A review of premature birth and subclinical infection

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Premature birth causes high rates of neonatal morbidity and mortality. There are multiple causes of preterm birth. This article reviews the evidence linking subclinical infection and premature birth. Although maternal genital tract colonization with specific organisms has been inconsistently associated with preterm birth and/or premature rupture of membranes, some infections have been consistently associated with preterm delivery. The association of histologic chorioamnionitis with prematurity is a consistent finding, but the mechanisms require further study. The relationship between histologic chorioamnionitis infection and the chorioamnionitis of prematurity requires additional research. A varying number of patients in "idiopathic" preterm labor have positive amniotic fluid cultures (0% to 30%), but it is not clear whether infection preceded labor or occurred as a result of labor. Evidence of subclinical infection as a cause of preterm labor is raised by finding elevated maternal serum C-reactive protein and abnormal amniotic fluid organic acid levels in some patients in preterm labor. Biochemical mechanisms for preterm labor in the setting of infection are suggested by both in vitro and in vivo studies of prostaglandins and their metabolites, endotoxin and cytokines. Some, but by no means all, antibiotic trials conducted to date have reported decreases in prematurity. These results support the hypothesis that premature birth results in part from infection caused by genital tract bacteria. In the next few years, research efforts must be prioritized to determine the role of infection and the appropriate prevention of this cause of prematurity. (Am J Obstet Gynecol 1992;166:1515-28.)

Key words: Premature birth, subclinical infection, biochemical mechanisms, chorioamnionitis, antibiotics

Preterm birth is the leading perinatal problem in the United States. The underlying cause of preterm birth is not evident in most cases, and currently, physicians are left with marginally effective attempts to delay delivery by arresting contractions. Breakthroughs in the prevention of preterm birth will require an improved understanding of its causes followed by appropriate strategies to prevent these causes. Evidence from several sources suggests a link between subclinical infection and prematurity. In August 1990 a symposium, sponsored by the Infectious Disease Society of Obstetrics and Gynecology, was held to discuss this evidence. This article summarizes the presentations and includes additional data presented since the symposium.

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Prematurity in perspective

The problems of being born premature or with low birth weight (LBW) are well documented. LBW leads to excessive neonatal mortality and morbidity. More than 60% of the mortality that occurs among infants without anatomic or chromosomal congenital defects is attributable to LBW.[1] A similar excess of morbidity also exists in the LBW group. The Surgeon General placed a high priority on the reduction of LBW by 1990. However, the rate of LBW has not appreciably changed in the United States over the past 40 years.[2] Approximately 6.7% of infants are born with LBW and 1% are born with very low birth weight (<1500 gm).[3] The United States ranks nineteenth in the world in infant mortality, behind virtually all European countries and in the same range as many third-world countries.

In contrast, the neonatal mortality rate has been dramatically reduced over the same 40 years in the United States,[4] caused mainly by improved care of LBW infants in neonatal intensive care units.[2] In fact, weight for

weight, an infant has the best survival rate in the United States. Thus, given a high LBW rate, infant mortality would be even higher in the United States were it not for high technology and expensive neonatal care. It is apparent that the emphasis of medical care for this problem in the United States has been placed on costly tertiary care rather than on prevention.

The main reason for a continued high LBW rate is a poor understanding of antenatal factors that contribute to an infant with LBW. One cannot expect to reduce LBW or even to appreciate the relative importance of various potential factors until the various causes are determined. Most investigators have assumed a final common pathway by which a variety of normal and pathologic processes eventually influence the human endocrinologic system that, in turn, causes uterine contractions and labor. While parts of this system have been identified in certain animals, processes important in human parturition are less clear. Without additional knowledge on the essential pathway, it has been difficult to identify the mechanisms by which various processes result in premature contractions or delivery.

To date, multiple factors have been associated with LBW. Determination of factors that are important causes of LBW awaits further study. Some factors appear highly associated with infant mortality and LBW because of consistent findings, a high relative risk ratio, or a high frequency of the factor in the population. Risk factors for both neonatal and postneonatal mortality include both low (<17 years) and high (>34 years) maternal age, black race, and low socioeconomic status.[2] These same three factors plus a history of an adverse pregnancy outcome, inadequate prenatal care, rupture of membranes, multiple pregnancies, medical conditions such as hypertension and diabetes, fetal anomalies, and drug abuse are some of the more important factors associated with LBW.[2] While some of these factors cause premature delivery, others such as socioeconomic status are only associated with preterm delivery.

Premature rupture of membranes is associated with about one third of LBW deliveries. Premature rupture of membranes has been reported to be the third leading cause of perinatal death in the collaborative perinatal project after "amniotic fluid infection" and abruptio placentae.[5] Factors associated with premature rupture of membranes include premature rupture of membranes in a prior pregnancy,[6] histologic chorioamnionitis,[7] abnormal bleeding, polyhydramnios, incompetent cervix, smoking,[8] and selected vaginal microorganisms[9] in the current pregnancy. Infection appears to be an important theme in premature rupture of membranes.

Recently, convincing evidence has associated LBW with infection. High rates of amniotic fluid infection, chorioamnion infection, and histologic chorioamnionitis have been reported in LBW pregnancies.[10] The highest proportion of these three infections occurs in the most premature pregnancies, an observation that adds to the impact of infection in LBW.

