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***Ureaplasma* species: Role in Diseases of Prematurity**

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SYNOPSIS

There is accumulating epidemiologic and experimental evidence that intrauterine and/or postnatal infection with *Ureaplasma* species is a significant risk factor for adverse pregnancy outcomes and complications of extreme preterm birth such as BPD and intraventricular hemorrhage. In a cohort of very low birth weight infants, *Ureaplasma* spp. were detected by culture or PCR in respiratory secretions, blood, and/or CSF of almost half of the subjects, suggesting that this organism is the most common pathogen affecting this population. This review will summarize the evidence supporting the hypothesis that *Ureaplasma*-mediated inflammation in different compartments (intrauterine, lung, blood, and/or brain) during a common developmental window of vulnerability contributes to preterm labor and lung and brain injury. Appropriate methods for detecting these fastidious organisms and potential strategies to prevent or ameliorate the effects of *Ureaplasma* infection are discussed.

Keywords

Ureaplasma urealyticum; *Ureaplasma parvum*; intrauterine infection; prematurity; bronchopulmonary dysplasia; intraventricular hemorrhage

Ureaplasma parvum (serotypes 1, 3, 6, and 14) and *U. urealyticum* (serotypes 2, 4, 5, 7–13) are closely related species of the *Mollicutes* class that are among the smallest free-living, self-replicating cells^{1–3}. All serovars lack cell walls, exhibit limited biosynthetic abilities, hydrolyze urea to generate ATP, and adhere to human mucosal surfaces³. *U. parvum* is the most common species isolated from clinical specimens^{3, 4}. Although *Ureaplasma* spp. are not typically considered pathogens in early onset neonatal sepsis, they have been implicated in complications of human pregnancy and neonatal outcomes. As observed by Volgmann *et al.*⁵ in the title, of a recent review, *Ureaplasma* is either a “harmless commensal or underestimated enemy of human reproduction”. This review will address this controversy and review evidence that the timing, duration, and intensity of the inflammatory response to *Ureaplasma* infection are major determinants of pregnancy and neonatal outcomes.

Epidemiology

Ureaplasma spp. are the most common bacteria implicated in human urogenital infections, including non-gonococcal urethritis in men and complications of pregnancy in women⁵. *Ureaplasma* spp. can be detected in vaginal flora in 40% of sexually inactive and 67% of

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sexually active women of reproductive age, and 25% of postmenopausal women⁶. Despite the detection of viable *Ureaplasma* on toilets for up to 2 hours⁷, infection from exposure to contaminated surfaces is unlikely. Known transmission routes involve sexual contact or maternal-infant transfer.

Impact of *Ureaplasma* Urogenital Tract Colonization on Reproduction

Because *Ureaplasma* is a commensal in the adult female genital tract, it has been considered of low virulence. However, *Ureaplasma* urogenital tract colonization has been causally linked to infertility^{8, 9}, early pregnancy loss¹⁰, stillbirth^{11, 12}, and preterm birth^{13–17}. The relationship with these outcomes remains controversial because a causal relationship has been difficult to prove for each condition due to 1) accuracy of differentiating the presence of bacteria as specimen contaminants vs. pathogens, 2) the polymicrobial nature of vaginal flora, 3) difficulty in detecting *Ureaplasma* and other fastidious microbes¹⁸, and 4) the contribution of other factors to the pathophysiology of these complex disorders. *In vitro* and *in vivo* experimental models have provided additional evidence in support of a role for *Ureaplasma* in these obstetrical disorders.

