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REVIEW

The Human *Ureaplasma* Species as Causative Agents of Chorioamnionitis

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SUMMARY The human *Ureaplasma* species are the most frequently isolated microorganisms from the amniotic fluid and placentae of women who deliver preterm and are also associated with spontaneous abortions or miscarriages, neonatal respiratory diseases, and chorioamnionitis. Despite the fact that these microorganisms have been habitually found within placentae of pregnancies with chorioamnionitis, the role of *Ureaplasma* species as a causative agent has not been satisfactorily explained. There is also controversy surrounding their role in disease, particularly as not all women infected with *Ureaplasma* spp. develop chorioamnionitis. In this review, we provide evidence that *Ureaplasma* spp. are associated with diseases of pregnancy and discuss re-

cent findings which demonstrate that *Ureaplasma* spp. are associated with chorioamnionitis, regardless of gestational age at the time of delivery. Here, we also discuss the proposed major virulence factors of *Ureaplasma* spp., with a focus on the multiple-banded antigen (MBA), which may facilitate modulation/alteration of the host immune response and potentially explain why only subpopulations of infected women experience adverse pregnancy outcomes. The information presented within this review confirms that *Ureaplasma* spp. are not simply "innocent bystanders" in disease and highlights that these microorganisms are an often underestimated pathogen of pregnancy.

KEYWORDS amniotic fluid, chorioamnionitis, multiple-banded antigen, neonate/fetus, pregnancy, *Ureaplasma*, virulence factors

INTRODUCTION

Chorioamnionitis refers to inflammation of the fetal membranes, which comprise the chorion and amnion. Although the chorioamnion is anatomically part of the placenta, it is derived from the zygote and is considered to be of fetal origin (see "Development, Structure, and Function of the Chorioamnion" below). The chorioamnion is also in contact with the decidua, a tissue of maternal origin, and together these form the maternal/fetal interface. Chorioamnionitis frequently occurs in parallel with microbial infection of the chorioamnion and amniotic fluid (1–3); however, it may also occur in the absence of demonstrable microorganisms (i.e., "sterile inflammation" [2, 4], which will not be discussed in this review). The clinical signs of chorioamnionitis include fever, uterine fundal tenderness, maternal tachycardia (>100 beats/minute), fetal tachycardia (>160 beats/minute), and purulent or foul-smelling amniotic fluid (5). However, it is becoming increasingly apparent that a large proportion of chorioamnionitis cases are subclinical and are not diagnosed until retrospective analysis of the placenta (6) (see "Diagnosis of Chorioamnionitis" below). Upon histological examination, acute chorioamnionitis is defined as diffuse influx of neutrophils into the chorioamnion/decidua, and the severity of the maternal and fetal immune response can be classified according to published standards (7). Chronic chorioamnionitis is less well defined but has been characterized by an infiltration of maternally derived mononuclear cells, usually macrophages and T lymphocytes, into the chorioamnion or chorionic plate (the fetal surface of the placenta that directly connects to the uterine wall, where the chorionic villi are formed) (7, 8).

Since amniotic fluid, but not the placenta, is accessible prior to delivery in women at risk for preterm labor, most clinical studies have correlated intra-amniotic infection or inflammation rather than chorioamnionitis with preterm labor/delivery. However, intra-amniotic infection, defined as microorganisms detected in the amniotic fluid (9), may not always be concordant with retrospective diagnosis of histological chorioamnionitis. Recently, a National Institutes of Health workshop recommended that the term "chorioamnionitis" be replaced with "intrauterine infection or inflammation or both" (abbreviated as "Triple I" and characterized as being either proven or suspected) or isolated maternal fever (10). For the purposes of this review, we have used the terms chorioamnionitis and intra-amniotic infection according to their traditional definitions, as described above.

Development, Structure, and Function of the Chorioamnion

The amnion develops from the ectoderm of the embryo 8 days after conception and surrounds the developing embryo to form an amniotic sac, which contains amniotic fluid. As the amniotic sac expands due to fetal growth and the production of amniotic fluid, the amnion makes contact with the chorion, which lines the decidua of the uterine wall, to form the chorioamnion at 10 to 12 weeks of gestation (11). The avascular chorioamniotic membranes persist until term in healthy pregnancies and perform critical barrier and container functions (12). The amnion comprises five layers: (i) a cuboidal epithelium, which is in contact with the amniotic fluid; (ii) an acellular basement membrane; (iii) a compact layer; (iv) a mesenchymal cell layer; and (v) a

spongy layer, which is in contact with the chorion (13). The amniotic epithelial cells and mesenchymal cells possess stem cell and immunomodulatory properties and have shown promising results for use in regenerative medicine (14). The chorion comprises four layers: (i) a cellular, fibroblast layer; (ii) a reticular layer; (iii) a pseudobasement membrane; and (iv) a trophoblast layer (13).

Diagnosis of Chorioamnionitis

The diagnosis of chorioamnionitis is currently based on clinical signs coupled with histological and microbiological analysis of the placenta after delivery of the newborn. Histologic grading of the placenta is considered the gold standard for the diagnosis of chorioamnionitis; however, this retrospective diagnosis is not useful in informing patient management throughout pregnancy, especially in the absence of clinical signs. Several studies have investigated the diagnostic value of amniotic fluid and maternal serum biomarkers for the detection of chorioamnionitis in pregnant women undergoing amniocentesis. Elevated inflammatory markers such as interleukin 6 (IL-6), IL-8, matrix metalloproteinase 8 (MMP-8), MMP-9, and monocyte chemotactic proteins within amniotic fluid are positive predictors of intra-amniotic inflammation and/or clinical chorioamnionitis (15–21); however, these markers may have poor positive predictive values for the detection of subclinical, histologic chorioamnionitis and may be variably expressed within the amniotic fluid and fetal membranes during chorioamnionitis (22–24). Recently, Liu et al. (25) reported that surface-enhanced laser desorption ionization–time of flight mass spectrometry (SELDI-TOF-MS) for the detection of human neutrophil defensin 1 (HNP-1) and HNP-2 and calgranulins A and C within amniotic fluid was highly accurate for the diagnosis of subclinical chorioamnionitis, but further studies with larger patient cohorts are required to validate these findings. Noninflammatory markers such as amniotic fluid lactate dehydrogenase and glucose were also recently investigated for the detection of histologic chorioamnionitis (26), but the diagnostic accuracy of these assays was low, suggesting that additional amniotic fluid biomarkers should be investigated for the diagnosis of chorioamnionitis.

CLINICAL PERSPECTIVES ON CHORIOAMNIONITIS AND ITS SIGNIFICANCE TO THE HEALTH OF THE PREGNANCY AND NEONATE

Clinical chorioamnionitis and histological chorioamnionitis affect 1 to 4% and 23.6% of term births (37 to 42 weeks of gestation), respectively (5, 27, 28). However, it has been well established that the frequency (29–31) and severity (31, 32) of chorioamnionitis are inversely related to gestational age at the time of delivery. In a study of 7,505 placentae from singleton pregnancies, Russell (29) reported that the frequency of chorioamnionitis in patients who delivered between 21 and 24 weeks of gestation was 94.4% (17/18 patients). More recently, Stoll et al. (30) demonstrated that histological chorioamnionitis was present in 70% (295/421) of pregnancies that delivered at 22 weeks of gestation. The frequency of histological chorioamnionitis was significantly higher in women who delivered after the spontaneous onset of labor than in those who had induction of labor at term or delivered via Caesarean section in the absence of labor (33, 34). Furthermore, the frequency of histological chorioamnionitis increases in patients with prolonged duration of labor (35) and premature rupture of membranes (36). Additional risk factors for chorioamnionitis include multiple digital examinations, nulliparity, bacterial vaginosis, alcohol and tobacco use, group B *Streptococcus* colonization, meconium-stained amniotic fluid, and epidural anesthesia (36–39).

