

## Preterm Birth 1

# Epidemiology and causes of preterm birth

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This paper is the first in a three-part series on preterm birth, which is the leading cause of perinatal morbidity and mortality in developed countries. Infants are born preterm at less than 37 weeks' gestational age after: (1) spontaneous labour with intact membranes, (2) preterm premature rupture of the membranes (PPROM), and (3) labour induction or caesarean delivery for maternal or fetal indications. The frequency of preterm births is about 12–13% in the USA and 5–9% in many other developed countries; however, the rate of preterm birth has increased in many locations, predominantly because of increasing indicated preterm births and preterm delivery of artificially conceived multiple pregnancies. Common reasons for indicated preterm births include pre-eclampsia or eclampsia, and intrauterine growth restriction. Births that follow spontaneous preterm labour and PPROM—together called spontaneous preterm births—are regarded as a syndrome resulting from multiple causes, including infection or inflammation, vascular disease, and uterine overdistension. Risk factors for spontaneous preterm births include a previous preterm birth, black race, periodontal disease, and low maternal body-mass index. A short cervical length and a raised cervical-vaginal fetal fibronectin concentration are the strongest predictors of spontaneous preterm birth.

### Introduction

Preterm deliveries are those that occur at less than 37 weeks' gestational age; however, the low-gestational age cutoff, or that used to distinguish preterm birth from spontaneous abortion, varies by location. In the USA, the preterm delivery rate is 12–13%; in Europe and other developed countries, reported rates are generally 5–9%.<sup>1,2</sup> The preterm birth rate has risen in most industrialised countries, with the USA rate increasing from 9·5% in 1981 to 12·7% in 2005 (figure 1),<sup>2</sup> despite advancing knowledge of risk factors and mechanisms related to preterm labour, and the introduction of many public health and medical interventions designed to reduce preterm birth.<sup>3</sup> Potential methods used to reduce preterm birth will be discussed in the second paper in this series.<sup>4</sup>

Preterm births account for 75% of perinatal mortality and more than half the long-term morbidity.<sup>5</sup> Although most preterm babies survive, they are at increased risk of neurodevelopmental impairments and respiratory and gastrointestinal complications. The outcome of preterm births will be discussed in detail in the third paper in this series.<sup>6</sup> Here, we explore the epidemiology, causes, and mechanisms leading to preterm births.

### Epidemiology

The obstetric precursors leading to preterm birth are: (1) delivery for maternal or fetal indications, in which labour is either induced or the infant is delivered by prelabour caesarean section; (2) spontaneous preterm labour with intact membranes; and (3) preterm premature rupture of the membranes (PPROM), irrespective of whether delivery is vaginal or by caesarean section (figure 2).<sup>7</sup> About 30–35% of preterm births are indicated, 40–45% follow spontaneous preterm labour, and 25–30% follow PPROM; births that follow spontaneous labour and PPROM are together designated spontaneous preterm births. The contribution of the causes of preterm births to

all preterm births differs by ethnic group. Spontaneous preterm birth is most commonly caused by preterm labour in white women, but by PPROM in black women.<sup>8</sup> Preterm births can also be subdivided according to gestational age: about 5% of preterm births occur at less than 28 weeks' (extreme prematurity), about 15% at 28–31 weeks' (severe prematurity), about 20% at 32–33 weeks' (moderate prematurity), and 60–70% at 34–36 weeks' (near term).

Much of the increase in the singleton preterm birth rate is explained by rising numbers of indicated preterm births (figure 3).<sup>9</sup> A high number of preterm multiple gestations associated with assisted reproductive technologies is also an important contributor to the overall increase in preterm births. Singleton pregnancies after in-vitro fertilisation are also at increased risk of preterm birth.<sup>10</sup> In the USA, increasing indicated preterm births mask a small, but

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This is the first in a *Series* of three papers about preterm birth

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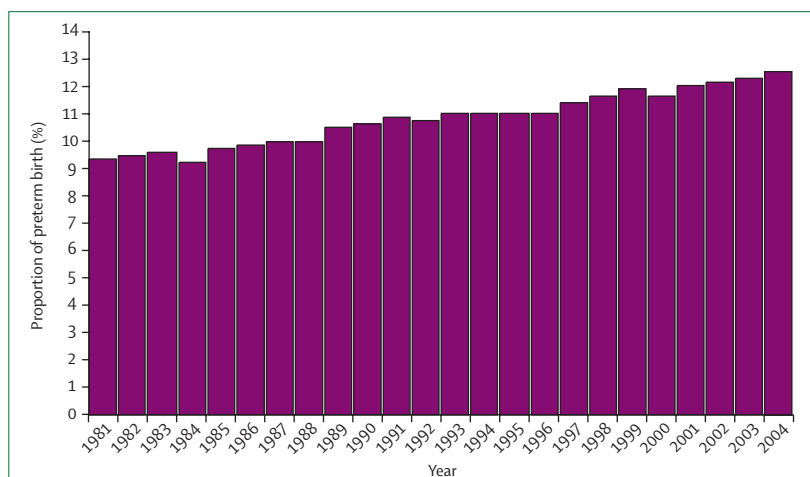


Figure 1: Percentage of all births classified as preterm in the USA, 1981–2004

Source: Martin JA, Kochanek KD, Strobino DM, Guyer B, MacDorman MF. Annual summary of vital statistics—2003. *Pediatrics* 2005; 115: 619–34.