Ureaplasma Intrauterine infection/ Chorioamnionitis

Intrauterine infection has been implicated as a major cause of preterm birth, especially in gestations < 30 weeks¹⁹. *Ureaplasma* spp. are the most common organisms isolated from amniotic fluid obtained from women who present with preterm labor (POL) with intact membranes^{20, 21}, preterm premature rupture of membranes (pPROM)²², and short cervix associated with microbial invasion of the amniotic cavity²³, and from infected placentas²². The prevalence of infected amniotic fluid with cultivated *Ureaplasma* as the only microbe ranges from 6–9% for pregnancies complicated by POL with intact membranes^{21, 24} to 22% for a cohort of women with POL or pPROM²⁵. Detection of cultivated *Ureaplasma* in placental chorion in pregnancies producing VLBW infants ranges from 6–10% in homogenized frozen tissue^{26, 27} to 28% in fresh tissue and is inversely related to gestational age¹³. Recovery of *Ureaplasma* from the chorion increased with duration of rupture membranes, suggesting an ascending route of infection¹³. However, *Ureaplasma* has also been detected in 22% of placentas with duration of rupture of membranes less than one hour, suggesting the possibility of a pre-existing infection. Indeed, *Ureaplasma* species have been detected in amniotic fluid as early as the time of genetic amniocentesis (16–20 weeks) in up to 13% asymptomatic women^{16, 28–30}. Placentas with the lowest rate of *Ureaplasma* recovery were from women delivered for preeclampsia or intrauterine growth restriction¹³. Recent analysis of amniotic fluid from women with preterm labor and intact membranes using advanced molecular techniques has demonstrated a greater prevalence (15%) and diversity of microbes including those uncultivated and uncharacterized compared to culture or species-specific PCR studies¹⁸. In this study, only half of *Ureaplasma*-positive amniotic fluid samples were identified by culture with increased detection by combined culture and PCR.

The presence of *Ureaplasma* as the only identified microbial isolate in the upper genital tract is significantly associated with adverse pregnancy outcomes, including premature delivery, neonatal morbidity, and perinatal death^{13, 21, 22, 25}. Experimental models of intrauterine *Ureaplasma* infection in mice³¹, sheep^{32, 33}, and non-human primates³⁴ have been described. Intra-amniotic inoculation of *U. parvum* did not stimulate preterm labor in mice or sheep, but did stimulate progressive uterine contractions and preterm delivery in Rhesus macaques inoculated at 136 d gestation (80% term)³⁴, suggesting species differences in the host response or serovar differences in virulence. The Rhesus macaque model is the first experimental model to definitely show a causal link between *Ureaplasma* intrauterine infection and POL.

***Ureaplasma*-mediated Inflammation and Preterm Labor**

There is accumulating evidence that intrauterine infection-induced preterm labor is the result of an inflammatory cascade initiated by bacterial interaction with host pattern recognition receptors (PRR) such as the toll-like receptors³⁵. Following engagement with PRRs, there is a sequential rise in inflammatory cytokines such as IL-1 β , TNF- α , IL-6, and IL-8 followed by leukocyte recruitment, increases in prostaglandins such as PGE2 and PGF2- α and matrix metalloproteinases. These mediators contribute to uterine contractions, cervical dilatation and effacement, and membrane rupture³⁵.

In the presence of pPROM, cultivated *Ureaplasma* as the sole microbe was associated with increased leukocytes and proinflammatory cytokines (IL-6, IL-1 β and TNF- α) in amniotic fluid and increased cord blood IL-6 concentrations, indicating a robust inflammatory response to this infection. While the majority of women in whom subclinical *Ureaplasma* amniotic cavity infection is detected midtrimester deliver at term¹⁶, those with elevated amniotic fluid IL-6 levels have increased risk for adverse pregnancy outcome including fetal loss and preterm delivery³⁶. In the Rhesus *U. parvum* intrauterine infection model, uterine activity was preceded by a rise in amniotic fluid leukocytes, inflammatory cytokines, prostaglandins PGE2 and PGF2 α and matrix metalloproteinase (MMP)-9, demonstrating that *Ureaplasma* alone stimulates the mediators of preterm labor³⁴. In contrast, heat-killed *U. parvum* serotype 1 failed to stimulate a significant increase in cytokine and PGE2 response in fetal membrane explants derived from term placentas. High inoculum (10⁶ color changing units (CCU)/ml), but not low inoculum (10² to 10⁴ CCU/ml) heat-killed *U. urealyticum* serotype 8 stimulated TNF- α , IL-10 and PGE2 production by choriodecidual explants *in vitro*. The apparent low virulence of *Ureaplasma* serotypes in these *in vitro* studies may be due, in part, to the use of laboratory reference strains from the American Tissue Culture Collection (ATCC, Manassas, VA) rather than more virulent clinical isolates, or killed rather than live organisms. Alternatively, a decreased capacity to stimulate an inflammatory response in the intrauterine compartment may allow *Ureaplasma* infections to persist for long periods of time. Figure 1 summarizes the inflammatory pathways initiated by *Ureaplasma* infection that may contribute to preterm labor.

