Infectious Comorbidities Encountered in Obstetrics and Neonatology

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Chapter: The Microbiology of Preterm Birth

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Introduction and Incidence of Preterm Birth

Preterm birth is best defined as delivery of a pregnancy before 37 weeks of gestation that is best determined based on an accurate last menstrual cycle or measurement of a first-trimester crown-rump length. In the United States, congenital malformations and preterm birth account for a majority of infant mortality and morbidity [1]. Preterm birth can occur spontaneously due to either premature rupture of membranes before 37 weeks of gestation, or preterm labor. This encompasses approximately 75% of all cases of preterm birth, almost 400,000 births per year [2]. Unfortunately, in the United States of America, with our advances in medical technology and education, the overall incidence of babies being born before 37 weeks of gestation exceeds 12% of all births. Some authors estimate that the non-Hispanic black population account for 18% of these births [2]. This estimation has plateaued over the past 5-10 years, but still remains a major public health problem [3]. Among survivors, approximately 60% of preterm births result in neonatal sepsis, intraventricular hemorrhage, necrotizing enterocolitis and bronchopulmonary disease, including respiratory distress syndrome [4].

Approximately one to two million neonatal deaths occur globally each year secondary to babies being born prematurely. It also accounts for an important contributor to child and adult morbidities. The highest proportions of preterm births occur amongst the low and middle income countries. Most of these causes are multi factorial in nature and vary by gestational age, as well as geographic and ethnic contexts. Although many interventions have been evaluated, few have moderate-to high-quality evidence for decreasing preterm birth. Focus on smoking cessation and progesterone treatment in women who are considered high risk of preterm birth, such as those with prior preterm births, has been targeted. Multiple different interventions during the antepartum and postnatal periods, such as antepartum maternal steroid administration or kangaroo mother care, have been shown to improve preterm neonatal survival after birth. Unfortunately, these interventions have not been implemented globally.

The problem of preterm birth spans many continents and ethnicities. In 2005, there were an estimated 12.9 million preterm births, representing almost 10% of total births worldwide [5]. By far, Africa and Asia carry the greatest burden of disease, with 11 million (85%) of these preterm births [5]. The lowest rate of preterm birth is in Europe (6.2%) while the rate in Latin America and the Caribbean is estimated to be 6.9% [5]. In Canada, the rates of preterm births have rose from a prevalence of 6.3% in the 1980s to 7.6% of live births in 2000 [6]. In Australia, birth before 37 weeks of gestation occurred in 7% of pregnancies during 2002, with 2.6% of all births occurring before 34 weeks of gestation and almost 70% of the total perinatal mortality [7].

Etiologies of preterm birth

Preterm delivery is the result of three natural processes occurring simultaneously before 37 weeks of gestation. These three processes commence a cascade resulting in an increase in uterine contractility, membrane activation and cervical ripening (Figure 1). The sequence of order differs amongst different circumstances; however, in general, uterine contractility tends to initiate leading to premature activation of the other two processes.

Figure 1: Premature Activation Cascade Leading to Preterm Birth.
Preterm birth is multifactorial in origin, but can broadly be divided into three basic etiologies: indicated, preterm labor (PTL), and preterm premature rupture of membranes (PPROM) (Table 1). Indicated (iatrogenic) preterm birth, from such causes as maternal medical conditions, accounts for 25% of premature deliveries in the United States [8]. The remaining 75% accounts for spontaneous preterm birth, of which 50% is thought to be from preterm labor and 25% from PPROM. Intrauterine infection accounts for approximately 25–40% of preterm births [8,9]. It is important to keep in mind that many of these infections are likely to present as a subclinical scenario; therefore, the pathogens responsible for these infections may be difficult to detect with conventional culture techniques [10,11].