Prevention of LBW and premature rupture of membranes can be targeted (1) to prevent the initiation of labor or premature rupture of membranes or (2) to inhibit preterm labor after it is recognized. Programs for prevention of preterm labor have had only limited success. The targeted treatment of cervical or vaginal infection has led to only a modest reduction of preterm labor and premature rupture of membranes, and the overall impact on preterm labor may be limited. Treatment of premature labor with tocolysis also has had limited impact on LBW and premature rupture of membranes.

Experimental difficulties exist in establishing the role of infection in LBW and premature rupture of membranes. Many LBW deliveries occur without placental or amniotic fluid infection. Antibiotic treatment of uninfected pregnancies would have no effect and inclusion of uninfected pregnancies into such treatment studies will usually mask any positive antibiotic effect. The detection of infection requires sensitive techniques because most maternal infection is subclinical. Upper genital tract infection most directly influences preterm delivery and sampling of the upper genital tract is difficult, particularly before premature labor. Whereas amniotic fluid can be sampled before delivery, the majority of infections occur in the placenta and currently it is not possible to sample the placenta before delivery. A cause-and-effect relationship is even harder to establish after an event has transpired.

Specific microorganisms associated with preterm birth

The first evidence that suggested a connection between infection and premature birth occurred with an association of a variety of cervicovaginal microorganisms with preterm delivery, LBW, or premature rupture of membranes (Table I). The next question was whether those lower maternal genital tract isolates were related to upper genital tract infection and, further, whether the relationship between upper genital tract infection and preterm birth, LBW, and premature rupture of membranes was a cause or an effect.

Syphilis has been related to preterm birth for many years. Untreated primary or secondary syphilis resulted in a 50% rate of prematurity compared with an 8% rate among controls.[11] Early latent syphilis resulted in a 20% prematurity rate. The association of syphilis with premature delivery has been reconfirmed during the current epidemic of congenital syphilis in which mean gestational age for newborns with congenital syphilis was 32.3 weeks. Twelve (39%) of 33 infants with congenital

syphilis and none of the controls had premature rupture of membranes.[12]

Untreated *N. gonorrhoeae* has been associated with preterm delivery.[13-16, 16A] In addition, treatment of *N. gonorrhoeae* reduces rates of preterm delivery and low birth weight similar to that of control populations, suggesting a causative role of *N. gonorrhoeae* in these events.

Asymptomatic group B streptococci bacteriuria has been consistently associated with preterm delivery. Preterm delivery rates of 20% versus 8.5%[17] and 7.2% versus 4.9%[18] occurred among women with bacteriuria with and without group B streptococci, respectively. A sevenfold decrease in preterm delivery resulted when antibiotics were administered (5.4%), compared with placebo (37.5%).[19] The extraordinarily high rate of prematurity in the bacteriuric group suggests that additional risk factors for prematurity may have been present.

In comparison to the consistent association with bacteriuria, the relationship of maternal group B streptococci genital colonization and adverse pregnancy outcome has been inconsistent. Five of the six studies assessing genital tract group B streptococci colonization with preterm labor or delivery demonstrated no significant association.[20-25] However, a significant association was found in three of four studies assessing the relationship of genital group B streptococci colonization and in preterm premature rupture of membranes[21, 22, 26, 27] (Table II).

In case-control studies genital tract mycoplasmas, especially *U. urealyticum*, have been associated with LBW.[28, 29] A subgroup of pregnant women with serologic evidence of invasive *U. urealyticum* infection (more than a fourfold rise in antibody) has been associated with LBW.[30] The mean birth weight was 233 gm lower in the group with an antibody response (3006 ± 565 vs 3229 ± 474 gm, $p = 0.009$). The clinical significance of a 200 gm difference at a 3 kg birth weight is questionable. A larger proportion of newborns weighing <2500 gm were born to mothers with a fourfold rise in antibody to *U. urealyticum* (38%, 10/27) compared with infants weighing >2500 gm (15%, 33/219). However, recent studies have failed to confirm such an association. None of the 11 cohort studies performed[31-41] support an association between *U. urealyticum* and prematurity or LBW. The treatment of *U. urealyticum* with erythromycin had no impact on LBW or premature rupture of membranes.[42] *M. hominis* has been inconsistently related with LBW; the relationship has been positive in some[25, 26, 31, 33, 42A] but not other[28, 35, 38] studies. Thus the majority of investigations have failed to provide evidence supporting an association between colonization of the maternal genital tract with *U. urealyticum* and preterm delivery or LBW. Data regarding *M. hominis* are less clear in relating this organism to prematurity.

Table I. Microorganisms or infections associated with preterm labor, preterm delivery, and preterm premature rupture of the membranes

Organisms
Treponema palladium
Neisseria gonorrhoeae
Group B streptococci
Ureaplasma urealyticum
Mycoplasma hominis
Chlamydia trachomatis
Trichomonas vaginalis
Bacteroides sp.
Infections
Bacterial vaginosis
Pyelonephritis
Bacteriuria