Chorioamnionitis: a Major Predictor of Preterm Birth

Preterm birth, defined as delivery at $<$37 weeks of gestation, is the leading cause of neonatal death worldwide (40). In addition, complications arising from preterm birth are a leading cause of death in children under the age of 5, second only to pneumonia (41). Microbiological studies have demonstrated that intrauterine infection may be responsible for 25 to 40% of preterm births (42); however, this is likely to be underreported due to difficulties in detecting fastidious microorganisms using conventional

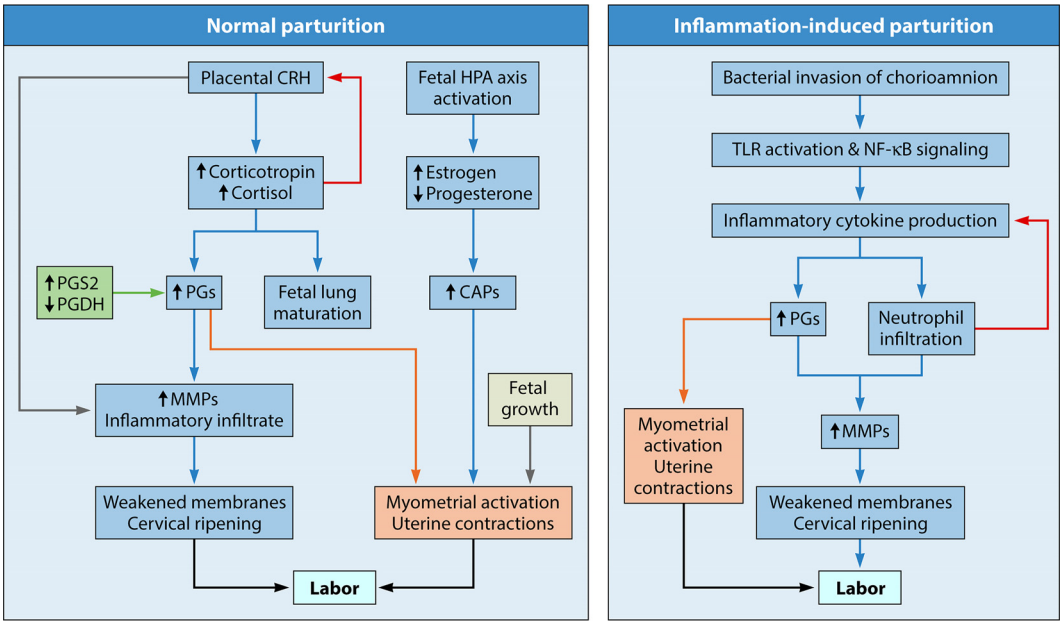

FIG 1 Comparison of key events involved in normal parturition and inflammation-induced parturition. Normal parturition is initiated by the increased placental synthesis of CRH at term, which causes the production of cortisol. Cortisol induces the production of prostaglandin E2 and prostaglandin F2α and works in a positive-feedback loop to further stimulate placental CRH production. Prostaglandins induce the production of matrix metalloproteases, which facilitate membrane rupture and cervical remodeling. In concert, activation of the fetal HPA axis leads to a functional progesterone withdrawal and production of contraction-associated proteins, which cause myometrial activation and uterine contractility. During chorioamnionitis, inflammatory cytokines and chemokines produced in response to microbial invasion of the chorioamnion and/or amniotic fluid stimulate prostaglandin production and neutrophil infiltration, leading to the synthesis of matrix metalloproteases and subsequent membrane weakening. Recognition of pathogen-associated molecular patterns by pattern recognition receptors (such as TLRs) is critical for the initiation of inflammation-induced parturition. CAPs, contraction-associated proteins; CRH, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; MMPs, matrix metalloproteases; NF-κB, nuclear factor kappa B; PGDH, prostaglandin dehydrogenase; PGs, prostaglandins; PGS2, prostaglandin-endoperoxide synthase 2; TLR, Toll-like receptor. The direction of the arrows within boxes represents either an increase or a decrease in expression.

culture methods. Histological chorioamnionitis complicates 40 to 70% of all preterm births (5), suggesting that chorioamnionitis may be an important, and potentially preventable, antecedent of preterm birth.

Parturition in Normal Pregnancy versus Chorioamnionitis

Figure 1 compares the key events that occur during normal parturition and inflammation-induced preterm delivery. The normal initiation of parturition in humans is a complex process that involves fetal hypothalamic-pituitary-adrenal (HPA) axis activation and increased placental synthesis of corticotropin-releasing hormone (CRH) (Fig. 1). Maternal CRH plasma levels increase throughout the duration of pregnancy and peak at term (43). Increased CRH levels drive the production of corticotropin and cortisol in the mother and fetus, which promotes fetal lung maturation and prostaglandin (PG) synthesis (e.g., PGE2 and PGF2α) within the amnion (44). PG production is enhanced by the concomitant downregulation of prostaglandin dehydrogenase (PGDH) within the chorion (45) and the production of prostaglandin-endoperoxide synthase 2 (PGS2, formerly cyclo-oxygenase 2) (46). Both CRH and PGE2 stimulate the release of MMPs (47, 48) (e.g., MMP-2 and MMP-9), which weaken the chorioamnion and facilitate membrane rupture and cervical ripening. In parallel, activation of the fetal HPA axis and uterine stretching caused by fetal growth lead to the upregulation of contraction-associated proteins and myometrial activation (44). Progesterone withdrawal coupled with increased estrogen production is also a key feature of parturition and further promotes uterine contractility (49–51).

In patients with chorioamnionitis, parturition may be accelerated by a maternal and/or fetal inflammatory response, which is thought to be mediated by Toll-like

receptor (TLR) signaling (Fig. 1). A recent prospective study of human pregnancies demonstrated that the expression of TLR-1 and TLR-2 was significantly increased in chorion obtained from preterm deliveries with histological chorioamnionitis compared to chorion from preterm deliveries without histological chorioamnionitis (52). Similar results were reported in separate studies by Moço et al. (53) and Kim et al. (54), suggesting that the upregulation of TLRs plays an important role in the pathogenesis of chorioamnionitis.

Bacterial endotoxins, such as lipopolysaccharide (LPS) (55), and live microorganisms (56) have been shown to upregulate placental/chorioamnion TLRs, which are expressed by amnion epithelial cells, decidual cells, intermediate trophoblasts in the chorion, macrophages, and neutrophils (54). *In vitro* studies have demonstrated that human primary amnion epithelial cells express functional TLR-2, TLR-4, TLR-5, and TLR-6 and that stimulation with TLR-5 and TLR-2/6 agonists leads to activation of nuclear factor kappa B signaling and the production of proinflammatory cytokines, MMP-9, and PGS2 (57). These findings are consistent with human studies and animal models of chorioamnionitis/intrauterine infection, which demonstrate an increase in IL-1β and IL-6 (58, 59), IL-8 (52), tumor necrosis factor alpha (TNF-α) (60), monocyte chemotactic proteins (61), and granulocyte colony-stimulating factor (G-CSF) (62) in preterm fetal membranes, amniotic fluid, and/or cord blood. These inflammatory cytokines and chemokines stimulate PG production (63, 64) and neutrophil infiltration and the release of MMPs (65), thus leading to cervical ripening and weakening/rupture of the fetal membranes. Indeed, the levels of MMPs (66) and PGs (56, 67) are significantly increased within the amniotic fluid and fetal membranes during chorioamnionitis.

