**In Focus**

**Infection and preterm birth**

Preterm birth (PTB) before 37 weeks’ gestation remains an important cause of perinatal morbidity and mortality, despite modern advances in obstetric and neonatal care. The causes of spontaneous PTB are multi-factorial; however, infection has been implicated as a significant cause of both PTB and late miscarriage, often with no visible signs or symptoms.

The most common source for microorganisms gaining access to the uterine cavity and placenta is the lower genital tract, although it is unclear under what circumstances these organisms ascend into the amniotic cavity causing preterm labour, often with chorioamnionitis. It is thought preterm labour may also be initiated by a cascading cytokine host response to vaginal pathogens. Abnormal vaginal flora is more likely to cause ascending infection and preterm labour than normal lactobacillary flora. Bacterial vaginosis (lack of normal vaginal lactobacilli with overgrowth of mixed anaerobic bacteria) in early pregnancy has been consistently associated with a two-fold or more increase in PTB rate.

Since antibiotic treatment usually eradicates bacterial vaginosis, a number of randomised, controlled trials have been undertaken to determine whether treatment during early/mid-pregnancy would lower the PTB rate. However, meta-analysis of these trials showed that treatment, while effectively eradicating bacterial vaginosis, failed to decrease the risk of PTB. Studies indicate that treatment earlier in pregnancy may be more successful.

Other vaginal microorganisms, such as genital mycoplasmas, have also been implicated in adverse pregnancy outcome. Group B Streptococcus and Escherichia coli are well-known causes of neonatal sepsis, and group B Streptococcus is a major pathogen in unexplained late miscarriage. Recent studies have focused on identifying women who are at highest risk of infection-associated PTB, for whom preventive treatment may be more beneficial. Genetic studies have identified gene polymorphisms in immunoregulatory genes which influence susceptibility to chorioamnionitis and PTB. Maintenance/restoration of normal lactobacillary flora is important in prevention of PTB.

**Vaginal flora**

The vaginal flora constitutes a dynamic and complex ecosystem, with many different aerobic and anaerobic organisms present at any one time and at different concentrations. Lactobacillus spp., including the important hydrogen peroxide-producing lactobacilli, are the dominant species in normal vaginal flora, maintaining the vaginal pH between 4.0-4.5. During pregnancy the vaginal flora changes as a result of the substantial hormone increases during the first trimester; the concentration of lactobacilli is ten-fold higher in pregnant women.

**Microbiological findings in preterm labour**

Comprehensive case-control studies revealed that two groups of bacteria, bacterial vaginosis organisms (Gardnerella vaginalis and Bacteroides spp.), and an enteropharyngeal group (E. coli, Klebsiella spp. and Haemophilus influenzae/parainfluenzae), were significantly more common in the genital tract of women in preterm labour (often with chorioamnionitis) than labour at term (Table 1).

In placental and amniotic fluid studies, these and other pathogens such as Group B Streptococcus were significant causes of chorioamnionitis, and Group B Streptococcus and E. coli are well-known as major causes of neonatal sepsis. Ureaplasma urealyticum is more common in women with ruptured membranes, and is a cause of chronic respiratory disease in very low birth weight neonates. The earlier the gestation of PTB, the stronger are the statistical associations between these organisms and adverse pregnancy outcome. Invasive maternal infection with Listeria monocytogenes is known to carry a high risk of preterm labour, although the pathogenesis is not due to ascending lower genital tract flora but is generally bloodborne from gastrointestinal infection.

**Microbiological findings in early pregnancy and risk of PTB**

Prospective vaginal flora studies of women in early pregnancy have shown significant associations between carriage of certain microorganisms and increased risk of PTB and preterm labour rupture of membranes (Table 1). The most consistent finding has been the association between PTB and bacterial vaginosis (or bacterial vaginosis organisms) in early pregnancy. Unlike the findings of studies in labour, there was no association between vaginal carriage of enteropharyngeal organisms in early pregnancy and increased risk of PTB.