The presence of *C. trachomatis* in the cervix at the initial prenatal visit was associated with spontaneous abortion, stillbirth, preterm delivery, and LBW.[38] The mean gestational age was 35.9 weeks in those with and 39.2 weeks in those without *C. trachomatis* ($p < 0.01$).[38] LBW was also associated with *C. trachomatis* in this study. Subsequent studies by Thompson et al.,[39] Harrison et al.,[31] and Hardy et al.[40] failed to confirm an overall association between chlamydia infection and adverse pregnancy outcome. However, Harrison et al. reported that a subgroup of women with cervical *C. trachomatis* and elevated levels of IgM antibody against *C. trachomatis* (suggestive of recent acquistion or active infection) had a significantly increased risk of premature rupture of membranes, LBW, and preterm delivery.[31] Sweet et al.[35] confirmed these findings. After controlling for the presence of other sexually transmitted disease organisms in a multivariate analysis, the association was no longer significant.[35] In another investigation *C. trachomatis* remained associated with LBW and premature rupture of membranes in a multivariate analysis controlling for bacterial vaginosis and obstetric factors related to prematurity.[43] Several other recent investigations support an association of *C. trachomatis* with preterm delivery.[25, 26, 37, 42A] Thus further large-scale studies that also use serologic assessment will be required to resolve the controversy over the role of *C. trachomatis* in preterm delivery and premature rupture of membranes.

The association of *T. vaginalis* with preterm delivery has also been a source of controversy. *T. vaginalis* was not associated with LBW,[44] and treatment was not associated with an increase in birth weight or gestational age in a placebo-controlled trial.[45] However, among adolescents, the incidence of LBW was 18% in patients with versus 6.7% in those without *T. vaginalis*

Table II. Genital tract colonization of group B streptococci and preterm delivery

Series	*Preterm labor*		*Preterm delivery*		*Premature rupture of membranes*	
	GBS pos. (%)	*GBS neg. (%)*	*GBS pos. (%)*	*GBS neg. (%)*	*GBS pos. (%)*	*GBS neg. (%)*
Baker et al.[20]	11	6.3	–	–	–	–
Regan et al.[21]	–	–	5.4	1.26	15.3	7*
Minkoff et al.[22]	17	8.7	–	–	10	8.8*
Hastings et al.[23]	–	–	6.5	6.4	–	–
Bobitt et al.[26]	–	–	–	–	5.6	1.7*
Lamont et al.[24]	6	0	–	–	–	–
Martius et al.[25]	21	11	–	–	–	–
Alger et al.[27]	–	–	–	–	16	4*

GBS, Group B streptococci.
*Statistically significant difference.

($p = 0.06$).[40] Further, in the large Vaginal Infections and Prematurity Study, *T. vaginalis* carriage at midpregnancy was significantly associated with LBW and premature rupture of membranes after adjustment for confounding factors and other microorganisms (Cotch MF. Personal communication, ICAAC abstracts, 1990). Finally, the incidence of premature rupture of membranes at term was 27.5% in women with positive cultures compared with 12.8% in those with negative cultures for *T. vaginalis* ($p < 0.03$).[22]

Bacteroides sp. have also been implicated in preterm delivery and/or premature rupture of membranes. In a stepwise logistic regression analysis, women colonized with *Bacteroides sp.* at their initial prenatal visit had a 40% increased preterm delivery rate compared with that for noncolonized women ($p = 0.003$).[22] Nonfragilis *Bacteroides sp.* were more often recovered from the cervices of women in spontaneous preterm labor (21% of 72) compared with those women not in labor but at a similar gestational age (4% of 26, $p = 0.03$).[25] Black-pigmented *Bacteroides sp.* were more frequently recovered from women in preterm labor who were delivered at <37 weeks (20% of 61) compared with control women at term (8% of 155, $p < 0.05$).[26] More recently, vaginal *Bacteroides sp.* has been associated with a 60% increased risk of preterm delivery, and *Bacteroides bivius* $>10^4$ colony-forming units per milliliter was associated with a twofold increased risk of delivery at ≤34 weeks' gestation.[46, 47]

Bacterial vaginosis is a clinical condition in which the normal vaginal flora, characterized by high concentrations of lactobacilli, is replaced by high concentrations of anaerobic bacteria, especially *Bacteroides sp.* and *Mobiluncus sp.*, *Gardnerella vaginalis*, and *M. hominis*.[48] Several studies have suggested that bacterial vaginosis is associated with LBW and premature rupture of membranes.[10, 26, 43, 49] Premature rupture of membranes occurred significantly more commonly in the bacterial vaginosis group (46%) than in those without bacterial vaginosis (4%).[49] In a cohort of pregnant women, bacterial vaginosis in the second or third trimesters of pregnancy was associated with LBW and premature rupture of membranes by univariate analysis. Bacterial vaginosis remained significantly associated with preterm labor (odds ratio 2.0, $p < 0.05$) and preterm premature rupture of membranes (odds ratio 3.0, $p = 0.03$) in a multivariate analysis controlling for *C. trachomatis* and risk factors for premature delivery. No significant association was present between bacterial vaginosis and LBW in the multivariate analyses. In other studies, bacterial vaginosis with preterm labor[43] or preterm delivery[26] with an odds ratio of 2 to 3. In the only cohort study in which patients were enrolled early in pregnancy (first prenatal visit), bacterial vaginosis was associated with a trend for an association with preterm labor (50% vs 29%), but these results were not statistically significant.[22]