Association of *Ureaplasma* spp. and Neonatal Outcomes

The timing and duration of fetal exposure to *Ureaplasma* and the intensity of the maternal and fetal inflammatory responses may be variable, but these factors likely contribute to the impact of *Ureaplasma* on neonatal outcomes. The rate of *Ureaplasma* respiratory tract colonization increases with duration of rupture of membranes^{37, 38}, indicating that for the majority of cases, *Ureaplasma* is likely vertically transmitted from mothers to their infants just prior to or at the time of preterm birth as the result of an ascending infection from the lower genital tract. However, in a prospective cohort from our institution, we observed that 23% of preterm infants with *Ureaplasma* respiratory tract colonization (unpublished observations), and 28% of infants with invasive *Ureaplasma* detected in blood or CSF by PCR³⁹ experienced rupture of membranes <1 h, suggesting that colonization/infection in these infants was the result of pre-existing (intrauterine) infection^{40, 41}. This is in agreement with Dammann *et al.*⁴⁰ who noted that rupture of membranes was <1 h in 31% of pregnancies with *Ureaplasma* culture-positive placentas. In our cohort, 78% of placentas of infants with *Ureaplasma* respiratory tract colonization and duration of ROM <1 h showed evidence of histologic chorioamnionitis, in contrast to 36% of culture-negative infants with duration of ROM <1 h. The placentas of *Ureaplasma*-positive infants in this group were more likely to have advanced maternal and fetal stages, indicating a more long-standing infection⁴².

These data suggest that fetal exposure to *Ureaplasma* spp may occur early in pregnancy and may be sustained during critical periods of development. We propose that *Ureaplasma*-induced injury to the preterm lung and brain occurs during a common developmental-dependent

window of vulnerability and is mediated by the inflammatory response in placental, blood, lung, and CNS compartments⁴³. During the period from 23 to 32 weeks gestation, the lung and brain share a vulnerability to injury mediated by infection/inflammation^{44–46}. During this period of saccular lung development^{45, 46} and predominance of the oxidative stress sensitive pre-oligodendrocyte in the fetal brain^{47–49}, exposure of the fetus to intrauterine *Ureaplasma* infection-mediated inflammation and post-natal interactions with other inflammatory stimuli may alter developmental signaling. The evidence supporting this hypothesis and potential mechanisms are reviewed in the following sections.

***Ureaplasma* spp and Neonatal Lung Injury**

The rate of respiratory tract colonization with *Ureaplasma* in infants <1500g birth weight ranges from 20–45%, depending on study entry criteria, frequency of sampling and detection methods^{50, 51}. In a recent cohort of infants <33 wks gestation, *Ureaplasma* spp were detected during the first week of life in tracheal aspirates or nasopharyngeal specimens in 35% of infants³⁹. *Ureaplasma* respiratory tract colonization is associated with a peripheral blood leukocytosis⁵² and early radiographic emphysematous changes of bronchopulmonary dysplasia (BPD)^{53, 54}. These findings may be explained, in part, by an *in utero* onset of the inflammatory response and lung injury.