Symptomatic bacterial vaginosis is characterised by a grey, watery vaginal discharge, often with a fishy odour. Microbiologically, bacterial vaginosis is described as an imbalance of vaginal flora with a reduction or absence of lactobacilli, and an overgrowth of mixed anaerobic flora, including G. vaginalis and often Mycoplasma hominis and Mobiluncus spp. (Figure 1). However, 50% of pregnant women with bacterial vaginosis are asymptomatic. Why these organisms multiply, many of which are normally present in small concentrations in the vagina, while the usually prevalent...
lactobacilli decrease, is not clear. The role of hydrogen peroxide-producing lactobacilli appears to be important in preventing overgrowth of anaerobes in normal vaginal flora.

Other organisms in pregnancy have also been associated with increased risk of PTB and adverse pregnancy outcome such as heavy vaginal carriage/overgrowth of group B Streptococcus, *M. hominis* (usually with bacterial vaginosis present), *Trichomonas vaginalis* and cervical *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. It has been known for many decades that asymptomatic bacteriuria is associated with adverse pregnancy outcome. Screening and treatment for asymptomatic bacteriuria at the first antenatal visit is routine in obstetric protocols in the western world. However, *Candida albicans* is not associated with increased risk of adverse pregnancy outcome.

**Intervention studies**

To determine if the risk of infection-associated PTB can be reduced, many studies of antibiotic treatment of bacterial vaginosis, using metronidazole or clindamycin during early to mid-pregnancy, have been undertaken. The Cochrane Database of Systematic Reviews reports a meta-analysis of randomised, placebo-controlled trials of antibiotic treatment of bacterial vaginosis in pregnancy. Although antibiotic therapy was effective in eradicating bacterial vaginosis, there was little evidence that screening and treating all pregnant women with asymptomatic bacterial vaginosis would prevent PTB and its consequences.

The gestation of treatment (early rather than mid pregnancy) appears to be important. In the five trials using treatment before 20 weeks, the use of antibiotics showed a significant reduction in risk of PTB.

It is known that women with a previous PTB are at higher risk of a subsequent PTB. Screening and treatment for bacterial vaginosis in early pregnancy has been advocated in these women since several trials have shown a significant reduction in PTB in this group. Although a recent meta-analysis did not confirm this, there was a reduction in the risk of preterm prelabour rupture of membranes in two trials.

**Intermediate vaginal flora**

Recent studies have focused on women with abnormal or ‘intermediate’ vaginal flora (by Gram-stain microscopy) not fitting the description of bacterial vaginosis. This intermediate flora is characterised by a reduction in normal lactobacilli, but overgrowth is by aerobic facultative pathogens not usually found in bacterial vaginosis (mainly group B streptococci or occasionally intestinal microorganisms such as *E. coli*, enterococci). Unlike bacterial vaginosis, vaginal leukocytosis is present. Several studies have shown an increased risk of adverse pregnancy outcome in women with intermediate flora. Two trials of antibiotic treatment of women with intermediate flora in early pregnancy found a significantly lower risk of PTB before 37 weeks.

**Table 1. Vaginal microorganisms and adverse pregnancy outcome.**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Risk of preterm birth: Sampled in midtrimester</th>
<th>Sampled in labour</th>
<th>Risk of preterm prelabour rupture of membranes: Sampled in midtrimester</th>
<th>Sampled in labour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial vaginosis</td>
<td>++</td>
<td>++</td>
<td></td>
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<tr>
<td><em>G. vaginalis</em></td>
<td>+</td>
<td></td>
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<tr>
<td>Bacteroides / <em>Prevotella</em> spp.*</td>
<td>++</td>
<td>+</td>
<td></td>
<td>+/-</td>
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<tr>
<td><em>M. hominis</em></td>
<td>+</td>
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<tr>
<td><em>U. urealyticum</em></td>
<td>+</td>
<td></td>
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<td>+/-</td>
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<tr>
<td>Group B Streptococcus*</td>
<td>+</td>
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<td>+</td>
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<tr>
<td><em>E. coli</em></td>
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<td></td>
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<tr>
<td>Klebsiella spp.</td>
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<tr>
<td><em>H. influenzae</em></td>
<td></td>
<td>+/-</td>
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<td>+/-</td>
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<tr>
<td><em>C. trachomatis</em></td>
<td>+</td>
<td></td>
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<td>+/-</td>
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<tr>
<td><em>N. gonorrhoeae</em></td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td><em>T. vaginalis</em></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Asymptomatic bacteriuria</td>
<td>++</td>
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</tbody>
</table>