Untreated acute pyelonephritis is consistently associated with approximately a 30% risk of preterm labor and delivery. Asymptomatic bacteriuria (other than with group B streptococci) has been less consistently associated with an increased risk of LBW.[50-67] Only five of the 17 cohort studies that assessed birth weight reported a significantly increased incidence of prematurity (LBW) in women with asymptomatic bacteriuria.[51, 53, 54, 61, 63] However, in a meta-analysis in which weighting of studies was performed, women with asymptomatic bacteriuria had a 60% higher rate of LBW than nonbacteriuric women (95% confidence interval 1.4 to 1.9).[68] Similarly disagreement exists among the four cohort studies that assessed gestational age as a complication of asymptomatic bacteriuria.[52, 59-61] Two studies demonstrated a significantly increased preterm delivery rate in patients with asymptomatic bacteriuria,[59, 60] while two others did not.[52, 61] In a meta-analysis of all four studies, women with asymptomatic bacteriuria had a 90% higher preterm delivery rate than nonbacteriuric women (95% confidence interval 1.3 to 2.9).[68]

A large number of studies have addressed the issue

Table III. Association of chorioamnion infection* with histologic chorioamnionitis

Series	No.	*Chorioamnion infection*		Odds ratio	95% Confidence interval
		Chorioamnionitis present (%)	*Chorioamnionitis absent (%)*		
Hillier et al.[10]	112	61	23	3.8	1.6-9.9
Pankuch et al.[72]	75	82	15	14	3.6-60
Svensson et al.[73]	87	70	45	2.8	0.6-15.4
Quinn et al.[74]	43	71	28	6.6	1.3-35.3
Zlatnick et al.[75]	95	51	27	3.4	1.2-10.0

*Defined as a positive culture.

of whether maternal genital tract colonization is associated with preterm labor, preterm delivery, or premature rupture of membranes. The results are often conflicting. *N. gonorrhoea* and pyelonephritis have been highly related to preterm birth but their occurrence is infrequent. *U. urealyticum* in the genital tract is not associated with premature delivery. For the remaining lower genital tract microorganisms mentioned and for asymptomatic bacteriuria, the association with prematurity, where found, is low (relative risk 1.4 to 2) and perhaps confined only to subsets. For all of these microorganisms, a more convincing cause-and-effect relationship would exist if the microorganism could be isolated from the amniotic fluid or placenta if women who are delivered of LBW infants have premature rupture of membranes.

Histologic chorioamnionitis and prematurity

Histologic chorioamnionitis, defined as inflammation of the extraplacental membrane, has been consistently linked with prematurity, LBW, and premature rupture of membranes. Histologic chorioamnionitis is detected in 19% to 74% of placentas from preterm deliveries and in 4% to 16% of placentas from term deliveries.[10, 69-71] Although mothers with histologic chorioamnionitis usually have no clinical evidence of infection, their infants have increased rates of sepsis and death.[70] The role of infection in the etiology of histologic chorioamnionitis has been investigated in several recent studies. In both preterm and term placentas, microorganisms have been recovered in 51% to 71% of placentas with 23% to 45% of those without histologic chorioamnionitis (Table III).[10, 72-75] The relationship between histologic chorioamnionitis and infection (positive cultures) of the chorioamnion is strongest among preterm deliveries[10] while it is less strong in term placentas.[76]

In spite of the strong statistical associations between chorioamnionitis and placental membrane infection, 18% to 49% of placentas with histologic evidence of chorioamnionitis are culture negative. This may reflect lack of culture sensitivity or a nonmicrobial cause of inflammation. From 15% to 45% of infected membranes are free of inflammation (Table III). Chorioamnion infection without inflammation may be related either to infection that starts at or near the time of delivery (before an inflammatory response is produced) or to contamination by vaginal bacteria. The infrequency of lactobacilli (a common vaginal contaminant) in placental cultures argues against frequent contamination (Table IV).

Microorganisms most commonly isolated from the chorioamnion include *U. urealyticum*, facultative and anaerobic gram-positive cocci, *G. vaginalis*, and *Bacteroides sp.* (Table IV). Surprisingly, coliforms are uncommon isolates. Likewise, *Haemophilus influenzae* and *N. gonorrhoeae* are rarely recovered from the chorioamnion even though these organisms can cause chorioamnionitis when they are present.[77, 78] In spite of widespread attempts, *C. trachomatis* is rarely recovered from the placenta. Fastidious anaerobes including *Mobiluncus* and *Fusobacterium sp.* may be underrepresented in current studies because of difficulties in isolating these organisms. Thus species of the vagina are found among microorganisms causing placental infection, but many such species appear to have tropism for the placenta.

While the association of histologic chorioamnionitis and prematurity has been well described, the effects of chorioamnion infection on the neonate are less understood. Histologic chorioamnionitis has been associated with an increased perinatal death rate, and infants born to women with chorioamnionitis have had a high prevalence of elevated antibody to *U. urealyticum*.[74] The effects of chorioamnion infection on neonatal outcome require further study to determine which placental pathogens present the greatest risk for poor neonatal outcome.