Neonatal Sequelae of Chorioamnionitis

During chorioamnionitis, the fetus may be directly exposed to microorganisms and inflammatory mediators within infected amniotic fluid. The fetus inspires, swallows, and is bathed in amniotic fluid; therefore, the fetal lungs (68, 69), gastrointestinal tract (70, 71), and skin (72) are primary sites of inflammation-mediated injury. Exposure to inflammatory mediators may also occur via the placental-fetal circulation, resulting in immunomodulation within the fetal blood (73–75), lymphoid tissues (76–78), and distant organs such as the brain (79, 80). The systemic response of the fetus to chorioamnionitis, termed the fetal inflammatory response syndrome (FIRS), is a severe inflammatory condition that is characterized by elevated inflammatory cytokines within fetal plasma, particularly IL-6 (81, 82), and increased fetal plasma white blood cell counts (83). FIRS is associated with multiorgan injury and with severe neonatal morbidity and mortality (82). The fetal immune response to chorioamnionitis has been reviewed in detail elsewhere (84, 85).

In human studies, chorioamnionitis has been associated with neonatal death (27, 86), early-onset neonatal sepsis (86–88), intrauterine growth restriction (89), poor neonatal growth (90), neurologic impairment/injury (91, 92), intraventricular hemorrhage (86), bronchopulmonary dysplasia (93–95), patent ductus arteriosus (86, 89, 93, 96), retinopathy of prematurity (89, 97, 98), cardiovascular abnormalities (99, 100), necrotizing enterocolitis (101, 102), and dermatitis (103). However, low gestational age is often a significant contributing factor (104–106), and therefore, it is difficult to attribute these sequelae solely to chorioamnionitis. Nonetheless, when controlling for gestational age in a multivariable analysis, a recent study of 3,082 extremely preterm infants ($<$27 weeks of gestation) demonstrated that fetal exposure to histological chorioamnionitis and clinical chorioamnionitis was associated with an increased risk of cognitive impairment at 18 to 22 months of corrected age compared to infants exposed to no chorioamnionitis or histological chorioamnionitis alone (107). When adjusting for gestational age, other studies have confirmed that chorioamnionitis is an independent risk factor for early-onset neonatal sepsis (108, 109), bronchopulmonary dysplasia (95), adverse neurodevelopmental outcome at 3 years (110), and necrotizing enterocolitis (108). Interestingly, the severity of chorioamnionitis has been shown to correlate with an increased frequency of chronic lung disease

and necrotizing enterocolitis (111) but has an inverse relationship with the development of respiratory distress syndrome (112).

HOST DEFENSES AND PATHWAYS OF MICROBIAL INVASION OF THE CHORIOAMNION AND AMNIOTIC FLUID

Traditionally, the normal intrauterine environment is considered to be a sterile site with the chorioamnion representing the major physical and immunological barrier to the developing fetus. The chorioamnion expresses TLRs, which detect pathogen-associated molecular patterns and signal to coordinate cellular immune responses. The chorioamnion also secretes numerous natural antimicrobial peptides and defensins to protect against microbial invasion (113). *In vitro*, human chorion and amnion from healthy pregnancies that delivered at term inhibited the growth of a wide range of pathogenic bacteria, including group B *Streptococcus*, group A *Streptococcus*, *Staphylococcus aureus*, and *Staphylococcus saprophyticus* (114). Parthasarathy et al. also reported that human fetal membranes possess strong antimicrobial effects against *Escherichia coli*, *Shigella* spp., and the fungal pathogens *Aspergillus niger* and *Aspergillus nidulans* (115). Nonetheless, a wide range of microbes are capable of invading the fetal membranes and amniotic cavity and causing chorioamnionitis. Specific routes by which microorganisms are thought to access the upper genital tract during pregnancy include (i) retrograde spread from the peritoneal cavity (via the Fallopian tubes), (ii) hematogenous dissemination via the placenta and maternal blood supply, (iii) iatrogenic contamination at the time of invasive medical procedures (such as chorionic villus sampling or amniocentesis), and (iv) ascending invasive infections from the lower genital tract (42). While other studies have suggested that bacteria (specifically, *Ureaplasma* spp.) may also gain access to the upper genital tract attached to spermatozoa (116, 117), the most widely accepted route is that microorganisms originating from the lower genital tract ascend through the cervix into the choriodecidual space and cross the chorioamnion membrane, thereby reaching the amniotic fluid and fetus (118).

Recent deep-sequencing studies have demonstrated that the placental parenchyma harbors a unique microbiome comprising nonpathogenic bacteria from the *Firmicutes*, *Tenericutes*, *Proteobacteria*, and *Fusobacteria* phyla, with distinct similarities to the adult oral microbiota (119). Furthermore, whole-genome shotgun sequencing of placental membranes (fetal chorion and/or villous placental membranes) from term deliveries without chorioamnionitis demonstrated the presence of a diverse range of bacteria, including *Enterobacter* spp., *E. coli*, *Acinetobacter lwoffii*, *Acinetobacter johnsonii*, and *Lactobacillus crispatus* (120). These findings redefine our understanding of the placental microenvironment and challenge the view that the fetus exists normally within a sterile compartment. It is therefore possible that the commensal microorganisms of the placental parenchyma and fetal membranes represent a previously unrecognized source of bacteria, which, under certain conditions, may initiate an inflammatory response leading to chorioamnionitis. This may also be important for the establishment of the fetal/neonate microbiota (119) and normal immune development of the fetus (121).

CAUSATIVE AGENTS OF CHORIOAMNIONITIS

A range of microorganisms, including bacteria, viruses, and (less frequently) yeast and fungi, have been implicated in chorioamnionitis. The bacterial pathogens that are most frequently isolated in cases of chorioamnionitis include the human *Ureaplasma* species (*Ureaplasma parvum* and *Ureaplasma urealyticum*), *Fusobacterium* spp., *Streptococcus* spp., and, less frequently, *Gardnerella* spp., *Mycoplasma* spp., and *Bacteroides* spp. (1, 62, 120, 122–124). Other studies have identified that the sexually transmitted pathogens *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, along with the uropathogen *E. coli* and yeast *Candida* spp., are also infrequently associated with chorioamnionitis (122, 125–128). Viral etiologies of chorioamnionitis include adenovirus, cytomegalovirus, enterovirus, and, less frequently, respiratory syncytial virus and Epstein-Barr virus (129–132). Of the microorganisms associated with chorioamnionitis, the human *Ureaplasma* spp. are consistently identified as the most common microorganisms

within the amniotic fluid and placentae of women with chorioamnionitis (1, 62, 123, 133, 134), funisitis (120, 135, 136), and preterm birth (1, 137).

THE HUMAN *UREAPLASMA* SPP.

The human *Ureaplasma* spp. were first discovered in 1954 in agar cultures of urethral exudates from male patients with nongonococcal urethritis (138). Due to their small colony size (5 to 20 μm) and their resemblance to the human *Mycoplasma* spp., *Ureaplasma* spp. were initially identified as tiny-form pleuropneuomonia-like organisms and referred to as T-mycoplasmas (138). However, *Ureaplasma* can be distinguished from *Mycoplasma* spp. (139) by the presence of a urease enzyme, which hydrolyzes urea to produce 95% of its energy requirements. The hydrolysis of urea produces ammonia, which leads to an increase in proton electrochemical potential and *de novo* ATP synthesis (140). The production of ammonia is a distinguishing feature for the identification of *Ureaplasma* spp. in culture, and these tiny bacteria are detected not by turbidity within broth but by an alkaline shift and pH indicator color change in both broth and agar culture media (141, 142). Due to this distinctive urease activity, the *Ureaplasma* spp. were reclassified into their own genus within the *Mycoplasmataceae* family in 1974 (139). As members of the class *Mollicutes*, *Ureaplasma* spp. do not possess a cell wall and are surrounded only by a plasma membrane. Due to this lack of structural integrity, the *Ureaplasma* spp. are pleomorphic, and individual organisms can range in size from 100 nm to 1 μm (143). As such, the *Ureaplasma* spp. are considered to be among the smallest self-replicating microorganisms.