The contribution of *Ureaplasma* respiratory tract colonization to the development of BPD has been debated; however, a meta-analysis of 17 clinical studies published before 1995 supported a significant association between *Ureaplasma* respiratory tract colonization and development of BPD defined as oxygen dependence at 28 to 30 d postnatal age⁵⁰. In a meta-analysis of 36 published studies involving ~3000 preterm infants, Schelonka *et al.*⁵¹ observed a significant association between *Ureaplasma* respiratory colonization and development of BPD whether defined as oxygen-dependence at 28 d or at 36 weeks post menstrual age (PMA). Studies published since the last meta-analysis support the *Ureaplasma* respiratory colonization-BPD association^{55, 56}, particularly for the subset of *Ureaplasma*-colonized infants exposed to chorioamnionitis and leukocytosis at birth⁵⁶.

Evidence from studies of human preterm infants^{57–59}, and intrauterine infection models in mice³¹, sheep^{32, 33}, and non-human primates^{34, 60} support that *Ureaplasma* infection is pro-inflammatory and pro-fibrotic and results in a BPD phenotype. In a review of lung pathology of archived autopsy specimens from *Ureaplasma*-infected preterm infants, the most striking findings were 1) the presence of moderate to severe fibrosis, 2) increased myofibroblasts, 3) disordered elastin accumulation, and 4) increased numbers of tumor necrosis factor- α (TNF- α) and transforming growth factor β_1 (TGF β_1)-immunoreactive cells in all *Ureaplasma*-infected infants compared to gestational controls and infants who died with pneumonia from other causes^{58, 59}. Experimental murine intrauterine *U. parvum* exposure stimulated fetal lung cytokine expression and augmented hyperoxia-induced lung injury³¹. In the 125 d immature baboon model, we observed extensive fibrosis (Fig. 2), an increase in the myofibroblast phenotype and increased expression of pro-inflammatory (TNF- α , interleukin (IL)-1 β) and pro-fibrotic cytokines (TGF β_1 ; oncostatin M) in lungs of antenatal *Ureaplasma*-infected animals compared to gestational controls or non-infected ventilated animals⁶⁰. In a study of rhesus macaques, histologic changes in the fetus' lungs depended on the duration of intrauterine exposure to *U. parvum*³⁴. Infection exposure duration less than 136 h resulted in neutrophil infiltration without epithelial injury. With progressive duration of exposure there was an influx of neutrophils and macrophages, epithelial necrosis, and type II cell proliferation. For exposure duration >10 d, increased collagen and thickened alveolar walls were evident. These data confirm that *Ureaplasma* infection contributes to chronic inflammation and fibrosis in the preterm lung. Moreover, these data suggest that *Ureaplasma* acts as a co-inflammatory

stimulus by causing an augmented, dysregulated inflammatory response to subsequent inflammatory insults such as hyperoxia and volutrauma.

Insights into how *Ureaplasma* infection-mediated inflammation produces the BPD phenotype are provided by studies of transgenic mice overexpressing pro-inflammatory cytokines. In transgenic mice, over-expression of TNF- α , IL-6, and IL-11 each inhibited alveolarization, indicating that prolonged exposure of the preterm lung to a pro-inflammatory environment may contribute to abnormal alveolar septation, the hallmark of the 'new BPD'⁶¹. In a recently developed bitransgenic CCSP-rtTA-(tetO)-CMV-IL-1 β mouse, IL-1 β was expressed under conditional control in airway epithelial cells in the fetal and neonatal lung⁶². IL-1 β expression increased on E14.5 and was maximal by E16.5 (pseudoglandular period) and decreased postnatally. In this model, postnatal growth was impaired and mortality was higher in the newborn mice expressing IL-1 β . The lungs of these newborn mice demonstrated many features of the BPD phenotype, including disrupted alveolar septation and capillary development, and disordered α -smooth muscle actin and elastin deposition in alveolar septa of distal airspaces⁶². These observations suggest that an early and prolonged exposure to *Ureaplasma*-mediated inflammation may be necessary to adversely affect lung development (see Fig. 1).