How does chorioamnion infection lead to premature rupture of membranes and/or preterm delivery? Schoonmaker et al.[79] have demonstrated that group B streptococci significantly decreased placental membrane integrity (i.e., lower bursting tension, less work to rupture, and less elasticity) in vitro, suggesting that placental infection could predispose to membrane rupture. Bernal et al.[80] have demonstrated that the fetal membranes from women with chorioamnionitis produce significantly higher levels of prostaglandin E_2 in vitro. It is likely that production of prostaglandin E_2 is

Table IV. Microorganisms recovered from chorioamnion infection

	Chorioamnion culture (%)					
	Hillier et al.[10] *(n = 112)*	*Pankuch et al.*[72] *(n = 75)*	*Svensson et al.*[73] *(n = 87)*	*Quinn et al.*[74] *(n = 43)*	*Zlatnick et al.*[75] *(n = 95)*	*Overall (N = 412)*
Aerobes						
Group B streptococci	4	5	3	2	5	4
Enterococci	0	5	1	0	1	2
Viridans streptococci	2	0	14	0	4	5
Staphylococci	0	12	16	2	0	6
G. vaginalis	13	0	0	0	4	4
Lactobacilli	1	0	0	0	0	0.2
Coliforms	0	1	2	2	1	0.7
Anaerobes						
Bacteroides sp.	4	7	2	4	4	4
Peptostreptococcus sp.	4	20	6	0	8	8
Genital mycoplasmas						
M. hominis	6	ND	ND	2	4	5
U. urealyticum	29	ND	ND	28	22	26
Yeast	0	1	2	0	0	0.7

ND, Not done.

significantly higher in women with chorioamnion infection. Lamont et al.[81] have demonstrated the *Bacteroides*-conditioned media–stimulated production of prostaglandin E_2 by human amnion cells in vitro. These authors suggested that bacterial phospholipase releases arachidonic acid from the amnion, leading to prostaglandin E_2 synthesis. Whereas further studies of how chorioamnion infection may cause preterm delivery are needed, the published data suggest that chorioamnion infection is strongly related to inflammation and weakening of the placental membrane and to stimulation of prostaglandin production by the amnion.

Amniotic fluid culture in premature labor

Further evidence to link prematurity with infection comes from amniotic fluid cultures of patients with intact membranes in preterm labor. In view of differences in sample size, in definition of preterm labor, and in microbiologic techniques, it is not surprising that the percent of patients with positive cultures varied widely from 0% to 30%[82-91] (Table V). In a recent large series, positive cultures were present in 11% of 264 women in preterm labor but in 22% of 111 women who were delivered within 24 to 48 hours of admission.[87] Patients in premature labor and with a positive amniotic fluid culture are usually delivered within 24 to 48 hours in contrast to culture-negative patients who tend to be delivered >30 days later.[87, 89]

These data provide strong circumstantial, but not strong causal, evidence that infection causes the preterm delivery. Difficulties in demonstrating a cause-and-effect relationship would require providing evidence (1) that infection preceded labor rather than vice versa, (2) that cultures are less frequently positive in comparable patients in term labor, and (3) that antibiotic therapy of those infected reduces preterm delivery.

Indirect "markers of infection" in premature labor

Amniotic fluid cultures may be an insensitive test to detect infection that begins in the decidua or amnion. In fact, a high rate of positive chorioamnion cultures occurs in preterm births.[10] Other "markers" of infection that have been assessed in patients in preterm labor include elevated C-reactive protein levels in maternal sera and short-chain organic acids (produced by bacterial metabolism) in amniotic fluid, detected by gas-liquid chromatography. Elevated serum C-reactive protein levels are part of the acute-phase reaction. Most women (86% to 88%) in preterm labor and with high C-reactive protein values do not respond to tocolytics.[92-94] In contrast, most patients with a normal C-reactive protein level responded to tocolytics (77% to 94%).[92-94]

Detection of abnormal organic acids in amniotic fluid was reported in all of six women in preterm labor, but in comparison none of five normal women not in labor had abnormal amniotic fluid acid levels. Only one had a positive culture. The authors suggested that the origin of these abnormal organic acids is bacterial growth in the uterine cavity.[95] The report of Wager et al.[96] did not confirm the value of the gas-liquid chromatography in this setting.

Other biochemical markers are discussed in the next section.

Biochemical mechanisms linking prematurity and infection

Prostaglandins. Until recently, prostaglandins have been considered the universal mediators of parturition in mammalian species.[97-101] Traditional evidence that supports the participation of prostaglandins in the mechanism of labor in humans includes the following: (1) Administration of prostaglandins results in abortion

or labor; (2) treatment with prostaglandins inhibitors delays the process of midtrimester abortion and the onset of labor and can arrest preterm labor; (3) parturition at term is associated with elevated amniotic fluid and maternal plasma concentrations of prostaglandins; (4) arachidonic acid (prostaglandin precursor) concentrations in the amniotic fluid increase during labor; (5) intraamniotic administration of arachidonic acid results in labor.

The evidence supporting a role for prostaglandins in the mechanisms responsible for preterm labor is less firm than for term labor.[101-111] In both plasma and amniotic fluid, levels of prostaglandins have been either normal[100-102] or increased[103, 104] in women in preterm labor. The discrepancies between these studies may be attributed to the heterogeneous nature of diseases causing preterm labor. Patients with preterm labor and microbial invasion of the amniotic cavity have significantly higher amniotic fluid concentrations of prostglandin E_2 and $F_{2\alpha}$ and their stable metabolites (bicycloprostaglandin E_2 and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$) than women in preterm labor with negative amniotic fluid cultures.[105-111] In contrast, amniotic fluid concentrations of prostaglandins E and $F_{2\alpha}$ are not different between women in preterm labor with negative amniotic fluid cultures and women without labor with negative cultures at similar gestational ages.[108] These findings are consistent with the observation that the production of prostaglandins by amnion and chorion-decidua is higher in patients with preterm labor and histologic chorioamnionitis than in patients with no evidence of placental inflammation. These data suggest an increased bioavailability of prostaglandins in preterm parturition associated with infection. It remains to be proved whether prostaglandins play a role in preterm labor not associated with infection.