Taxonomic Classification

The human *Ureaplasma* spp. are divided into two species, which contain at least 14 serovars: *U. parvum* (serovars 1, 3, 6, and 14) and *U. urealyticum* (serovars 2, 4, 5, and 7 to 13) (144). *U. parvum* possesses a smaller genome (0.75 to 0.78 Mbp) than *U. urealyticum* (0.84 to 0.95 Mbp) (145), and these two species can also be distinguished based on restriction fragment length polymorphisms, DNA-DNA hybridization, multilocus sequence typing, and sequences of 16S rRNA, multiple-banded antigen (*mba*), and urease genes (146–151). While this taxonomic classification was formally accepted in 2002, it has not been universally adopted within the literature, and often the 14 serovars are still erroneously referred to as *U. urealyticum*.

Several methods for serotyping *Ureaplasma* spp. have been described, including growth inhibition tests (152, 153), immunoperoxidase tests (154), enzyme-linked immunosorbent assays (155, 156), and colony indirect epi-immunofluorescence (157), which utilize rabbit antisera. These tests performed poorly due to a lack of standardized reagents and the presence of multiple cross-reactions between serovars. These approaches also poorly discriminate clinical samples containing more than one *Ureaplasma* serovar. Therefore, serotyping of *Ureaplasma* for diagnostic and epidemiological purposes has historically been technically challenging. Molecular biology-based typing methods based on sequencing of the upstream region of the *mba* gene (151), conventional PCR of *mba* (158–160), and random amplified polymorphic DNA PCR (158) have also been described. However, these methods do not fully discriminate all 14 *Ureaplasma* serovars. In addition, the *mba* gene was recently shown to be part of a phase-variable gene superfamily (145), suggesting that its use as a diagnostic target may be limited.

Following the release of full genome sequences of *Ureaplasma* American Type Culture Collection (ATCC) strains, Xiao et al. designed 14 separate monoplex real-time PCR assays, which successfully typed all 14 ATCC type strains without cross-reactivity between serovars (161). However, when these real-time PCRs were used to type clinical human *Ureaplasma* isolates, 6% of isolates failed to amplify and could not be typed according to any of the known 14 serovars (162). Whole-genome shotgun sequencing of a selection of these isolates revealed that the gene targets for real-time PCR were completely absent or had been significantly modified, such that one of the primers was unable to bind. Even more intriguing was that following filtering and subculture of

single *Ureaplasma* colonies isolated from samples thought to contain mixtures of multiple serovars, several isolates continued to express loci from more than one serovar. DNA sequencing revealed that these isolates were in fact hybrids or genetic mosaics that carried multiple serovar markers. Screening of 271 clinical samples initially believed to contain multiple serovar mixtures demonstrated that 75 (28%) were hybrids, which carried markers of up to 4 different serovars (162). These data, in combination with recent comparative genome sequencing studies, demonstrate that there is extensive evidence of horizontal gene transfer (HGT) in *Ureaplasma* spp., suggesting that typing these microorganisms into defined serovar groups may be of limited value for diagnostic purposes (162) and that *Ureaplasma* organisms exist as quasispecies (145). On the other hand, it is possible that there are more-stable gene targets that have yet to be identified, which could be utilized for the discrimination of *Ureaplasma* serovars or pathogenic versus commensal subtypes. Large-scale comparative genome sequencing studies are required to clarify this issue.

Ureaplasma spp. Are Commensals of the Female Lower Genital Tract

Ureaplasma can be isolated from the mucosal surfaces of the vagina or cervix from 40 to 80% of sexually active females (163). *U. parvum* is isolated more frequently from the lower genital tract of females than *U. urealyticum* (158, 159, 164–166), and serovar 3 is the most common serovar isolated from females in the United States and Australia (116, 158, 163). *Ureaplasma* colonization of the female lower genital tract has been associated with numerous factors, including ethnicity (particularly African-American, Central/West African, and Indigenous Australian women) (123, 167, 168), age (most prevalent in the 14- to 25-year age group; carriage declines with increasing age) (165, 167), the number of recent sexual partners (123, 168), the use of nonbarrier contraceptives (123), level of education (167), age of first sexual intercourse (123), and intrauterine devices (167, 169). *Ureaplasma* spp. are considered to be commensal organisms within the female lower genital tract due to (i) their high prevalence and (ii) studies demonstrating no differences in the rates of endocervical *Ureaplasma* colonization between women of reproductive age with and those without symptoms of genital infection (165, 166). However, others have reported that *Ureaplasma* spp. can cause lower urogenital tract infections, such as symptomatic vaginitis (170, 171), cervicitis (172), bacterial vaginosis (173), pelvic infections (174, 175), and urinary tract infections (176–178).

Lower Genital Tract *Ureaplasma* Colonization Association with Chorioamnionitis and Adverse Pregnancy Outcomes

It has been proposed that the presence of *Ureaplasma* spp. in the female lower genital tract may be a risk factor for chorioamnionitis and adverse pregnancy outcomes, such as preterm birth (179–184). A prospective study of 2,471 women attending an antenatal clinic demonstrated that *Ureaplasma* spp. were isolated from vaginal swabs from 52/97 women (53.6%) who delivered preterm and that vaginal *Ureaplasma* colonization was an independent risk factor for preterm birth (odds ratio, 1.64; confidence interval, 1.08 to 2.48; $P = 0.02$). Despite this statistical association, it should be noted that, in the same study, *Ureaplasma* was also isolated from the lower genital tract of 783/1,891 women (41.1%) who delivered at term. Similarly, Kataoka et al. (179) demonstrated that *U. parvum* was detected in 16/21 women (76.2%) who delivered preterm and also in 440/856 women (51.4%) who delivered at term ($P = 0.024$). Other authors have reported equally high carriage rates in women who deliver at term, and the majority of studies conclude that lower genital tract *Ureaplasma* colonization is not a significant predictor of preterm birth or chorioamnionitis (185–190).

Ureaplasma Can Cause Ascending Asymptomatic Infections of the Upper Genital Tract

Although *Ureaplasma* spp. are (in most instances) considered to be commensals within the lower genital tract, these microorganisms are capable of causing ascending

asymptomatic infections of the upper genital tract. A recent study of fertile and infertile women undergoing diagnostic laparoscopy (who had no symptoms of genital tract infection) demonstrated that lower genital tract *Ureaplasma* colonization can lead to asymptomatic infection of the pouch of Douglas (191). Furthermore, *Ureaplasma* spp. have been isolated from the endometrium and Fallopian tubes of nonpregnant women in the absence of clinical symptoms or abnormal pathology (192, 193). While it was historically thought that the *Ureaplasma* spp. were of low virulence and that their presence in the upper genital tract might be of little consequence, there is now increasing evidence that these microorganisms are not simply innocent bystanders. The presence of *Ureaplasma* spp. in the upper genital tract of nonpregnant women suggests that these microorganisms may infect the embryo at the time of implantation (163). Moreover, they are capable of inducing chorioamnionitis, which can adversely affect the health of the pregnancy and neonate. Here, we discuss the role of the human *Ureaplasma* spp. as causative agents of chorioamnionitis.

UREAPLASMA SPP. AS ETIOLOGICAL AGENTS OF CHORIOAMNIONITIS

The first study to identify an association between *Ureaplasma* spp. and chorioamnionitis was published in 1975 and identified a link between carriage of *Ureaplasma* spp. in the lower genital tract and an increased incidence of chorioamnionitis (194). While the majority of studies since have demonstrated that lower genital tract colonization with *Ureaplasma* is not predictive of adverse outcomes during pregnancy, the role of *Ureaplasma* spp. in chorioamnionitis has remained controversial. Attempts to correlate infection with *Ureaplasma* spp. with the presence of chorioamnionitis have been made by a variety of studies and utilizing amniotic fluid, cord blood, or placental samples. These studies have demonstrated that *Ureaplasma* spp. are habitually found in placentae with chorioamnionitis (Table 1). Despite the fact that up to 100% of placentae infected with *Ureaplasma* spp. have evidence of histological chorioamnionitis (Table 1), a causative role for these microorganisms has not been satisfactorily explained and is complicated by a number of factors.