+/-, +, ++ General indication of strength of association and/or number of studies with significant findings
* When present in heavy concentrations
† In late miscarriage
Δ Cause of amnionitis but uncommon
Midtrimester miscarriage

The association between intra-uterine infection and late miscarriage (16-24 weeks’ gestation) has been largely unrecognised. In a study of placentas and fetuses in unexplained late miscarriage, group B Streptococcus was the most significant pathogen recovered, especially in women with intact membranes.

The remaining spectrum of microorganisms recovered was similar to that found in preterm labour at later gestations such as bacterial vaginosis organisms (Bacteroides/Prevotella spp., G. vaginalis), S. anginosus and U. urealyticum. There were no clinical signs suggestive of infection in 70% of women in this study, yet microorganisms were found in 62% of cases (placenta and/or fetus), and 61% had histological evidence of chorioamnionitis.

Identification of women at high risk of PTB

Since the host response to the presence of microorganisms may vary, studies of cytokine/inflammatory responses have been undertaken. Women with immunoregulatory gene polymorphisms which affect their inflammatory response to certain microorganisms may be at increased risk of adverse pregnancy outcome. Studies of cytokine gene polymorphisms have shown interleukin-10 (IL-10 -1082A/-819T/-592A) and mannose binding lectin (MBL2 codon 54Asp) single nucleotide polymorphisms were independently associated with histological chorioamnionitis and PTB before 29 weeks.

Pregnant women with periodontal disease may have a higher risk of PTB due to the potential to seed the bloodstream with Lactobacillus species indicate importance to a healthy vagina and reduction of PTB. Studies of the effects of Lactobacillus phages which may decimate anaerobic bacteria but also Lactobacillus suppositories to re-establish the normal flora, so essential in preventing infection-associated PTB.

References

22. Larsson, PG. et al. (2008) Human lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate: a 6-month, double-blind, randomized, placebo-controlled study. BMC Women’s Health 8, 3.

Helen McDonald is an emeritus microbiologist, Women’s & Children’s Hospital, North Adelaide, where she was the chief medical scientist, Diagnostic Microbiology Laboratories, until her retirement in 2004. Prior to merger with the Adelaide Children’s Hospital she was the microbiologist in charge of the Queen Victoria Hospital Microbiology Laboratories (1976-1995), and during this time she gained her Gr.DipHA, FASM and PhD. Her major research interests are the role of vaginal flora/infection in PTB and neonatal sepsis, and vaginal microbicides for prevention of HIV acquisition.

Maintenance of normal lactobacillary flora

Maintenance of normal lactobacillary flora is of primary importance to a healthy vagina and reduction of PTB. Studies of Lactobacillus species indicate L. crispatus, L. iners, L. gasseri and L. jensenii are most likely to be part of the normal flora in a healthy vagina. L. iners deserves close scrutiny, as it was not found in earlier studies due to its peculiar culture requirements, and phenotypic methods have not been able to separate the closely related Lactobacillus species of the vagina. Studies of changes in vaginal flora after treatment with metronidazole showed L. iners was the first Lactobacillus to colonise the vagina post treatment, suggesting L. iners is a dominant part of the vaginal flora when the flora is in a transitional stage.

Further studies using molecular tools are needed to elucidate the role of Lactobacillus species in maintaining normal vaginal flora. Studies of the effects of Lactobacillus phages which may decimate Lactobacillus populations, are also needed. Treatment of bacterial vaginosis may require not only antibiotics capable of eradicating anaerobic bacteria but also Lactobacillus suppositories to re-establish the normal flora, so essential in preventing infection-associated PTB.

Figure 1. Gram-stain of bacterial vaginosis including Mobiluncus (Gram-variable curved rods).