Arachidonate lipoxygenase metabolites. Metabolites of arachidonic acid derived through the lipoxygenase pathway involving leukotrienes and hydroxyeicosatetraenoic acids have also been implicated in the mechanisms of spontaneous parturition at term.[112-117] Arachidonate lipoxygenase products are inflammatory mediators, so it is possible that they also participate in the mechanisms of preterm labor associated with infection. Concentrations of 5-hydroxyeicosatetraenoic acid, leukotriene B_4, and 15-hydroxyeicosatetraenoic acid are increased in the amniotic fluid of women with preterm labor and with microbial invasion of the amniotic cavity.[118-120] Similarly, amnion from patients with histologic chorioamnionitis releases more leukotriene B_4 in vitro than amnion from women delivered preterm without inflammation.[121]

The precise role of arachidonate lipoxygenase products in parturition in association with infection remain to be determined. 5-Hydroxyeicosatetraenoic acid and leukotriene C_4 may stimulate uterine contractility, and leukotriene B_4 may recruit neutrophils to the site of infection and participate in the regulation of the cyclooxygenase pathway.[122, 123] Leukotriene B_4 has been shown to act as a calcium ionophore[124] and thus may increase phospholipase activity and enhance the rate of prostaglandin synthesis by intrauterine tissues.

Table V. Amniotic fluid culture results from patients in premature labor

Series and year	*No.*	*Positive (%)*
Harger et al.,[91] 1991	38	0
Weibel and Randall,[82] 1985	35	3
Wahbeh et al.,[83] 1984	27	4
Duff and Kopelman,[84] 1987	24	4
Skoll et al.,[90] 1989	127	6
Leigh et al.,[85] 1986	59	12
Miller et al.,[86] 1980	15	20
Romero et al.,[87] 1989	264	20
Bobitt et al.,[88] 1981	29	21
Gavett et al.,[89] 1986	54	24

Bacterial products. For the past decade the explanation of the onset of labor in the setting of infection has been that bacterial products directly stimulate prostaglandin biosynthesis[125, 126] and bacterially conditioned media stimulate prostaglandin production by human amnion.[127-129] Although the specific product(s) responsible for this effect has not been established, one candidate is endotoxin (lipopolysaccharide). Lipopolysaccharide is a component of the wall of gram-negative bacteria, and it can stimulate prostaglandin production by macrophages, amnion,[130] and decidua.[131] Lipopolysaccharide has been found in the amniotic fluid of women with intraamniotic infections,[132, 133] and in cases of preterm premature rupture of membranes, amniotic fluid concentrations of endotoxin are higher in patients in labor than in patients not in labor.[134] Other bacterial products that may stimulate prostaglandin production are peptidoglycans in cases with gram-positive bacterial infection.

However, bacterial products alone may not be responsible for the onset of preterm labor in the setting of infection. One third of women with preterm premature rupture of membranes without labor have organsims in the amniotic cavity, suggesting that the presence of microorganisms in the amniotic cavity per se is not sufficient to produce labor. Further, the effects of microbial products on prostaglandin production by intrauterine tissues are highly dose dependent and may even be inhibitory.[135] The quantitative aspects of these biologic effects appear important. For example, the concentrations of lipopolysaccharide required to elicit prostaglandin production by amnion cells are rarely present in the amniotic cavity.[134]

Cytokines. The traditional view has been that through the release of toxins or enzymes the microorganisms were directly responsible for the metabolic

derangements associated with infection. It has now been established that endogenous host products secreted in response to infection are responsible for many of the effects of infection. In endotoxin shock, for example, bacterial endotoxins exert their deleterious effect through the release of endogenous mediators such as tumor necrosis factor and interleukin-1.

Parturition in the setting of infection may be signaled by secretory products of macrophage activation (monokines), including interleukin-1, tumor necrosis factor, and interleukin-6. The first monokine implicated in the onset of labor in the setting of infection was interleukin-1: (1) Interleukin-1 stimulates prostaglandin production by amnion and decidua in vitro[136]; (2) human decidua can produce interleukin-1 in vitro in response to bacterial products[137, 138]; (3) amniotic fluid interleukin-1 bioactivity and concentrations are elevated in patients with preterm labor and bacteria in the amniotic cavity,[139] but interleukin-1 is not present in fluid from patients with preterm labor and no bacteria; (4) among patients with preterm premature rupture of membranes and bacteria in the amniotic cavity, amniotic fluid interleukin-1 bioactivity and concentrations are higher in patients with than in those without labor,[139] indicating it is not the presence of microorganisms per se but rather the host response that is associated with parturition; (5) in vitro perfusion of human uteri with interleukin-1 elicits uterine contractility (unpublished data); (6) administration of interleukin-1 to pregnant animals leads to abortion and preterm labor (Romero R, Mazor M, Tartakovsky B. Unpublished observations).

Tumor necrosis factor, a cytokine released during macrophage activation, is also capable of stimulating prostaglandin production by several cell types. Evidence supporting a role of tumor necrosis factor in human parturition in the setting of infection includes: (1) Tumor necrosis factor stimulates prostaglandin production in vitro by human amnion and decidua[131, 140]; (2) tumor necrosis factor is produced by human decidua in response to bacterial products[141, 142]; (3) tumor necrosis factor is absent in normal amniotic fluid but present in the amniotic fluid of patients with intraamniotic infection and preterm labor[140]; (4) tumor necrosis factor can induce premature labor when administered to pregnant mice (Romero R. Unpublished observations).