A factor which complicates the role of *Ureaplasma* spp. in chorioamnionitis is that not all women who are infected with these microorganisms develop chorioamnionitis or experience adverse pregnancy outcomes. Gerber et al. tested the amniotic fluid from 254 asymptomatic pregnant women at 15 to 17 weeks of gestation by PCR and detected *Ureaplasma* spp. in 29/254 (11.4%) subjects (137). Significantly, this study identified that 24% of women infected/colonized with *Ureaplasma* spp. delivered preterm, compared to 4.4% of women who were not infected with *Ureaplasma* spp. However, this study failed to comment on the vast majority (76%) of women in the study who were infected/colonized with *Ureaplasma* who went on to deliver at term with no apparent adverse outcomes. Similarly, Horowitz et al. detected intra-amniotic *Ureaplasma* infections in six pregnant women (2.8%), but only three (50%) of these women experienced preterm birth (195). Numerous studies have identified that the severity of upper genital tract *Ureaplasma* infection/inflammation in pregnant women is highly variable. Some studies have demonstrated that there may be immunological evidence of severe inflammation (196, 197), while in others there may be only moderate inflammation (198), or there may be no correlation between infection with *Ureaplasma* spp. and inflammation (199) (Fig. 2).

Although it remains unclear why some women infected with *Ureaplasma* spp. experience adverse pregnancy outcomes while others do not, some researchers have attributed these differences in sequelae to the virulence of the infecting serovar (200), the bacterial load present (201, 202), or genetic background/ethnicity (203, 204). However, these findings are not always consistent, with a recent study by our group demonstrating no correlation between the numbers of *Ureaplasma* present within placentae, the species/serovar present, or the ethnicity of women infected with *Ureaplasma* and the incidence or severity of histological chorioamnionitis (62). Furthermore, animal model studies in which *Ureaplasma* infections have been established with the same strain and dose of *U. parvum* resulted in divergent inflammatory responses within

TABLE 1 Incidence of *Ureaplasma* infection, polymicrobial infections, and chorioamnionitis in women delivering preterm, late preterm, or at term[g]

Author(s) of reference (yr)	Reference no.	GA (wk)	Specimen type	*n*	Incidence, no. positive/no. total (%)			
							Ureaplasma spp.	
					Ureaplasma infection	Polymicrobial infection	With chorioamnionitis	Without chorioamnionitis
Viscardi et al. (2008)	222	<33	S/CSF	313	74/313 (23.6)	—[a]	30/46 (65.0)	16/46 (35.0)
Hassanein et al. (2012)	310	<35	CB	30	13/30 (43.3)	No polymicrobial infections	7/13 (53.8)	6/13 (46.2)
Gray et al. (1992)	311	<28	AF	2,461	8/2,461 (0.4)	—[b]	8/8 (100.0)	0/8 (0.0)
Yoon et al. (1998)	60	≤36	AF	120	25/120 (20.8)	11/120 (9.0)	5/25 (20.0)	
Yoon et al. (2003)	312	≤35	AF	252	23/252 (9.1)	—[c]		
Park et al. (2013)	136	<34	AF	56	35/56 (62.5)	7/56 (12.5)	26/47 (55.31)[f]	0/3 (0.0)
Kacerovsky et al. (2014)	16	24–36	AF	124	26/124 (21.0)	5/124 (4.0)[d]		
Romero et al. (2015)	313	≤35	AF	59	6/24 (25.0)	10/24 (41.7)	3/6 (50.0)	2/6 (33.3)[f]
Stepan et al. (2016)	314	24–34	AF	122	33/122 (27.0)	8/122 (6.6)	29/33 (87.9)	4/33 (12.1)
Musilova et al. (2015)	315	24–36	AF	166	40/166 (24.1)	19/166 (11.4)	26/40 (65.0)	14/40 (35.0)
Stepan et al. (2016)	316	24–36	AF	386	103/386 (26.7)	32/386 (8.3)	70/103 (68.0)[f]	16/103 (15.5)[f]
Berger et al. (2009)	317	≤33	AF/PL	114	32/114 (28.1)	—[a]	11/25 (44.0)[f]	14/25 (66.0)[f]
Hillier et al. (1988)	1	<37	PL	112	32/112 (28.6)	—[c]	19/29 (65.5)[f]	10/65 (15.4)[f]
Stein et al. (1994)	318	Any GA	PL	182	21/182 (11.5)	—[e]	11/16[f]	5/16[f]
Van Marter et al. (2002)	319	<36	PL	206	58/155 (37.4)	—[e]	51/77 (66.2)	7/78 (9.0)
Miralles et al. (2005)	320	<33	PL	14	5/14 (35.7)	5/14 (35.7)	4/5 (80.0)	1/5 (20.0)
Egawa et al. (2007)	135	<32	PL	83	4/83 (4.8)	5/83 (6.0)[b]	4/4 (100.0)	0/4 (0.0)
Olomu et al. (2009)	321	<28	PL	866	52/866 (6.0)	21/52 (40.4)	34/52 (65.4)	18/52 (34.6)
Kasper et al. (2010)	202	<34	AF	118	32/118 (27.1)	—[a]	5/19 (26.3)[f]	14/19 (73.7)[f]
Namba et al. (2010)	134	≤32	PL	151	63/151 (41.7)	13/151 (8.6)	52/63 (82.5)	11/63 (17.5)
Roberts et al. (2012)	4	>37	PL	195	2/195 (1.0)	1/195 (0.5)	0/2 (0.0)	2/2 (100.0)
Kundsin et al. (1984)	322	Various	PL	801	156/801 (19.5)	18/801 (2.2)[b]	32/53 (60.4)[f]	21/53 (39.6)
Sweeney et al. (2016)	62	>32	PL	535	42/535 (7.9)	4/57 (7.0)	26/38 (68.4)	12/38 (31.6)
Cox et al. (2016)	133	<37	PL	57	13/57 (22.8)		9/24 (37.5)	4/33 (12.1)

[a]Only *Ureaplasma* spp. were tested for within the study.
[b]Only genital mycoplasmas (*Ureaplasma* spp. and *Mycoplasma hominis*) were tested for within this study.
[c]Study states that >1 organism may have been isolated, but prevalence of polymicrobial infections not stated.
[d]Only *Ureaplasma* spp., *Mycoplasma hominis*, and *Chlamydia trachomatis* tested for within this study.
[e]No comment on polymicrobial infections.
[f]Not all placentae in study were tested.
[g]The incidence of chorioamnionitis in *Ureaplasma*-infected women is frequently high, indicating that these microbes are associated with chorioamnionitis.
Abbreviations: AF, amniotic fluid; CB, cord blood; CSF, cerebrospinal fluid; GA, gestational age; PL, placenta; S, serum.

the chorioamnion (59, 205, 206) and within other genital tract tissues (207), suggesting that the development or magnitude of host immune responses may contribute to the severity of chorioamnionitis. Indeed, we have demonstrated that the human *Ureaplasma* spp. can undergo immune evasive behavior *in vivo* by varying the expression of their surface-exposed antigens and that the severity of chorioamnionitis is inversely related to the number of antigenically distinct subtypes detected within amniotic fluid (reviewed in detail below). Therefore, we hypothesize that the ability of some *Ureaplasma* strains to hide from the immune system may be an important predictor of outcomes and may potentially explain why some women do not develop chorioamnionitis despite high bacterial loads within the amniotic fluid and chorioamnion.