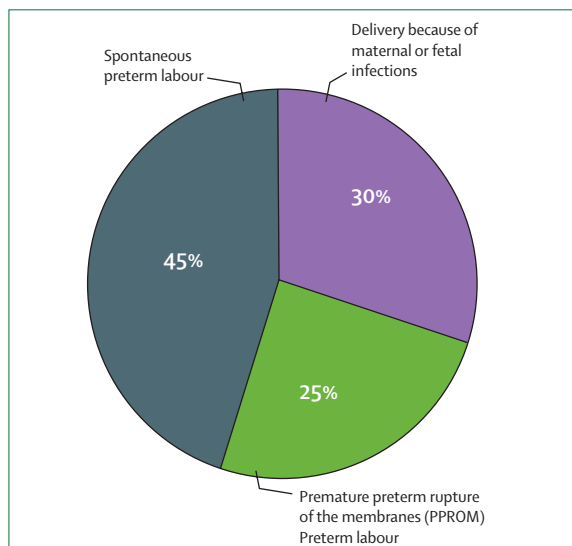


Figure 2: Obstetric precursors of preterm birth

important, reduction in spontaneous preterm births, especially in black women.<sup>11</sup>

PPROM is defined as spontaneous rupture of the membranes at less than 37 weeks' gestation at least 1 h before the onset of contractions. The cause of membrane rupture in most cases is unknown, but asymptomatic intrauterine infection is a frequent precursor. Risk factors for PPRM are generally similar to those for preterm spontaneous labour with intact membranes, although infections and tobacco exposure play important parts.<sup>12</sup> Most women with PPRM begin labour spontaneously within several days, but a small proportion of women remains undelivered for weeks or months. Since the membranes generally form a barrier to ascending infection, a common complication of PPRM is development of intrauterine infection and preterm labour.<sup>13</sup>

Preterm labour is usually defined as regular contractions accompanied by cervical change at less than 37 weeks' gestation. Pathogenesis of preterm labour is not well understood, but preterm labour might represent early idiopathic activation of the normal labour process or the results of pathological insults. A role for the fetus in determining the timing of the onset of labour has been proposed on the basis of studies in sheep.<sup>14</sup> Ablation of the fetal hypophysis or adrenal glands, or both, prevents the initiation of parturition; thus fetal cortisol is central to labour initiation in sheep. The same mechanism might be implicated in parturition in women, as suggested by reports of protracted pregnancies with an anencephalic fetus.<sup>15</sup> Theories proposed to explain the initiation of term labour are: (1) progesterone withdrawal, (2) oxytocin initiation, and (3) decidual activation. The progesterone withdrawal theory stems from studies in sheep.<sup>14,16</sup> As parturition nears, the fetal-adrenal axis becomes more sensitive to adrenocorticotrophic hormone, increasing the secretion of cortisol. Fetal cortisol stimulates placental

17 $\alpha$ -hydroxylase activity, which decreases progesterone secretion and increases oestrogen production. The reversal in the oestrogen/progesterone ratio results in increased prostaglandin formation, initiating a cascade of events that culminates in labour. In human beings, serum progesterone concentrations do not fall as labour approaches;<sup>16</sup> however, because progesterone antagonists—such as RU486—initiate preterm labour and progestational agents prevent preterm labour, a decrease in local progesterone concentrations or in the number of receptors is a plausible mechanism for initiation of labour.<sup>16–18</sup> Because intravenous oxytocin increases the frequency and intensity of uterine contractions, the assumption is that oxytocin plays a part in labour initiation. However, blood concentrations of oxytocin do not rise before labour and the clearance of oxytocin remains constant; thus oxytocin is unlikely to initiate labour.

An important pathway leading to labour initiation implicates inflammatory decidual activation.<sup>19</sup> Although at term, decidual activation seems to be mediated at least in part by the fetal-decidual paracrine system (perhaps through localised decreases in progesterone concentration), in many cases of early preterm labour, decidual activation seems to arise in the context of intrauterine bleeding or an occult intrauterine infection.