***Ureaplasma* spp. and other Neonatal Lung Disorders**

There have been case^{63, 64} and small series reports of culture-confirmed congenital *Ureaplasma* pneumonia in late preterm infants⁶⁵. In one series, 3 infants presented with clinical features of pneumonia and persistent pulmonary hypertension⁶⁵. In each infant, tracheal aspirates and blood samples were *Ureaplasma* culture-positive and postmortem lung cultures were positive for *Ureaplasma* in 2 infants who died. In a 5 year series of 159 perinatal autopsies, postmortem lung cultures were positive for *Ureaplasma* in 9% of cases. Forty-three percent of the autopsied cases were intrauterine deaths and 45% had histologic evidence of congenital pneumonia⁶⁶. This was in agreement with a larger series of 430 stillborn and neonatal autopsies in which 8% were *Ureaplasma*-positive in the lungs⁶⁷. These studies suggest that *Ureaplasma* species are uncommon causes of pneumonia in term and near-term infants, but cultures for these organisms should be considered in cases of unexplained congenital pneumonia or fetal demise.

Ureaplasma Invasive Disease

Although *Ureaplasma* spp. are most commonly isolated from respiratory secretions, the organisms have also been isolated from gastric aspirates⁶⁸, blood^{69, 70}, cerebrospinal fluid^{71, 72}, lung⁶⁵ and brain^{73, 74} tissue. Isolation of *Ureaplasma* from cord blood was first described in 1969⁷⁵ and isolation from cord or venous blood in association with pneumonia was documented in several other studies^{65, 69, 76}. In 2 recent large prospective cohorts, *Ureaplasma* was detected in 17% of cord blood cultures⁷⁰ and 23.6% serum and/or CSF PCR samples³⁹, but invasive disease was not associated with BPD at 36 weeks PMA in either cohort. *U. parvum* was the predominant species detected in serum and CSF. In infants for whom *Ureaplasma* respiratory status was known, 19% of serum and 11% CSF samples from infants with *Ureaplasma* respiratory colonization and 15% serum and 19% CSF samples from infants without *Ureaplasma* respiratory colonization were PCR-positive. In all cases, the *Ureaplasma* detected in respiratory samples was the same species as the one detected in serum or CSF from the same subject³⁹.

***Ureaplasma*-mediated inflammation and neonatal brain injury**

Although considerable evidence has been published linking *Ureaplasma* respiratory colonization with neonatal lung morbidity, less is known about possible links between

intrauterine *Ureaplasma* exposure, invasive disease, and neurologic morbidities such as intraventricular hemorrhage (IVH) and white matter injury. In a prospective series of infants with suspected sepsis/meningitis or hydrocephalus, *M. hominis*, a related genital mycoplasma, was isolated in 5 and *Ureaplasma* spp. in 8 CSF samples⁷¹. Six infants with *Ureaplasma*-positive CSF had severe IVH complicated by post-hemorrhagic hydrocephalus in 3. There are additional case reports of IVH complicated by hydrocephalus and/or death in association with *Ureaplasma*-positive CSF^{73, 77}. In our study of invasive *Ureaplasma*³⁹, the risk of severe IVH ($\geq$ Grade 3) was 2.5-fold higher in serum PCR-positive than PCR-negative infants after adjustment for gestational age, but there was no association of cranial ultrasound abnormalities with detection of *Ureaplasma* in CSF. This may have been due to selection bias of lumbar punctures more likely being performed in more stable infants. *U. parvum* was the species detected in all PCR-positive infants with severe IVH. There was a 5-fold increased risk for severe IVH in the presence of *Ureaplasma* PCR positive serum combined with elevated serum IL-1 β ³⁹, suggesting a possible link between invasive *Ureaplasma*, cytokinemia, and neonatal brain injury (see Figure 1).

A role for *Ureaplasma* in neonatal brain injury is supported by recent studies in experimental animal models. In the murine intrauterine *U. parvum* infection model, the brains of fetal and newborn mice showed evidence of microglial activation, delayed myelination, and disturbed neuronal development³¹. In antenatal- *U. parvum*-exposed immature rhesus macaques, CSF and brain tissue were culture-positive in 20%³⁴. Although there was no evidence of hemorrhage on gross and microscopic examinations of the brains of these animals, specific immunohistochemical analyses were not performed. Future studies in these models will provide additional insights into the mechanisms of *Ureaplasma*-mediated brain injury and could evaluate potential therapeutic interventions.