Table 1 summarizes human studies which have investigated the role of *Ureaplasma* spp. in chorioamnionitis. These studies showed that the rates of *Ureaplasma*-associated inflammation within the chorioamnion may vary between 0 and 100%, further highlighting the diversity of histological chorioamnionitis and why it is so difficult to confirm the role of these microorganisms as causative agents of chorioamnionitis. Additionally, the pathogenic role of *Ureaplasma* spp. is often unclear as the majority of these infections are clinically silent. *Ureaplasma* infections of the chorioamnion can persist asymptomatically for up to 2 months in humans (208), and *Ureaplasma*-infected placentae cannot be distinguished macroscopically from normal placentae (although there may be histological evidence of chorioamnionitis that is detected following delivery). Due to the predominantly asymptomatic nature of *Ureaplasma* infections,

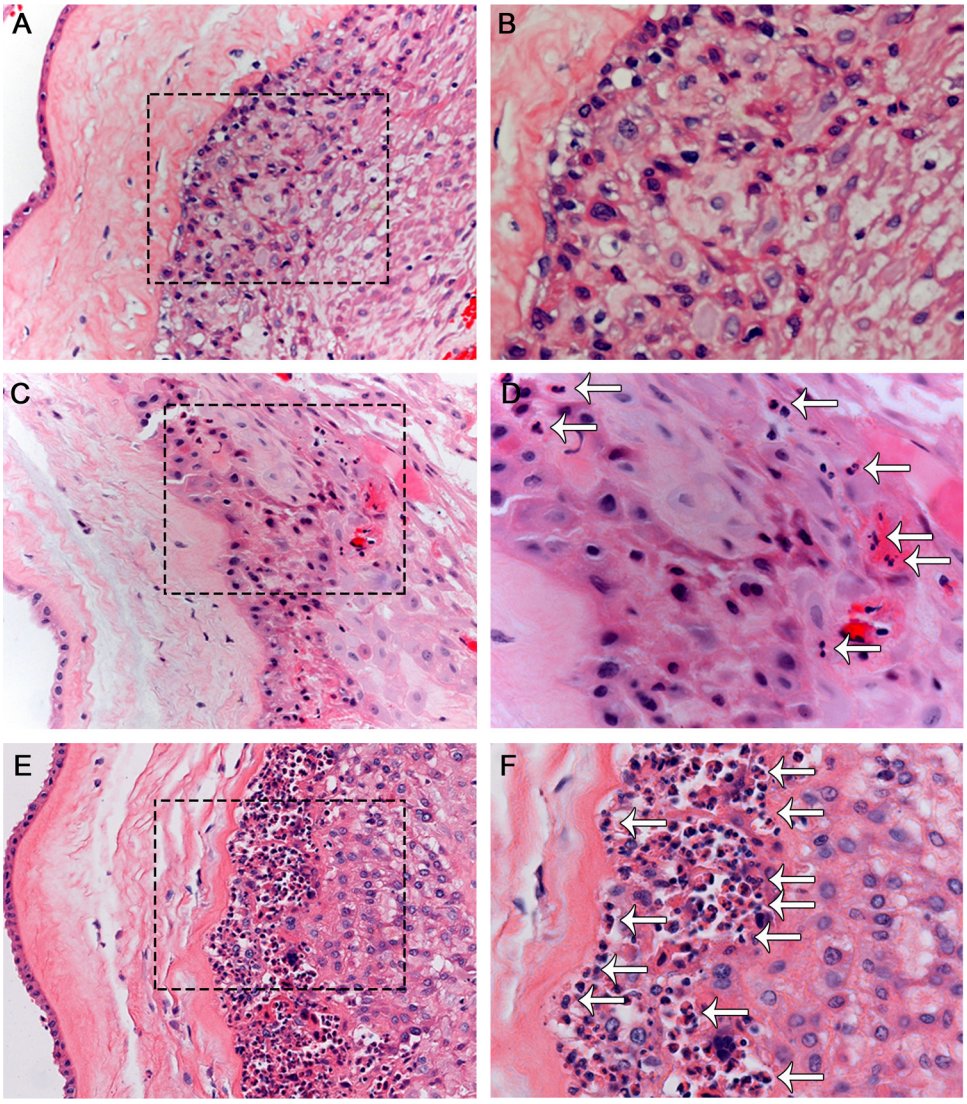

FIG 2 Differences in the presence of chorioamnionitis in *Ureaplasma*-infected women. Hematoxylin-and-eosin-stained chorioamnion tissue demonstrates that some women whose placentae are colonized with *Ureaplasma* spp. have no evidence of chorioamnionitis (A and B), while other women have mild/moderate (C and D) or severe (E and F) evidence of inflammation (demonstrated by neutrophil influx [arrows]) within their chorioamnion, despite high numbers of *Ureaplasma* spp. present within the tissue. Images are shown at ×200 (A, C, and E) and ×400 (B, D, and F) total magnifications; boxed areas in panels A, C, and E are shown in panels B, D, and F, respectively.

coupled with the fastidious growth requirements of these microorganisms, pregnant women are not routinely screened for *Ureaplasma* spp., and therefore, these tiny bacteria are not always suspected (and are, therefore, likely to be underreported) as causative agents of chorioamnionitis.

One of the major reasons why the role of *Ureaplasma* spp. in chorioamnionitis has remained unconfirmed is due to the polymicrobial nature of chorioamnionitis (5, 209). The majority of studies investigating chorioamnionitis focus specifically on very preterm (<28 weeks) and early preterm (28 to 32 weeks) pregnancies, and these studies have demonstrated that up to 67% of amniotic fluid or placental samples with chorioamnionitis contained at least two detectable microorganisms (often *Ureaplasma* spp. and another microorganism) (Table 1). Because of this, researchers have not been able to confidently claim that *Ureaplasma* spp. are true etiological agents of chorioamnionitis. However, a recent study by our research group demonstrated that infections within late preterm (32 to 36 weeks) and term (≥37 weeks) placentae typically harbored only a single microorganism (90.5%) and that the presence of *Ureaplasma*

spp. alone was significantly associated with histological chorioamnionitis, at any gestational age (62). Further investigations confirmed the finding that placental infections with *Ureaplasma* spp. are strongly associated with chorioamnionitis, using whole-genome shotgun sequencing of late preterm and term placentae (120). Similarly, another study has reported that preterm placentae infected with *Ureaplasma* spp. alone are independently associated with inflammation of the chorioamnion membranes. This study demonstrated that there were no differences in the incidence of chorioamnionitis in placentae infected with *Ureaplasma* spp. and other microorganisms compared to placentae infected with *Ureaplasma* spp. alone (210). Taken together, these recent data not only suggest that *Ureaplasma* spp. are likely to be a key etiological agent of chorioamnionitis in the absence of other microorganisms but also support a causal role for *Ureaplasma* in chorioamnionitis throughout pregnancy.