## Causes of preterm labour

### Risk factors

Preterm labour is now thought to be a syndrome initiated by multiple mechanisms, including infection or inflammation, uteroplacental ischaemia or haemorrhage, uterine overdistension, stress, and other immunologically mediated processes.<sup>19</sup> A precise mechanism cannot be established in most cases; therefore, factors associated with preterm birth, but not obviously in the causal pathway, have been sought to explain preterm labour. An increasing number of risk factors are thought to interact to cause a transition from uterine quiescence toward preterm labour or PPRM. Since many of the risk factors result in increased systemic inflammation, increasing stimulation of the infection or inflammation pathway might explain some of the increases in preterm births associated with multiple risk factors.<sup>20</sup>

Defining risk factors for prediction of preterm birth is a reasonable goal for several reasons. First, identification of at-risk women allows initiation of risk-specific treatment.<sup>21</sup> Second, the risk factors might define a population useful for studying specific interventions. Finally, identification of risk factors might provide important insights into mechanisms leading to preterm birth. There are many maternal or fetal characteristics that have been associated with preterm birth, including maternal demographic characteristics, nutritional status, pregnancy history, present pregnancy characteristics, psychological characteristics, adverse behaviours, infection, uterine contractions and cervical length, and biological and genetic markers.<sup>21</sup>

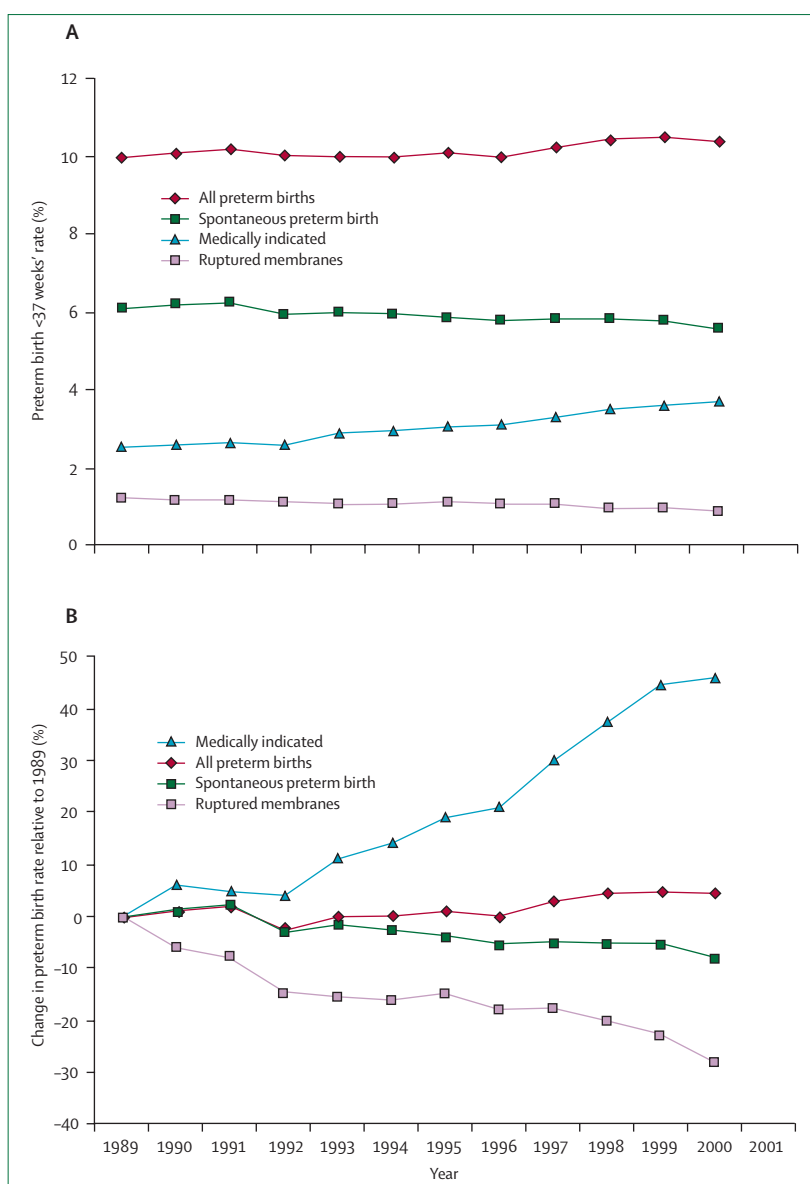
### Maternal risk factors

In the USA and in the UK, women classified as black, African-American, and Afro-Caribbean are consistently reported to be at higher risk of preterm delivery: preterm birth rates are in the range of 16–18% in black women compared with 5–9% for white women. Black women are also three to four times more likely to have a very early preterm birth than women from other racial or ethnic groups.<sup>22,23</sup> Part of the discrepancy in preterm birth rates between the USA and other countries might be explained by the high rate of preterm births in the USA black population. Over time, the disparity in preterm birth rates between black and white women has remained largely unchanged and unexplained, and contributes to a cycle of reproductive disadvantage with far-reaching social and medical consequences.<sup>24</sup> East Asian and Hispanic women typically have low preterm birth rates. Women from south Asia, including the Indian subcontinent, have high rates of low birthweight caused by decreased fetal growth, but preterm delivery does not seem to be substantially increased. Other maternal demographic characteristics associated with preterm birth include low socioeconomic and educational status, low and high maternal ages, and single marital status.<sup>25–27</sup> The mechanisms by which the maternal demographic characteristics are related to preterm birth are unknown.