The impact of intrauterine *Ureaplasma* infection on neurodevelopmental outcome is unknown. A recent study comparing outcomes of infants born < 33 weeks gestation with and without amniotic cavity infection with *Ureaplasma* or other microbes at time of delivery reported lower psychomotor developmental index scores on the Bayley Scales of Infant Development and a higher rate of cerebral palsy at 2 years adjusted age in infants exposed to intrauterine infection compared to non-exposed infants⁷⁸. Infants exposed to intrauterine *Ureaplasma* were more severely affected than those exposed to other bacteria. Additional studies are needed to assess the contribution of *Ureaplasma* to adverse neurodevelopment.

***Ureaplasma* spp. and Neonatal Meningitis**

The prevalence, clinical risk factors, and CSF parameters in *Ureaplasma* CNS infections have not been fully elucidated. Most series have focused on the preterm population, but there have been case reports of *Ureaplasma* isolation in CSF of term infants^{69, 79}. The rate of *Ureaplasma* culture-positive CSF in prospective series in preterm infants varied from 0.2% to 9%^{71, 72, 80–82}. The prevalence for *Ureaplasma* is higher than for other causes of bacterial meningitis (1–2%)^{83, 84}. Although CSF pleocytosis has been described, it is absent in most cases^{69, 82}. The utility of CSF parameters in the diagnosis of bacterial meningitis in preterm infants is debated⁸⁴. In our prospective series³⁹, the CSF white blood cell count, percent neutrophils, and glucose and protein concentrations did not differ between *Ureaplasma* PCR positive and negative samples (Table 1). Only 25% of *Ureaplasma*-positive CSF samples had a white blood cell count >20 cells/mm³ compared to 16% of *Ureaplasma*-negative samples. No other bacteria were detected in these samples. Taken together, these observations suggest that *Ureaplasma* infections may be the most common cause of CNS infections in neonates, but most infections are asymptomatic. Since most infections appear to resolve without therapy, it is unclear whether culture for these organisms should be included in the routine evaluation of sepsis in the newborn. Additional studies focused on long-term outcomes of *Ureaplasma*

CNS infections are warranted before specific recommendations can be made for clinical management.

***Ureaplasma* Infections in Infants and Children**

The duration of *Ureaplasma* respiratory tract colonization is unknown. In a study conducted in 1969, 12 of 21 *Ureaplasma*-colonized newborns remained culture positive for up to 10 months. In addition, 7 of 52 initially culture-negative infants converted to culture-positive during the same time period, confirming the importance of multiple cultures for definitive diagnosis as well as suggesting the possibility of horizontal transmission. Over a 10 year period, 31% of over 45,000 cultures at a national reference laboratory for mycoplasma cultures were positive for *Mycoplasma* spp. not including *M. pneumoniae*⁴. In children, the majority of *Ureaplasma* positive respiratory cultures were from children < 12 months (92%), but 4% were from children 1 to 7 years of age. In agreement, Matlow et al⁸⁵ reported that only 1.8% of non-neonatal respiratory samples submitted to a single institution laboratory were positive for *Ureaplasma*.

Ureaplasma respiratory tract colonization has also been proposed as an etiologic factor in reactive airway disease in young infants. Wheezing in infants and children less than 3 years of age has been associated with isolation of *Ureaplasma* from the upper respiratory tract⁸⁶. Interestingly, in a large study of almost 3000 women and their offspring in Sweden, maternal vaginal colonization with *Ureaplasma* during pregnancy was associated with a 2-fold increased risk for infant wheezing defined as one or more hospitalizations for asthma during the first 3 years of life⁸⁷. The investigators proposed that acquisition of microorganisms such as *Ureaplasma* spp. at birth affects the establishment of infant microflora and subsequent development of allergy and wheezing.