<table>
<thead>
<tr>
<th>Causes</th>
<th>Overall Percentage (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicated (iatrogenic)</td>
<td>25</td>
</tr>
<tr>
<td>Preterm Labor</td>
<td>50</td>
</tr>
<tr>
<td>Preterm Premature of Membranes</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

* Intrauterine infection accounts for approximately 25–40% of all preterm births

Table 1: Causes of Preterm Birth in the United States of America

Routes for intrauterine infection

Goldenberg et al. [8], analyzed different infectious routes that lead to preterm birth. They found that infection can culminate within the uterus and amniotic fluid via hematogenous spread, incidental introduction via a diagnostic or therapeutic amniocentesis or retrograde spread from the abdominal cavity through the fallopian tubes. However, they found that infection ascending from the vagina had the highest risk factor for preterm birth (Figure 2). Other authors have also found that genital tract infections account for a majority of preterm births [12,13]. Ascending infection involves a 4-step process consisting of: cervical and vaginal infection, choriodectidal infection, intra-amniotic infection, and fetal infection. One should understand that bacterial and protozoan infections ascending from the lower genital tract account for a significant cause of preterm births, not viral infections. Other infectious channels leading to intrauterine infections include maternal periodontitis [23], as well as asymptomatic bacteruria and systemic maternal infections [19,20].

Intrauterine infections account for roughly 50 percent of babies being born less than 28 weeks of gestation, defined as extreme preterm birth. Microbial chorioamnion invasion justifies approximately 73 percent of spontaneous preterm births before 30 weeks of gestation and only 16 percent among indicated preterm delivery without labor [14]. Histologic chorioamnionitis is inversely related to gestational age, which accounts for up to 90 percent of gestations ending at between 20 and 24 weeks [15] Standard tocolytic therapies in patients with evidence of microbial invasion of the amniotic fluid tend to be refractory to these therapies, resulting in rapid preterm delivery [16]. This suggests that the pathophysiology of infection-associated preterm labor differs from that of idiopathic preterm labor. Of special interest, two conditions are essentially needed for intrauterine infections to cause preterm birth. The microorganisms involved must first enter the amniotic cavity and when encountered by the host immune system, be recognized as a foreign invader. After this occurs, the second process involves an unquantified number bacterial species being able to breach some threshold level in order to trigger an intraamniotic inflammatory response. This in turn induces preterm labor.

Proinflammatory cytokine prostaglandin cascade is another key player in the pathogenesis of infection associated preterm birth [17]. Fetal membranes become weakened in response to bacteria or bacterial products that produce these inflammatory mediators. This weakening can result in preterm premature rupture of membranes (PPROM). Evidence has shown that elevated concentrations of cytokines and prostaglandins in amniotic fluid are found in patients with intraamniotic infection and preterm labor [18]. These same studies have proven that introduction of bacterial products into the amniotic fluid results in stimulation and production of proinflammatory cytokines by the uterine decidua. In turn, this stimulates the production of prostaglandins by the amnion and the decidua. These authors have also concluded that the administration of IL-1 to pregnant mice or non-human primates induces preterm labor, which can be prevented by the administration of IL-1 receptor antagonist protein [18].

Maternal systemic infection and maternal periodontal disease have both been well-proven to induce preterm birth [19-21]. Periodontal disease is considered an anaerobic bacterial infection of the mouth, affecting up to 50 percent of pregnant women. Maternal periodontal disease has been associated with several adverse pregnancy outcomes, including preterm birth, preeclampsia, and fetal loss [19-21]. In a review of approximately 20 case reports involving preterm birth, 15 to 18 of these case reports were found to have an association with periodontal disease [22]. The exact mechanism of why and how preterm birth results from periodontal disease is not completely understood; however, experimental evidence from studies with rabbits suggests that the oral pathogens associated with periodontitis can gain access to the systemic circulation and can be recovered from amniotic fluid[23]. These studies have also found these same pathogens within the placenta of the same hosts [23]. Of importance, gram-negative anaerobes have been widely associated with periodontitis secondary to
the release of lipopolysaccharide endotoxins. These endotoxins lead to increases in the levels of proinflammatory mediators, including cytokines and prostaglandins [23].

**Intrauterine infection and preterm birth**

Petit et al [24] looked at the relationship between preterm birth and intrauterine infection, which was demonstrated using animal models. His study focused on inducing their abortions by injecting bacteria or endotoxins. Similar studies have been introduced into pregnant humans which demonstrated that examination of the amniotic fluid showed the anteriority of infection over labor induction and the existence of a subclinical latency phase between these two phenomena. Several markers of the infection have been studied:

- Maternal leukocytosis >15,000/mm³
- C-Reactive-Protein (CRP)>20mg/l,
- An increase of fibronectin
- An increase in cervical Interleukin-6 (IL-6)
- Short cervical length especially before 32 weeks gestation
- Leukocytosis (>15,000/mm³) within the amniotic fluid with elevated interleukin concentrations.