Observational studies of the type of work and physical activity related to preterm birth have produced conflicting results.<sup>28–31</sup> Investigation of work-related risk is made difficult by confounding factors; however, even after accounting for population differences, working long hours and undertaking hard physical labour under stressful conditions are probably associated with an increase in preterm birth. The level of physical activity is not consistently related to the rate of preterm birth.

Whether differences in demographic, social, or economic risks, frequent absence of health insurance, and absence of a strong supportive economic and social safety net contribute to the disparity in preterm birth rates between the USA and other developed countries is unknown. Lower gestational age cutoffs for defining preterm birth used in the USA might explain part of the difference in preterm birth rates. What does seem clear, however, is that in many USA immigrant groups, the greater the length of time spent living in the USA, the higher the preterm birth rate; the explanation for this finding is also unknown.

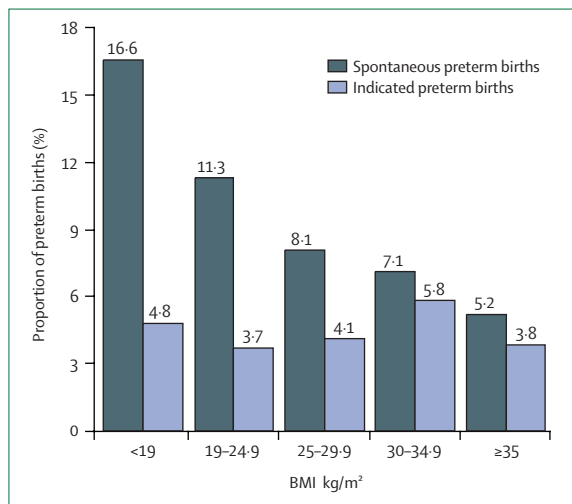
There is a raised risk of preterm birth in pregnancies arising within close temporal proximity to a previous delivery.<sup>32</sup> An interpregnancy interval of less than 6 months confers a greater than two-fold increased risk of preterm birth after adjustment for confounding variables.<sup>33</sup> Furthermore, women whose first birth was preterm are far more likely to have a short interval than women who had a term first birth, thus compounding the risk. Although the mechanism is not clear, one potential explanation is that the uterus takes time to return to its normal state, including resolution of the inflammatory status associated with the



**Figure 3: Temporal changes in singleton preterm births overall and temporal changes resulting from ruptured membranes, medically indicated preterm labour, and spontaneous preterm labour in USA, 1989–2000**  
A) Rates in each group by year. B) The percentage change in rates relative to 1989. Figure adapted from reference 9.

previous pregnancy. Maternal depletion might be another cause because pregnancy consumes maternal stores of essential vitamins, minerals, and aminoacids. A short interval decreases the opportunity to replenish these nutrients.

Nutritional status during pregnancy can be described by indicators of body size such as body-mass index (BMI), nutritional intake, and serum assessments for various analytes.<sup>34–36</sup> For example, a low prepregnancy BMI is associated with a high risk of spontaneous preterm birth, whereas obesity can be protective (figure 4).<sup>35</sup> Women with low serum concentrations of iron, folate, or zinc have more preterm births than those with measurements within the



**Figure 4: Comparison of spontaneous and indicated preterm birth by maternal body-mass index (BMI)**

Figure adapted from reference 35.

normal range.<sup>34,36</sup> There are many potential mechanisms by which maternal nutritional status might affect preterm birth—eg, spontaneous preterm birth can be caused by maternal thinness associated with decreased blood volume and reduced uterine blood flow.<sup>37</sup> Thin women might also consume fewer vitamins and minerals, low concentrations of which are associated with decreased blood flow and increased maternal infections.<sup>37,38</sup> Obese women are more likely to have infants with congenital anomalies, such as neural-tube defects, and these infants are more likely to be delivered preterm.<sup>39</sup> Obese women are also more likely to develop pre-eclampsia and diabetes, and have indicated preterm births associated with these disorders.

### Pregnancy history

The recurrence risk in women with a previous preterm delivery ranges from 15% to more than 50%, dependent on the number and gestational age of previous deliveries. Mercer and colleagues<sup>40</sup> reported that women with previous preterm deliveries had a 2.5-fold increased risk in their next pregnancy. The risk of another preterm birth is inversely related to the gestational age of the previous preterm birth. The mechanism for the recurrence is not always clear, but women with early spontaneous preterm births are far more likely to have subsequent spontaneous preterm births; women with indicated preterm births tend to repeat such births.<sup>41,42</sup> Persistent or recurrent intrauterine infections probably explain many repetitive spontaneous preterm births.<sup>41</sup> The underlying disorder causing indicated preterm births, such as diabetes, hypertension, or obesity, frequently persists between pregnancies.