Ureaplasma as a significant cause of sepsis or meningitis beyond the newborn period appears unlikely. In a series of infants less than 3 months of age evaluated for sepsis, *Ureaplasma* was not detected in any blood or CSF sample, but it was isolated from 6% urine cultures⁸⁸. Other diseases associated with *Ureaplasma* include pediatric joint disorders⁸⁶.

***Ureaplasma* Diagnostic Methods**

Ureaplasma spp. may be isolated from blood, amniotic fluid, CSF, sputum, bronchoalveolar lavage, pleural fluid, and semen. Since these organisms are susceptible to desiccation and are sensitive to temperature changes, attention to sample collection and processing are of utmost importance. Samples should be directly inoculated into 10B broth for transport on ice to the laboratory. The specimen should be inoculated in 10B broth with serial 1:10 dilutions and incubated under atmospheric conditions and on A8 agar and incubated in 5% CO₂ at 37°C. Cultures are observed for up to 7 days for broth color change from yellow to pink, indicating pH change due to urease activity in the absence of turbidity. Any broth with color change should be sub-cultured on A8 agar. Colonies of *Ureaplasma* spp. are identified presumptively by their characteristic brown appearance on A8 agar in the presence of the CaCl₂ indicator. *Ureaplasma* can be differentiated from *M. hominis* by the larger size, lack of precipitate, and "fried-egg" appearance of *M. hominis*. Cultures usually grow within 24–48 h. Since most clinical laboratories lack the expertise for culture of these organisms, cultures may need to be shipped for processing to a reference laboratory such as the University of Alabama Mycoplasma Diagnostic Laboratory.

PCR protocols for detection of the common *Ureaplasma* spp. mba or urease genes are available^{89–91} as well as species and serovar-specific primers^{89, 92, 93}. These methods have the advantage of more rapid results and potentially increased sensitivity compared to traditional culture.

Therapeutic Considerations

Interventions during pregnancy

Antibiotic therapy is now standard of care for management of pPROM⁹⁴. However, usual regimens failed to eradicate organisms including *Ureaplasma* or diminish inflammation in the amniotic cavity in pregnancies complicated by pPROM⁹⁵. In a small series of women with microbial invasion of the amniotic cavity with short cervix, parenteral azithromycin eradicated *Ureaplasma* in 3 of 4 cases and delivery occurred at term. Combination of antibiotics with anti-inflammatory drugs may improve efficacy. In an experimental *Ureaplasma* intraamniotic infection in Rhesus macaques, azithromycin alone or in combination with dexamethasone and indocin prevented fetal lung damage (Novy, MJ *et al.*, Maternal azithromycin (AZI) therapy for *Ureaplasma* intraamniotic infection (IAI) prevents advanced fetal lung lesions in rhesus monkeys, 2008 SGI Annual Scientific Meeting, March 26–29, 2008, San Diego, CA, Abstract 438). Whether this approach will be beneficial for CNS outcomes is unknown.

BPD Prevention?

Despite *in vitro* susceptibility of *Ureaplasma* to erythromycin⁹⁶, trials of erythromycin therapy in the first few weeks of life in *Ureaplasma* colonized preterm infants have failed to demonstrate efficacy to prevent BPD^{97, 98} or eradicate respiratory tract colonization⁹⁹. The failure to prevent BPD in these studies may have been due to the small sample size of each study, or to the initiation of erythromycin therapy too late to prevent the lung inflammation and injury that contribute to the pathogenesis of BPD. Demonstration of efficacy of antibiotic therapy to prevent BPD in *Ureaplasma*-colonized preterm infants will require carefully designed adequately powered studies.