Interleukin-6 is also a major mediator of the host response to infection. Interleukin-6 is produced by a variety of cells including macrophages and endometrial stromal cells. Major change in the biochemical, physiologic, and immunologic status of the host occurs from interleukin-6, including an acute-phase plasma protein response and the induction of other cytokines such as interleukin-1 and tumor necrosis factor. Women in preterm labor with intraamniotic infection had higher amniotic fluid levels of interleukin-6 than women in preterm labor without intraamniotic infection.[143, 144]

Macrophage colony-stimulating factor is a cytokine that is capable of regulating the number of macrophages and their level of activation. Macrophage-colony stimulating factor is produced by human decidual explants in response to lipopolysaccharide and it is present in the amniotic fluid of women in preterm labor with intraamniotic infection.

Noncytokine bioactive agents secreted during the inflammatory process may also participate in this process. Platelet-activating factor is a lipid that is present in the amniotic fluid of women with preterm labor. This compound is capable of stimulating prostaglandin E_2 production by amnion and of directly stimulating myometrial contractions.

This evidence suggests that the host products of macrophage activation (cytokines) play a role in the mechanism of human parturition in the setting of infection. Systemic maternal infections, such as pyelonephritis, or localized infections, such as deciduitis, could signal parturition through monocyte-macrophage activation in peripheral blood and decidua. Preterm labor can be viewed as an event occurring when the intrauterine or maternal environment is hostile and threatens the well-being of the fetus. The initiation of preterm labor in the setting of infection may be considered to have survival value.

Antibiotic trials to prevent premature birth

With the evidence that subclinical infection is a major cause of preterm birth, the logical extension is to conduct antibiotic intervention trials in an attempt to prevent prematurity. Trials to date have generally been of three designs: (1) antibiotic trials conducted antepartum in patients at high risk for preterm delivery; (2) antibiotic trials among women with premature labor but with intact membranes, as adjuncts to standard tocolytic therapy; and (3) antibiotic trials among women with preterm premature rupture of membranes but without preterm labor.

In the first antepartum antibiotic trial, patients with genital mycoplasmas in the lower genital tract were enrolled in a trial of erythromycin versus placebo for 6 weeks.[30, 145] Women in the erythromycin group, as compared with the placebo group, had a significantly higher mean birth weight (3331 vs 3187 gm, $p = 0.04$) and a lower rate of LBW (3% vs 12%, $p = 0.06$). In a study sponsored by the National Institutes of Health conducted at five centers, >900 patients with lower genital tract *U. urealyticum* were randomized to either erythromycin or placebo from the twenty-eighth through the thirty-fifth week of gestation.[42] Patients in the erythromycin group, compared with the placebo group, ex-

Table VI. Summary of antibiotic trials to delay delivery in preterm labor

Series and year	No.	Regimen	Significant results in antibiotic group			
			Delay in delivery	Increase in birth weight	Increase in term delivery	Decrease in perinatal mortality
McGregor et al.,[149] 1986	17	Erythromycin orally for 7 days	Yes	$p = 0.07$	No	No
Newton et al.,[150] 1989	95	Ampicillin intravenously for 2 days plus erythromycin for 4 days	No	No	No	No
Morales et al.,[151] 1988	150	Ampicillin orally or erythromycin orally, either for 10 days	Yes	No	Yes*	No
Winkler et al.,[152] 1988	19	Erythromycin orally for 7 days	Yes	No	Not reported	No

*For ampicillin versus placebo.

perienced no improvement in any of the following measures: rate of LBW, premature labor, preterm delivery, or preterm premature rupture of membranes. In the third study 229 patients at high risk for preterm delivery were randomized to a 7-day course of erythromycin or placebo.[146] No significant differences were present between the erythromycin and the placebo groups in preterm birth (7% vs 8%), mean birth weight (3132 ± 588 vs 3099 ± 548 gm), or preterm premature rupture of membranes (1.7% vs 4.6%). A significant reduction was observed in premature rupture of membranes at term between the groups (6% vs 16%, $p = 0.01$).

In two studies patients with cervical *C. trachomatis* were assessed for the effect of antibiotic treatment of pregnancy outcome. Patients successfully treated for *C. trachomatis* ($n = 244$) had significantly lower rates of premature rupture of membranes, premature labor, and small-for-gestational-age infants when compared with those who failed to respond ($n = 79$).[147] In a second report pregnancy outcome was studied in *C. trachomatis*–positive untreated patients ($n = 1110$), *C. trachomatis*–positive treated patients ($n = 1327$), and *C. trachomatis*–negative women ($n = 9111$). The untreated *C. trachomatis*–positive group had significantly increased rates of premature rupture of membranes and LBW and lower survival rates compared with those of both the treated *C. trachomatis*–positive group and those with negative cultures.[148] However, retrospective nonrandomized studies such as these are less convincing because of possible confounding factors.