### Pregnancy characteristics

Multiple gestations—accounting for only 2–3% of infants—carry a substantial risk of preterm delivery, and result in 15–20% of all preterm births. Nearly 60% of twins

are born preterm. About 40% of twins will have spontaneous labour or PPROM before 37 weeks' gestation, with others having an indicated preterm delivery because of pre-eclampsia, or other maternal or fetal disorders. Nearly all higher multiple gestations will result in preterm delivery. Uterine overdistension, resulting in contractions and PPROM, is believed to be the causative mechanism for the rate of increased spontaneous preterm births.<sup>19</sup>

Vaginal bleeding caused by placental abruption or placenta previa is associated with a very high risk of preterm delivery, but bleeding in the first and second trimesters that is not associated with either placental abruption or placenta previa is also associated with subsequent preterm birth.<sup>43</sup> Extremes in the volume of amniotic fluid—polyhydramnios or oligohydramnios—are associated with preterm labour and PPROM. Maternal abdominal surgery in the second and third trimesters can stimulate contractions culminating in preterm delivery. Maternal medical disorders, such as thyroid disease, asthma, diabetes, and hypertension, are associated with increased rates of preterm delivery, many of which are indicated because of maternal complications. History of cervical cone biopsy sample or loop electrocautery excision procedures secondary to premalignant cervical disorders have also been associated with an increase in spontaneous preterm delivery, as have various anomalies of the uterus itself—such as the presence of a septum.<sup>44</sup>

Mothers experiencing high levels of psychological or social stress are at increased risk of preterm birth (generally <2-fold) even after adjustment for the effects of sociodemographic, medical, and behavioural risk factors.<sup>45,46</sup> Furthermore, exposure to objectively stressful conditions, such as housing instability and severe material hardship, has also been associated with preterm birth.<sup>47</sup> Although the mechanism underlying the association between psychological or social stress and increased risk of preterm birth is unknown, a role for corticotropin releasing hormone has been proposed.<sup>48–50</sup> Women exposed to stressful conditions also have increased serum concentrations of inflammatory markers—such as C-reactive protein—an observation not accounted for by other established risk factors for inflammation.<sup>51</sup> These findings suggest that systemic inflammation might be a pathway by which stress could increase the risk of preterm birth.

Clinical depression during pregnancy has been reported in up to 16% of women, with up to 35% having some depressive symptoms.<sup>52,53</sup> Although the results are inconsistent, several reports suggest a relation (risks generally rose <2-fold) between depression and preterm birth.<sup>54–55</sup> Depression is associated with an increase in smoking, and drug and alcohol use; therefore, the relation between depression and preterm birth might be mediated by these behaviours.<sup>56–58</sup> Nevertheless, in some studies that adjusted for smoking and drug and alcohol use, the association between depression and preterm birth persisted, suggesting that this relationship might be caused by more than confounding. Although, the

mechanism(s) underlying the association of depression and preterm birth is unknown, there is an association between depressed mood and a reduction in natural killer cell activity, and higher plasma concentrations of pro-inflammatory cytokines and their receptors.<sup>59</sup> Inflammation, therefore, might also partly mediate the relation between depression and preterm birth.

In the USA, about 20–25% of pregnant women smoke, and, of these, 12–15% continue throughout pregnancy.<sup>60–62</sup> Tobacco use increases the risk of preterm birth (<2-fold) after adjustment for other factors.<sup>61,62</sup> The mechanism(s) by which smoking is related to preterm birth is unclear. There are more than 3000 chemicals in cigarette smoke and the biological effects of most are unknown;<sup>63</sup> however, both nicotine and carbon monoxide are powerful vasoconstrictors, and are associated with placental damage and decreased uteroplacental blood flow. Both pathways lead to fetal growth restriction and indicated preterm births. Smoking is also associated with a systemic inflammatory response and can increase spontaneous preterm birth through that pathway.<sup>64,65</sup> Although heavy alcohol consumption has been associated with preterm birth, neither mild nor moderate alcohol use is generally regarded as a risk factor for preterm birth. Cocaine and heroin use have been associated with preterm birth in several studies.

Intrauterine infection is a frequent and important mechanism leading to preterm birth.<sup>66,67</sup> The mechanisms by which intrauterine infections lead to preterm labour are related to activation of the innate immune system.<sup>68</sup> Microorganisms are recognised by pattern-recognition receptors—eg, toll-like receptors, which in turn elicit the release of inflammatory chemokines and cytokines—such as interleukin 8, interleukin 1 $\beta$ , and tumour necrosis factor (TNF)  $\alpha$ . Microbial endotoxins and proinflammatory cytokines stimulate the production of prostaglandins, other inflammatory mediators, and matrix-degrading enzymes. Prostaglandins stimulate uterine contractility, whereas degradation of extracellular matrix in the fetal membranes leads to PPROM.<sup>67,68</sup>

Microbiological studies suggest that intrauterine infection might account for 25–40% of preterm births;<sup>67</sup> however, 25–40% might be a minimum estimate because intrauterine infection is difficult to detect with conventional culture techniques.<sup>69</sup> Several investigators have shown additional microbial footprints in the amniotic cavity by using molecular microbiological techniques<sup>70–72</sup>—eg, women with a positive amniotic fluid *Ureaplasma urealyticum* PCR, but a negative culture, have similar rates of preterm birth to women with positive cultures for the same microorganism.<sup>73</sup> Furthermore, since the rate of microbial colonisation of the chorioamnion is twice that seen in the amniotic cavity, rates of intrauterine infection based only on amniotic fluid cultures substantially underestimate the level of association.<sup>74</sup>