The new 14-membered macrolides that are derivatives of erythromycin and the related 15-member azalides have immunomodulatory effects, including effects on neutrophil function (e.g. chemotaxis, cell adhesion, oxidative burst and phagocytosis) and inhibition of cytokine release (e.g., IL-1 β , IL-8, TNF α)¹⁰⁰ and nitric oxide production *in vitro*¹⁰¹. Macrolide antibiotics may exert immunomodulatory anti-inflammatory effects in the setting of infection, and these may occur independently of a direct bactericidal effect¹⁰². In addition, azithromycin exhibits higher potency than erythromycin against clinical *Ureaplasma* isolates *in vitro*¹⁰³. Pharmacokinetic studies in mice and humans have shown that azithromycin is preferentially concentrated in pulmonary epithelial lining fluid and alveolar macrophages^{104–106}. Since neutrophil recruitment and activation has been implicated in BPD pathogenesis^{107, 108}, the experimental effects observed with azithromycin *in vitro* and *in vivo* indicate that this drug may be beneficial in the treatment of *Ureaplasma* infection and the prevention of BPD in preterm infants.

Since *Ureaplasma*-mediated lung injury may be initiated *in utero* and augmented postnatally by exposure to mechanical ventilation and hyperoxia, therapy to prevent BPD should be initiated as soon as possible after birth in infants at risk. Recently, Walls *et al.*¹⁰⁹ demonstrated that azithromycin, but not erythromycin prophylaxis improved outcomes and reduced inflammation in a murine neonatal *Ureaplasma* infection model. This suggests that azithromycin may be effective if administered immediately after birth. However, until appropriate pharmacokinetics and efficacy trials are conducted, a dosing regimen for azithromycin in neonates cannot be recommended.

Summary

Ureaplasma species may indeed be an “underestimated enemy of human reproduction”. However, there are many questions yet to be answered before recommendations for changes in clinical practices can be recommended. We need to learn more about the relative contribution

of *Ureaplasma* virulence factors, host immune factors that affect susceptibility to these pathogens and the variability in the inflammatory response, and interactions with environmental factors such as oxygen exposure and mechanical ventilation. Routine antibiotic use should be limited to clinical trials until clear benefit can be established. However, culture or PCR for these organisms should be considered in cases of unexplained fetal demise, congenital pneumonia, or meningitis unresponsive to broad-spectrum antibiotics. Follow-up cultures should be obtained in any treated infant since effective clearance has proven difficult in erythromycin trials.

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Figure 1. Overview of potential inflammatory pathways involved in *Ureaplasma*-mediated preterm labor, and common neonatal morbidities

Intrauterine *Ureaplasma* infections may develop by ascending infection or transplacental route. Engagement with host pathogen recognition receptors initiates a maternal and fetal inflammatory cascade. In the amniotic cavity, there is sequential upregulation of inflammatory cytokines, recruitment of leukocytes, and release of prostaglandins, and metalloproteinases leading to uterine contractions, cervical dilatation and effacement, and membrane rupture^{34, 35}. In the fetal lung, prolonged exposure to inflammatory cytokines inhibits alveolar development¹¹⁰. Systemic invasion via the umbilical cord stimulates a cytokinemia in blood and/or CNS resulting in microglia activation and pre-oligodendrocyte and neuronal injury³¹.

Figure 2. Collagen staining is increased in lungs from antenatal *U. parvum*-infected baboons
Lung specimens from 125d gestation baboon newborns ventilated for 14d that were either infected antenatally with *U. parvum* serovar 1 (D) or noninfected (C) were stained with trichome for collagen and compared to stained lung sections from 125d (A) and 140 d (B) gestational controls. The most marked fibrosis occurred in the *U. parvum* infected lungs (arrows). Magnification x200.

Table 1CSF Characteristics in infants with invasive *Ureaplasma*

CSF PARAMETER	CSF PCR (+) N=36	CSF PCR (-) N=153
WBC /mm ³ *	4 (0–172)	3 (0–193)
% neutrophils	31 (0–92)	17 (0–87)
% lymphocytes	14 (0–100)	16 (0–100)
% monocytes	23 (0–100)	42 (0–100)
Glucose (mg/dL)	47 (26–105)	52 (0–171)
Protein (mg/dL)	154 (64–527)	152 (47–558)

* CSF white blood cell counts were corrected for red blood cells. Data are expressed as median with range in parentheses.