In the first trial of the adjunctive use of antibiotics in patients in premature labor with intact membranes, a subgroup of patients with cervical dilatation between 1 and 5 cm who received adjunctive erythyromycin, as compared with the placebo group had significant prolongation of pregnancy (32 ± 11 vs 22 ± 7 days, $p = 0.02$), a significant improvement in patients delivered at term (87% vs 33%, $p = 0.04$), and an improvement in birth weight (2943 ± 483 vs 2615 ± 459 gm, $p = 0.07$).[149] Three additional studies of similar design have been reported.[150-152] In patients in preterm labor who received either ampicillin plus erythromycin for 4 days or placebo, there was no decrease in preterm birth (38% in the antibiotic group vs 44% in the placebo group).[150] In patients in idiopathic preterm labor randomized to one of the following regimens: ampicillin orally for 10 days, erythromycin orally for 10 days, or no antibiotic,[151] those receiving either ampicillin or erythromycin had significant prolongation of the pregnancy (31.7 days for ampicillin vs 28.5 days for erythromycin and 16.6 days for controls, $p < 0.05$) and patients receiving ampicillin compared with those receiving placebo also had a significantly higher rate of term delivery (45% vs 15%, $p < 0.05$). Among patients in idiopathic preterm labor randomized to either erythromycin for 7 days or placebo, a significant prolongation of the pregnancy occurred in the erythromycin group versus the placebo group (43 ± 25 vs 10 ± 19 days, $p < 0.05$).[152] Although there may be several reasons to explain the inconsistency of antibiotics in delaying delivery, there appear to be differences regarding the investigators' "intent to treat." For example, in the only "negative study" a high percentage of enrolled women were analyzed[150] whereas in the "positive studies" the percentage of enrolled patients ultimately analyzed was small.[149, 151] Even when antibiotic treatment has led to a prolongation of pregnancy, its use has not been accompanied by a consistent increase in birth weight and has not led to a decrease in perinatal mortality (Table VI).

Several recent randomized antibiotic trials have been conducted among patients with preterm premature rupture of membranes (Table VII). The hypothesis of these studies was that subclinical infection either causes premature rupture of membranes as a primary event

Table VII. Summary of antibiotic trials to delay delivery after preterm premature rupture of membranes

Series and year	No.	Regimen	Significant results in antibiotic group			
			Delay in delivery	Increase in birth weight	Increase in term delivery	Decrease in perinatal mortality
Amon et al.,[153] 1988	83	Ampicillin intravenously, then orally until delivery	Yes	No	No	No
Morales et al.,[154] 1989	82	Ampicillin intravenously "pending cultures"	Yes	No	No	No
Johnston et al.,[155] 1990	85	Mezlocillin intravenously, ampicillin orally until delivery	Yes	Yes	Yes	No
Christmas et al.,[156] 1990	56	Ampicillin gentamicin, clindamyin intravenously for 1 day, amoxicillin and clavulanic acid for 7 days	Yes	No	No	No

or causes the premature labor that ensues after preterm premature rupture of membranes. A significant increase was observed in pregnancies delivered >1 week after premature rupture of membranes in the ampicillin group versus the no-ampicillin group (47% vs 26%, $p = 0.05$).[153] This prolongation of pregnancy was not accompanied by a significant difference between groups in birth weight, gestational age at delivery, or hospital stay >30 days. In another report patients with preterm premature rupture of membranes were given betamethasone and randomized to ampicillin or no ampicillin.[154] No significant difference was found between those who had and those who had not received ampicillin in birth weight or in survival (93% vs 89%). Patients receiving ampicillin compared with those not receiving ampicillin had significantly less clinical amnionitis (4% vs 26%, $p < 0.01$). In the third study neither corticosteroids nor tocolytics were given to patients with preterm premature rupture of membranes.[155] Patients randomized to antibiotics versus placebo had a significant prolongation of pregnancy ≥7 days (45% vs 18%, $p < 0.01$), a significant increase in birth weight (1897 ± 600 vs 1587 ± 592 gm, $p < 0.05$), and a decrease in neonatal hospital stay >30 days (18% vs 38, $p < 0.05$). No significant differences were reported in perinatal mortality. In a smaller study antibiotic therapy compared with expectant management of patients with preterm premature rupture of membranes was associated with prolongation of pregnancy ≥8 days (36% of antibiotic groups vs 10% of expectant group, $p < 0.05$) but with no significant difference in mean birth weight, perinatal mortality, or respiratory distress syndrome.[156] These authors concluded that intervention with antibiotics delayed labor but offered no appreciable perinatal advantage.

In addition to these randomized trials there have been several interesting, innovative reports regarding the use of antibiotics in premature rupture of membranes. Successful eradication of "bacterial colonization" of the amniotic fluid was achieved by parenteral maternal antibiotic therapy in a preterm gestation with premature rupture of the membranes at 29 weeks' gestation.[57] Transcervical infusion of antibiotics into the amniotic cavity has occurred in patients with preterm premature rupture of membranes.[158, 159] Substantial antibiotic concentrations were achieved in the amniotic fluid, but the safety and efficacy of this approach have not been established.

In conclusion, current antibiotic trials allow no definite conclusion as the efficacy of antibiotics in prolonging pregnancy in patients with either preterm labor or preterm premature rupture of membranes. These discordant results may result from population differences. Antibiotics would be expected to have no effect, for example, among the subset of women with preterm labor–premature rupture of membranes if the cause is not infection. Differences in analysis of eligible patients also may have been important. For example, a positive study might be more likely if the investigators limited analysis to patients who completed versus those who did not complete the antibiotic course. Additional trials seem appropriate in view of results to date. However, randomized trials need to be conducted in selected groups at high risk for preterm delivery and/or infection or preferably in groups with specific infections or with evidence of preexisting infection such as histologic chorioamnionitis.

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