Accumulating evidence suggests that intra-amniotic infection is a chronic process.<sup>67</sup> Women with positive

*U urealyticum* amniotic fluid cultures or who are PCR-positive for *U urealyticum* at the time of midtrimester genetic amniocentesis often have spontaneous preterm labour or PPROM weeks after the procedure.<sup>75–77</sup> Importantly, the earlier the gestational age at which women present with preterm labour, the higher the frequency of intrauterine infection.<sup>78,79</sup> At 21–24 weeks' gestation, most spontaneous births are associated with histological chorioamnionitis compared with about 10% at 35–36 weeks.<sup>78,79</sup>

The microorganisms most commonly reported in the amniotic cavity are genital *Mycoplasma* spp, and, specifically, *U urealyticum*, but many other organisms have been identified.<sup>80–83</sup> Some common lower genital tract microorganisms, such as *Streptococcus agalactiae*, are rarely seen in the amniotic cavity before membrane rupture.<sup>67,84</sup> The genital mycoplasmas and other organisms detected in the uterus before membrane rupture are typically of low virulence, probably accounting for both the chronicity of intrauterine infections and the frequent absence of overt clinical signs of infection.<sup>67</sup>

Intrauterine infection can be confined to the decidua, extend to the space between the amnion and chorion, and reach the amniotic cavity and the fetus.<sup>67,68</sup> The amniotic cavity is usually sterile for bacteria, but the significance of microorganisms in the membranes is less clear. Bacteria have been cultured from the chorioamnion in 15% of non-labouring women with intact membranes undergoing indicated caesarean delivery.<sup>67</sup> Fluorescence in-situ hybridisation with a DNA probe specific for conserved regions of bacterial DNA (the 16S ribosomal RNA) has detected bacteria in the membranes of up to 70% of women undergoing elective caesarean section at term.<sup>85</sup> These findings suggest that the presence of bacteria in the chorioamnion alone cannot be sufficient to cause an inflammatory response, preterm labour, and preterm birth.<sup>85</sup> Nevertheless, bacteria in the membranes and an associated inflammatory response in the amniotic fluid have been identified in more than 80% of women in early preterm labour with intact membranes who underwent caesarean section.<sup>68</sup> Thus, bacterial infection probably predisposes to preterm birth.

Microorganisms can gain access to the amniotic cavity by: (1) ascending from the vagina and the cervix; (2) haematogenous dissemination through the placenta; (3) accidental introduction at the time of invasive procedures; and (4) by retrograde spread through the fallopian tubes (figure 5).<sup>67,86</sup> The most common pathway is the ascending route. Although most investigators believe that ascent happens during the second trimester, the timing is unknown; some women have asymptomatic endometrial colonisation before pregnancy.<sup>87</sup> Irrespective of when colonisation occurs, the hypothesis is that only when the membranes become tightly applied to the decidua at about 20 weeks' gestation, essentially forming an abscess, do colonised women become symptomatic and progress to early preterm birth.<sup>67</sup>