The main marker for the newborn is the CRP, but other markers can also be used for an early diagnosis of an infection, especially interleukin-6.

Trivedi et al. [25] looked at spontaneous labor at term versus preterm and they found that in laboring term women, a cascade resulting in the activation of the fetal adrenal axis and placental corticotropin-releasing hormone was associated. However, the exact cascade leading to preterm labor in extreme preterm births was not known. Among neonates delivered before 28 weeks of gestation, as referred to earlier in section 5.0 as extreme preterm birth, spontaneous delivery was associated with more frequent signs of placental inflammation and infection but less expression of the placental corticotropin-releasing hormone. They concluded that infection and inflammation, rather than premature activation of the fetal adrenal axis, is a big cause of extremely preterm birth in pregnant humans.

**Which species of bacteria infect amniotic fluid leading to preterm birth?**

Mycoplasma hominis one of the most commonly isolated microorganisms associated with intraamniotic infection. Ureaplasma urealyticum and Fusobacterium sp. are also commonly isolated from the amniotic cavity in pregnant women who present with intact membranes and preterm labor [26,27]. Other microorganisms found in the amniotic fluid are listed in Table 2. Up to 50% of patients can present with more than 1 type of species causing intraamniotic infections.

<table>
<thead>
<tr>
<th>Class of Microorganism</th>
<th>Species</th>
</tr>
</thead>
</table>
| Gram Positive          | - Mycoplasma hominis*  
|                        | - Streptococcus agalactiae  
|                        | - Peptostreptococcus species  
|                        | - Staphylococcus aureus  
|                        | - Streptococcus viridans  
|                        | - Lactobacillus species  
|                        | - Enterococcus faecalis |
| Gram Negative          | - Ureaplasma urealyticum*  
|                        | - Fusobacterium species*  
|                        | - Prevotella species  
|                        | - Delfia species  
|                        | - Neisseria species  
|                        | - Escherichia coli |
| Gram-Variable          | - Gardnerella vaginalis  
|                        | - Bacteroides species  
|                        | - Mobiluncus species |

* Most commonly isolated from the amniotic cavity of women with preterm labor and intact membranes.

**The role of lactobacilli in the vaginal microbiome**

The normal human female vaginal flora consists of an ecosystem of multiple different microorganisms. Of special note, Lactobacillus species are a group of Gram-positive facultative anaerobic organisms most commonly found in post pubertal asymptomatic female patients. On average, there are approximately 107 different lactobacilli microorganisms per gram of vaginal secretion. Figure 3 illustrates the more common Lactobacillus species, with more than 1 type of species potentially being present in any one individual [28].

![Figure 3: Lactobacillus Species Commonly Isolated From Vaginal Specimens.](image-url)
Bacterial vaginosis

Bacterial vaginosis is felt to be secondary to a breach in a female’s normal vaginal ecosystem. This results from a reduction in the number of Lactobacillus species with a replacement of anaerobic organisms [31]. Clinically, the diagnosis of bacterial vaginosis can be made using a different number of criteria available (Table 3). Most physicians use the Amsel’s criteria [32] as it is easier to utilize. Alternatively, bacterial vaginosis can be diagnosed based on the assessment of cellular morphologies of the bacteria observed in samples using criteria first introduced by Spiegel et al. [33] and then modified by Nugent et al. [34]. Nugent criteria is based on a numerical scoring system (0–10). The score reflects the relative abundance of curved gram-variable rods (Mobiluncus), large gram-positive rods (Lactobacillus) and lastly the small gram-variable rods (G. vaginalis/Bacteroides spp.).