ANIMAL MODELS HAVE HELPED TO ELUCIDATE THE PATHOGENESIS OF *UREAPLASMA* CHORIOAMNIONITIS

Studies in experimental animal models have confirmed that *Ureaplasma* spp. can cause chorioamnionitis and fetal inflammation following intrauterine inoculation. Using a nonhuman primate model, Novy et al. (211) inoculated 10^7 CFU of *U. parvum* serovar 1 into the amniotic fluid of pregnant rhesus macaques at day 132 to 147 of gestation (term = 155 to 172 days) via an indwelling catheter. Intra-amniotic *U. parvum* caused a significant influx of leukocytes into the amniotic fluid and significant increases in the amniotic fluid levels of (i) TNF-α, IL-1β, IL-1ra, IL-6, and IL-8; (ii) PGE2 and PGF2α; and (iii) latent (92-kDa) and active (83-kDa) MMP-9 compared with preinoculation baseline values. A progressive increase in uterine activity was also observed following *U. parvum* intra-amniotic inoculation, and the mean inoculation-to-labor onset period was significantly reduced in *U. parvum*-infected animals compared to those inoculated with sterile medium or saline. Histological examination of fetal membranes revealed acute chorioamnionitis that was characterized by edematous thickening of the chorioamnion, neutrophil infiltration, denudation of amnion epithelial cells, and necrosis and microabscess formation in chorion trophoblast cells (211). Similarly, intra-amniotic injection of *U. parvum* serovar 1 into the amniotic cavity of pregnant baboons at day 122 to 123 of gestation (term is 185 days) resulted in elevated levels of amniotic fluid IL-6 and IL-8 at the time of preterm delivery (125 days) and histological evidence of acute chorioamnionitis (212). In contrast, more-recent studies in rhesus macaques demonstrated that despite the presence of high numbers (3.9×10^7 CFU/ml) of *U. parvum* serovar 1 within the amniotic fluid, no chorioamnionitis was detected after acute durations (3 days and 7 days) of infection (213).

While nonhuman primate models exhibit the closest resemblance to humans with respect to gestational length, uterine anatomy, and parturition, experimental intrauterine infection causes preterm delivery (211, 214), and therefore, it is only possible to study acute chorioamnionitis in these models. In contrast, sheep do not experience inflammation-induced preterm birth, as intra-amniotic infection/inflammation does not cause significant activation of the fetal HPA axis, cortisol production, and subsequent progesterone withdrawal, which are required for the initiation of labor in many species (215–217). This enables the study of chronic, asymptomatic intrauterine infection and chorioamnionitis, which is not possible using other animal models. In addition, fetal sheep are similar in size to human fetuses, which enables instrumentation of the ewe and fetus (217), and thus makes the ovine model very useful for the study of fetal development and neonatal outcomes following chorioamnionitis exposure.

We have demonstrated that human *U. parvum* clinical isolates injected into the amniotic cavity of pregnant sheep at 55 days (term is 150 days) can chronically colonize the amniotic fluid and fetus (59, 205, 215, 218). Following an intra-amniotic injection of 2×10^4 CFU of *U. parvum* serovar 6 at 55 days of gestation, temporal analysis demonstrated that the peak of amniotic fluid infection occurred between 87 days and 101 days of gestation and that the number of CFU per milliliter remained high (approximately 10^7 CFU/ml) until the time of surgical delivery at 140 days (59). These

data demonstrate that *Ureaplasma* can chronically colonize the amniotic fluid for at least 85 days and suggest that amniotic fluid, a rich source of urea, can support the long-term growth of these microorganisms. We further demonstrated that *U. parvum* was consistently isolated from the chorioamnion and fetal lung following chronic intra-amniotic infection (205, 215, 218–220) and was also isolated from the umbilical cord and other fetal tissues, including cerebrospinal fluid, gut, kidney, liver, and spleen (205). These findings are consistent with human studies that have reported that *Ureaplasma* spp. may systemically infect the fetus, leading to neonatal morbidity and mortality (221–228).

Both chronic and acute intrauterine *Ureaplasma* infections were capable of causing histological chorioamnionitis in pregnant sheep (59, 205, 206, 218, 219). Intra-amniotic *U. parvum* infection was also associated with increased expression of IL-1β, IL-6, and IL-8 mRNA within the chorioamnion (59, 219) and an influx of neutrophils, monocytes/macrophages, and lymphocytes (59, 205, 218), compared to medium (vehicle) controls. Generally, the severity of chorioamnionitis correlated with increased duration of intra-amniotic *Ureaplasma* exposure (206); however, variability in the severity of inflammation was a notable feature of these sheep studies (205, 206), consistent with findings from human pathological investigations. Despite 100% of chorioamnion samples being infected with *U. parvum*, the severity of chorioamnionitis ranged from moderate (characterized by inflammatory cell infiltrate, fibrosis, scarring, sloughing of the amnion epithelium, and disruption of the normal tissue architecture) to no histological evidence of chorioamnionitis (205). The severity of chorioamnionitis was not related to the bacterial load within the chorioamnion at the time of delivery, the inoculating serovar, or the initial dose of *U. parvum* (205).

In an attempt to explain the differences in severity of *Ureaplasma* chorioamnionitis and address whether some *Ureaplasma* isolates are inherently more virulent than others, we infected the amniotic cavity of pregnant sheep with clonal *U. parvum* serovar 6 isolates (59), derived from placental isolates, which had caused severe histological chorioamnionitis (virulent strain-derived strain) or no chorioamnionitis (avirulent strain-derived strain) in a previous ovine study (205). Regardless of the inoculating clonal strain, moderate to severe chorioamnionitis was observed in experimentally infected animals and there were no differences in the chorioamnion expression of TLR-1, TLR-2, TLR-6, IL-1β, IL-6, IL-8, IL-10, and TNF-α between animals infected with the avirulent strain-derived strain and those infected with the virulent strain-derived strain. Similarly, there were no differences in the numbers of *U. parvum* isolated from the amniotic fluid, chorioamnion, cord, or fetal lung at 140 days (59). In the same study, we demonstrated that only a subpopulation of infected ewes from each group generated a serum IgG response to intrauterine *U. parvum* infection. When cytokine expression was compared between animals with and without anti-*Ureaplasma* serum IgG, the expression of IL-1β, IL-6, and IL-8 was significantly increased in the chorioamnion of anti-*Ureaplasma* IgG$^+$ animals. In addition, maternal anti-*Ureaplasma* serum IgG was associated with a significant increase in meconium-stained amniotic fluid (59). These findings are also consistent with human studies that have demonstrated that patients with anti-*Ureaplasma* antibodies are at a higher risk for adverse pregnancy and neonatal outcomes than are those who do not develop a humoral immune response (229, 230). Taken together, this suggests that *Ureaplasma* strains are not likely to be inherently virulent or avirulent but that the host response to infection may affect the pathogenesis of chorioamnionitis.

The Immune Response to *Ureaplasma* Chorioamnionitis: Harmful or Helpful?

Studies in BALB/c and C57BL/6 mice have provided unique insights into the potentially harmful immune responses that may occur during *Ureaplasma* chorioamnionitis. BALB/c mice typically display a Th1/M1-dominant immune profile, whereas the immune profile of C57BL/6 mice is consistent with a Th2/M2 bias (203). These differences have enabled researchers to examine the immunopathogenic role of a skewed Th1/M1 or Th2/M2 response in *Ureaplasma* chorioamnionitis. In a model of experimental intra-

uterine infection, von Chamier et al. injected 10^7 CFU of *U. parvum* into the uterine horns of pregnant BALB/c and C57BL/6 mice at 14 days (203). Examination of the fetal membranes at 72 h postinfection demonstrated that C57BL/6 mice exhibited mild-moderate chorioamnionitis, whereas BALB/c mice displayed severe necrotizing chorioamnionitis and extensive neutrophil infiltration. These differences could not be attributed to differences in bacterial load; however, the placental expression levels of cytokines and calgranulins were markedly different between the strains (203). In a separate study, it was demonstrated that intrauterine *U. parvum* infection increased the expression of TLR2 and CD14 on neutrophils in BALB/c but not C57BL/6 mice (56). TLR/CD14-mediated signaling triggered by bacterial lipoproteins has been shown to extend the survival of apoptotic neutrophils in infected tissues, thereby increasing the duration of inflammation (231). It is therefore possible that TLR2/CD14 signaling plays a role in the extensive neutrophil infiltration and severe chorioamnionitis observed in BALB/c mice. Interestingly, increased levels of soluble CD14 are also observed in the amniotic fluid of women with intrauterine *Ureaplasma* infection (232), suggesting that CD14 signaling may be an important area for future research. Combined, these studies demonstrate that the host immune response may be a key factor that modulates the pathogenesis of acute *Ureaplasma* chorioamnionitis. Further studies using genetically modified/knockout mouse lines may significantly improve our understanding of protective versus pathogenic immune responses to intrauterine *Ureaplasma* infection.