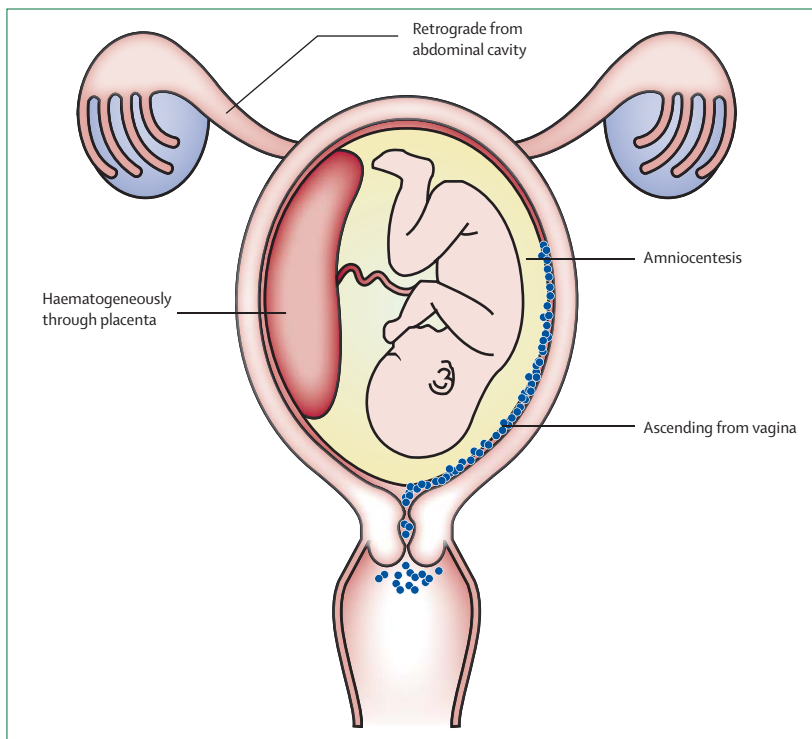


Figure 5: Potential routes of intrauterine infection

The most advanced and serious stage of ascending intrauterine infection is fetal infection. Carroll and colleagues<sup>88</sup> reported that fetal bacteraemia is present in 33% of fetuses with positive amniotic fluid cultures versus 4% with negative cultures. In another study, genital mycoplasma species were detected in 23% of umbilical cord cultures from infants born at less than 32 weeks' gestation.<sup>89</sup> Both studies suggest that subclinical fetal infection is far more common than traditionally recognised. Microbial invasion of the amniotic cavity is frequently associated with intra-amniotic inflammation and a fetal inflammatory response.<sup>90,91</sup> The fetal inflammatory response has been linked to the onset of preterm labour, and fetal injury and long-term handicap—including periventricular leucomalacia, cerebral palsy, and chronic lung disease.<sup>92–94</sup>

Bacterial vaginosis—a disorder defined by a change in the microbial ecosystem of the vagina—is diagnosed clinically by the presence of clue cells, a vaginal pH greater than 4.5, a profuse white discharge, and a fishy odour when the vaginal discharge is exposed to potassium hydroxide.<sup>95</sup> In the laboratory, bacterial vaginosis is defined by the Nugent criteria in which gram-stained smears are scored on the basis of numbers of lactobacilli, which tend to be low, and the presence of organisms resembling mobiluncus and bacteroides, the numbers of which tend to be high.<sup>96</sup> A score of 7–10 is used to diagnose bacterial vaginosis, and has been associated with a 1.5-fold to 3-fold increase in the rate of preterm birth.<sup>97,98</sup> Black women in

both the USA and the UK are three times more likely to have bacterial vaginosis than are white women, and this difference might explain 50% of the excess preterm births in black women.<sup>21,99,100</sup> The mechanism by which bacterial vaginosis is associated with preterm birth is unknown, but microorganisms that cause the infection probably ascend into the uterus before or early during pregnancy.<sup>101,102</sup>

Whether other genital infections are causally associated with preterm birth is not always clear.<sup>103,104</sup> For many infections, a range of associations has been reported, varying from none to strong. Women with genital infections generally have other risk factors, and many studies have not considered confounding variables. Nevertheless, trichomoniasis seems to be associated with preterm birth with a relative risk (RR) of about 1.3.<sup>105</sup> Chlamydia is probably associated with preterm birth only in the presence of a maternal immune response, and most probably with a RR of about 2.<sup>106</sup> Syphilis and gonorrhoea are probably associated with preterm birth with a RR of about 2.<sup>107</sup> Vaginal group B streptococcus, *U urealyticum*, and *M hominus* colonisations are not associated with increased risk of preterm birth.<sup>103,104</sup>

Several non-genital tract infections, such as pyelonephritis and asymptomatic bacteriuria, pneumonia, and appendicitis, are associated with, and probably predispose to, preterm birth.<sup>103,108</sup> Periodontal disease has received widespread scrutiny with some case-control studies suggesting an increased risk independent of other factors.<sup>109,110</sup> One potential explanation for the relation is that gingival crevice organisms, by way of maternal bacteraemia and transplacental passage, result in an intrauterine infection;<sup>111</sup> however, after adjustment for other factors, periodontal disease associated with preterm birth was not related to increased intrauterine bacterial colonisation or histological chorioamnionitis.<sup>112</sup> The biological pathway underlying the relation between periodontal disease and preterm births remains elusive.

Compared with bacterial infections, there is sparse evidence that viral infections predispose to preterm birth; however, when the mother is severely ill, such as with varicella pneumonia or severe acute respiratory syndrome, a preterm delivery might occur.<sup>113,114</sup> In several studies, the viral DNAs—identified by PCR techniques—in the amniotic fluid of asymptomatic women undergoing genetic amniocentesis were generally unrelated to subsequent preterm births;<sup>115</sup> therefore, it seems unlikely that maternal viral infection plays an important part in preterm birth, but controversy persists, and with little information, further study is needed.<sup>116</sup>

Several studies have shown an association between uterine contraction frequency and preterm birth,<sup>117–119</sup> however, uterine contractions do not predict preterm birth well in singletons because of the wide variation in frequency in normal pregnancy and the large overlap in frequency between women who do and do not deliver preterm.<sup>119</sup> Newman and colleagues<sup>120</sup> reported similar results in a study in twins; however, women admitted with

a diagnosis of preterm labour, if they do not deliver, remain at increased risk of subsequent preterm labour and PPRM.