**Table 3: Diagnostic Criteria for Bacterial Vaginosis (BV).**

<table>
<thead>
<tr>
<th>Amsel’s Criteria</th>
<th>Hay/Ison’s Criteria</th>
<th>Nugent Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Clue cells.</td>
<td>Grade 1 (Normal): Mainly Lactobacillus.</td>
<td>0–3 negative</td>
</tr>
<tr>
<td>2) Fishy odor after adding 10% potassium hydroxide (KOH) solution.</td>
<td>Grade 2 (Intermediate): Lactobacilli present, but Gardnerella or Mobiluncus morphotypes also present.</td>
<td>4–6 intermediate</td>
</tr>
<tr>
<td>3) Homogeneous discharge being thin, white or yellow.</td>
<td>Grade 3 (Bacterial Vaginosis): Predominantly Gardnerella and/or Mobiluncus morphotypes. Absent or reduced amount of Lactobacillus.</td>
<td>7+ positive for BV</td>
</tr>
<tr>
<td>4) pH of vaginal fluid &gt;4.5</td>
<td>Requires three of the following four signs or symptoms</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4: The Microorganisms Associated with Bacterial Vaginosis.**

<table>
<thead>
<tr>
<th>Bacterial Vaginosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Gardnerella vaginalis</td>
<td></td>
</tr>
<tr>
<td>2) Prevotella</td>
<td></td>
</tr>
<tr>
<td>3) Peptostreptococcus</td>
<td></td>
</tr>
<tr>
<td>4) Mobiluncus</td>
<td></td>
</tr>
<tr>
<td>5) Bacteroides</td>
<td></td>
</tr>
</tbody>
</table>

Should asymptomatic bacterial vaginosis be treated during pregnancy?

The decision on whether pregnant women with asymptomatic bacterial vaginosis be treated with antimicrobial agents to prevent preterm birth has always been a challenging question to answer. Bacterial vaginosis is associated with intraamnionic infection and therefore is considered a risk factor for preterm delivery. However, according to the Cochrane Reviews, these do not support the concept of widespread screening for bacterial vaginosis and treatment to prevent premature delivery [40–42]. It is important to note that the most significant clinical factor in identifying women at risk for preterm birth is a history of a prior preterm birth [43-44].

Many prominent leaders in the field of perinatology and neonatology have recommended that only patients at high risk for preterm delivery should be treated with antibiotics if they are found to have bacterial vaginosis [45]. These included pregnant women with a previous history of a spontaneous preterm birth. Routine screening and treatment of BV in low-risk patients should wait until best practices for timing and methods of screening, as well as treatment of infection, have been established. According to the Centers for Disease Control and Prevention (CDC), suggestions for the treatment of BV during pregnancy are listed in Table 5 [46]. Patients should be reevaluated one month after treatment.

**Table 5: Treatment of Bacterial Vaginosis During Pregnancy.**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>500 mg twice daily for seven days</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>250 mg three times daily for seven days</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>300 mg twice daily for seven days</td>
</tr>
</tbody>
</table>

Conclusion

The association between intrauterine infection and preterm birth is most commonly associated with lower genital tract infections, systemic infections and periodontal disease. One must keep in mind that not all pregnant women with these infections delivery prematurely; hence, the host inflammatory response to a potential pathogen must play a critical role in preterm birth. Cytokines play a major role in this inflammatory cascade, being brought on by the introduction of bacteria or bacterial products, such as lipopolysaccharide, into the amniotic fluid. Research around the world is being conducted to target these cytokines in animals and humans, with efforts to decrease the overall percentage of preterm births. As this percentage decreases, both neonatal mortality and morbidity will secondarily decrease. Further research is needed to better understand these context-specific pathways leading to preterm birth, as well as to develop appropriate and efficacious prevention strategies and interventions to improve survival of neonates born prematurely.
In the United States of America, we can come a long way in understanding some of the key infectious causes of preterm birth; however, I believe that our current understanding of normal vaginal microbiota, bacterial vaginosis, and the relationship to intrauterine infection and preterm birth is limited. Our understanding and knowledge on the human microbiome and its role in preventing intrauterine infections have evolved around certain medical technologies, such as DNA sequencing, allowing us to gain better assessments on the diversity and function of the microbial communities. This in turn provided a greater insight to microbial community structure, function, dynamics, and the interspecies interactions that are central to explaining how the human microbiota functions to maintain host health or predispose individuals to diseases. Lower genital tract infections, such as bacterial vaginosis, are notorious in leading to different obstetrical complications, such as preterm birth. Even though there is a debate on whether these specific patients should receive antibiotic therapy during pregnancy if they are asymptomatic, the CDC has made it very clear that all patients should receive therapy, regardless of their obstetrical history.

References


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