* may simply reflect its fastidious nature and the sometimes long median interval between delivery and culture, or the use of antibiotics in labor. *S. anginosus*, a microorganism that commonly occurs with *G. vaginalis* and *Bacteroides/Prevotella* in bacterial vaginosis, was recovered in seven cases and was very strongly associated with fetal pneumonitis, which has not been previously reported. The role of bacterial vaginosis microorganisms in late miscarriage was also shown in a study of 783 women screened in early pregnancy, and a higher risk of preterm birth and late miscarriage (16–24 weeks of gestation) was found in women with bacterial vaginosis compared to normal flora<sup>17</sup> (7.0% of 57 women with bacterial vaginosis had late miscarriage compared to 1.0% of 384 with normal flora).

Enteropharyngeal microorganisms (*E. coli*, *P. mirabilis*, *H. influenzae*, *S. aureus*) were found in 38% of cases in which microorganisms were recovered, mostly in mixed infections with anaerobes and in women with ruptured membranes. This supports the findings of our previous study showing a higher risk of preterm birth in women with vaginal enteropharyngeal bacteria in labor. *U. urealyticum* was the most common microorganism recovered; however, insofar as most isolates were found in the placenta and not in the fetus, and usually from cases with

ruptured membranes, its pathogenic role in this setting is uncertain.

It is not possible using this study design to demonstrate a cause and effect relationship between infection of the placenta and fetus and spontaneous midgestation fetal loss; an unavoidable limitation of the study was the absence of control cases of similar gestation. A further limitation was the impact of the varying time interval between death and autopsy on recovery of fastidious microorganisms, which was difficult to estimate. However, the isolation of *B. fragilis* from the subamniot and *Peptostreptococcus* and *U. urealyticum* from the fetal lung in a case with an interval of  $\geq 100$  hr between death and culture would suggest this may be only a minor effect.

The role of mixed infections in the aetiology of preterm labor requires further study. These results suggest that certain pathogenic bacteria such as GBS can primarily invade the chorioamniot and amniotic fluid, gain entry to the fetus, and initiate labor, leading to spontaneous miscarriage. Containment of such intraamniotic bacterial colonization or progression to infection depends on the effectiveness of the amniotic fluid antibacterial mechanisms and the number and pathogenicity of the colonising bacteria.<sup>18</sup>

Our data indicate that amniotic fluid infection is a major cause of spontaneous midgestation fetal loss and that this may occur in the presence of intact membranes and without clinical signs of sepsis. The patterns of microorganisms recovered in this group also suggest that this may represent the extreme end of the spectrum of microbiological influence on preterm labor. Therefore, microbiological studies are important in explaining fetal loss. These results indicate that GBS is a major pathogen in midgestation fetal loss with and without intact membranes and suggest a role as initiator/trigger of preterm labor.

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