As labour approaches, the cervix shortens, softens, rotates anteriorly, and dilates. Both digital and ultrasound examinations of the cervix have shown that cervical shortening is a risk factor for preterm delivery.<sup>121–123</sup> Ultrasound has proven especially useful in two circumstances: the first is in asymptomatic women, whereas the second is in those presenting with contractions. In asymptomatic women, at 24 weeks' gestation, a cervical length of less than 25 mm defines increased risk of preterm birth. The shorter the cervix, the greater the risk.<sup>122,123</sup> Women with preterm contractions often present a clinical dilemma, since nearly 60% of such women will, without treatment, deliver at term. Such women are usually observed for several hours before a decision is made to initiate tocolytic treatment, give corticosteroids, or discharge the patient. Cervical length can discriminate between women not in labour and those who carry a pronounced risk of early delivery. With a cervical length greater than 30 mm, the likelihood of delivering in the next week is about 1%, and most women can be safely discharged without treatment.<sup>124</sup>

Cervical insufficiency caused by congenital cervical weakness, surgery, or trauma has been implicated as causal for some preterm births; however, distinguishing cervical insufficiency from cervical shortening attributable to other causes has proven difficult, and the exact contribution to preterm birth is unknown.<sup>125</sup>

### Biological and genetic markers

Biological fluids (eg, amniotic fluid, urine, cervical mucus, vaginal secretions, serum or plasma, or both, and saliva) have been used to assess the value of biomarkers for the prediction of preterm birth.<sup>21,126</sup> Cytokines, chemokines, oestriol, and other analytes have been assessed, and many, especially those related to inflammation, are associated with preterm birth. Studies of biomarkers have improved the understanding of the mechanisms of disease leading to spontaneous preterm birth, but few biomarkers have shown clinical usefulness.<sup>21</sup> An important consideration is that, besides the specific nature of the analyte and its origin, an understanding of timing related to gestational age of collection and delivery is also necessary. For example, the concentration of matrix metalloproteinase-9 in serum rises substantially about 24 h before labour initiation.<sup>127</sup> Such late prediction is of little value in prevention, but can aid in understanding the pathophysiology of preterm labour. Salivary oestriol concentration predicts late preterm birth quite well, but is not especially useful for the prediction of earlier preterm births.<sup>128</sup> Prediction of late preterm births is of little importance because morbidity in these births is low.

The most powerful biochemical preterm birth predictor identified to date is fetal fibronectin—a glycoprotein that when present in cervicovaginal fluid is a marker of

choriodecidual disruption.<sup>129</sup> Typically, fetal fibronectin is absent from cervicovaginal secretions from 24 weeks' until near term; however, 3–4% of women undergoing routine screening at 24–26 weeks' are positive, and are at substantially increased risk of preterm delivery. For clinical care, an important characteristic of the fetal fibronectin test is its negative predictive value. In questionable cases of preterm labour, only about 1% of women with a negative test deliver in the next week.<sup>130</sup>

Each mechanism of disease responsible for preterm labour and PPRM has the potential for a genetic component. Women with sisters who gave birth preterm have an 80% higher risk of delivering preterm themselves.<sup>131</sup> Grandparents of women having a preterm birth are significantly more likely to have been born preterm themselves.<sup>132</sup> Genetic association studies have been used to identify single-nucleotide polymorphisms in several genes associated with preterm labour and PPRM.<sup>133–135</sup> The fetal and maternal genotypes modify the risk of preterm delivery.<sup>135</sup> A gene-environment interaction has been shown with maternal carriage of an allele of the *TNFA* gene and bacterial vaginosis.<sup>136</sup> Although neither characteristic alone was associated with spontaneous preterm birth, the combination increased the risk of preterm birth. Similarly, maternal carriage of a polymorphism in the *IL6* gene did not result in increased risk of spontaneous preterm birth for white or black women;<sup>137</sup> however, black women who were carriers of the *IL6* allele and had bacterial vaginosis had a two-fold greater risk of preterm birth than did those who carried the variant but did not have such infection. An interaction between maternal smoking and gene polymorphism on birthweight has also been described.<sup>138</sup>

The data provide evidence for gene-environment interactions in spontaneous preterm birth. Technological advances and completion of the HapMap project have made possible the conduction of whole-genome association studies;<sup>139</sup> therefore, genetics is evolving from a candidate gene approach in which the DNA variants of biologically interesting genes are studied to a true genomic approach that aims to examine the entire genome. High-density arrays now allow simultaneous examination of 500 000 or more DNA variants in the same individual. Such studies have not been undertaken in relation to preterm birth.

The proteome is the entire set of proteins encoded by the genome, and proteomics is the study of the global set of proteins.<sup>140</sup> Analysis of amniotic fluid and serum from women with preterm labour and PPRM has been undertaken to identify biomarkers for preterm labour and PPRM. In one study, bacteria were inoculated into the amniotic fluid of rhesus monkeys and the proteomic response monitored over time;<sup>141</sup> several novel infection markers were identified. The same proteins were identified in amniotic fluid of pregnant women with chorioamnionitis-associated preterm labour. Thus, proteomic techniques can be used to identify biomarkers in women with premature labour and PPRM.

Additional research that clearly defines the mechanisms by which risk factors are related to preterm birth is crucial. Improved understanding of these mechanisms should allow clinicians to design appropriate interventions so that the incidence of preterm birth and related fetal and neonatal morbidity and mortality will be reduced.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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