Immune Effects of *Ureaplasma* spp. on the Fetus

Animal model studies from our research group have investigated the fetal immune responses to *U. parvum* exposure during gestation. In a series of experiments in pregnant sheep, it was demonstrated that chronic (69 days), but not acute (7 days), *in utero* infections with *U. parvum* suppressed innate immune responses in fetal sheep. Fetuses were challenged with *E. coli* LPS 2 days prior to delivery, and the fetuses that were chronically exposed to intra-amniotic *Ureaplasma* spp. demonstrated significant decreases in pro- and anti-inflammatory cytokine expression, as well as fewer $CD3^+$ T lymphocytes and myeloperoxidase$^+$ cells within the fetal lung, compared to the fetuses that were intra-amniotically exposed to sterile culture medium (vehicle). Blood monocytes obtained from these same animals also had a significantly decreased response to LPS *in vitro* (121), demonstrating that fetal exposure to *U. parvum in utero* can markedly alter the neonatal immune responses following delivery. Similarly, chronic exposure to *U. parvum* alone (with no LPS challenge) was sufficient to augment the presence of transforming growth factor beta (TGF-β) within the fetal lung, which may also contribute to the development of lung pathologies, such as bronchopulmonary dysplasia (233).

In both rhesus macaque and sheep models, intra-amniotic *U. parvum* infections decreased the populations of $CD4^+$ $FOXP3^+$ regulatory T cells (Tregs) in the preterm fetus, in both the thymus and the periphery (213, 234). Furthermore, a gamma interferon response was seen in Tregs exposed to *U. parvum* during gestation, and this response was absent in Tregs of fetuses exposed to control (medium) intra-amniotic injections. Since it is well established that Tregs are potent anti-inflammatory T cells (235), these results suggest the existence of a subset of Tregs that can develop a Th1 phenotype early in life and suggest that this response may be increased in the presence of inflammation (e.g., chorioamnionitis).

MANIPULATION OF HOST CELLS BY *UREAPLASMA* SPP.

Compared to other *Mycoplasma* spp., the cytadherence of *Ureaplasma* has not been investigated in detail. *In vitro* studies have demonstrated that *Ureaplasma* spp. are adherent to erythrocytes (236), placental endothelial cells (237), and human epithelial cells (238); however, the adhesion mechanisms are unknown. Pretreatment of HeLa cells and erythrocytes with neuraminidase significantly reduced ureaplasmal adherence (238), suggesting that *Ureaplasma* may bind to receptors containing sialic acid. In contrast, the adhesion of *Ureaplasma* to spermatozoa is thought to be mediated by

sulfogalactoglycerolipid, which is expressed by the mammalian male germ cell membrane (239).

The human *Ureaplasma* spp. have been shown to alter/manipulate host cells in several ways. Allam et al. reported that *U. parvum* significantly increased filamin A phosphorylation at serine 2152 in human benign prostate cells and altered its intracellular distribution (240). Filamin A is an actin-binding protein that regulates the cytoskeleton and is involved in antimicrobial signaling pathways (241). Further investigation into the upstream and downstream signaling events may therefore reveal novel insights into *Ureaplasma*-host interactions. In endothelial cells isolated from normal and preeclamptic placentae, *U. urealyticum* significantly reduced cell viability, altered the expression of heat shock protein 70, and significantly increased the intracellular concentration of calcium and iron. It was suggested that these events occurred as part of the cellular stress response to infection and may indicate that cells are progressing toward apoptosis (237). Additional studies have demonstrated that *U. urealyticum* induces apoptosis in other cell types, including human lung epithelial cells (A549) and THP-1-derived macrophages (242). *Ureaplasma*-infected cells demonstrated an altered morphology and underwent DNA fragmentation and translocation of phosphatidylserine to the outside surface of the cell (as determined by annexin V staining and flow cytometry) (242). *Ureaplasma* spp. further manipulate host cells by suppressing innate host defense pathways. A recent study demonstrated that *Ureaplasma* infection decreased the expression of antimicrobial peptide genes in THP-1 cells *in vitro*, in association with a significant decrease in histone H3K9 acetylation (243). These findings suggest that *Ureaplasma* may downregulate antimicrobial/host defense genes via epigenetic modifications (243), which may be an important factor contributing to the ability of these microorganisms to cause persistent infections. Further studies using a combination of *ex vivo* and *in vivo* approaches are required to elucidate the host-pathogen interactions that occur during *Ureaplasma* chorioamnionitis.

UREAPLASMA VIRULENCE FACTORS

While *Ureaplasma* spp. were traditionally portrayed as microorganisms of low virulence, they are now recognized as the cause of serious disease. As such, *Ureaplasma* spp. have evolved specific virulence mechanisms that contribute to their survival and disease pathogenesis. Five proposed virulence factors have been identified: the multiple-banded antigen (MBA), phospholipases A and C, IgA protease, and the urease gene of *Ureaplasma* spp. Genetic manipulation of these microorganisms has remained elusive, and thus definitive roles for these proposed virulence factors have not been determined. Furthermore, recent genome sequencing studies have questioned the presence of some of these proposed virulence factors.

Multiple-Banded Antigen (MBA)

The MBA was first described by Watson et al. (244) and has since been identified as one of the major virulence factors of the human *Ureaplasma* spp. The *mba* gene, which encodes the MBA protein, contains no homology to any other known prokaryotes and is unique to *Ureaplasma* spp. (245). The MBA protein is the major antigen that is recognized by the host during infection and elicits the production of cytokines by activating nuclear factor kappa B via TLR-1, -2, and -6 (246–248). The MBA protein consists of three major domains: a typical prokaryotic signal peptide, an N-terminal transmembrane domain that is conserved among all 14 serovars of *Ureaplasma* spp., and a C-terminal (surface-exposed) variable domain that is composed of multiple repeating units, with both serovar-specific and cross-reactive epitopes (249, 250). The C-terminal region of the MBA has been shown to alter by switching on/off the gene (antigenic phase variation) and more commonly to vary in size (antigenic size variation) (59, 205, 249–252). *U. urealyticum* serovar 13 is the only *Ureaplasma* serovar that does not contain any tandem repeat units in the C-terminal variable domain of *mba* (145).

While some studies demonstrated differences in the size of the MBA protein (giving rise to the name of the protein itself as the multiple-banded antigen) (244, 252), the first

study to characterize MBA size variation demonstrated that differences in the size of the MBA protein directly correlated with the number of tandem repeating units within the *mba* gene (149, 253). More recently, Knox et al. identified *mba*/MBA size variation *in vivo* using an ovine model (205). Pregnant ewes were chronically infected for 69 days with a nonclonal *U. parvum* isolate, and the size of *mba*/MBA was assessed. This study demonstrated that the number of *mba*/MBA size variants was inversely correlated with the severity of inflammation within the chorioamnion: when >9 *mba*/MBA size variants were identified, there was little or no chorioamnionitis; however, when <5 *mba*/MBA size variants were identified, there was severe histological chorioamnionitis (205). Other ovine studies have identified that variation in the size of the *mba*/MBA was not seen after 3 days of intra-amniotic infection, while some slight variation was seen after 7 days of infection (206) and significant *mba*/MBA size variation was seen after 69 days of chronic intra-amniotic *U. parvum* infection (59, 205, 206). Dando et al. (59) also demonstrated the ability of *Ureaplasma* spp. to vary their *mba*/MBA size throughout the course of gestation and suggested that size variation of *mba*/MBA (presumably by slipped-strand mispairing) may be a mechanism by which *Ureaplasma* spp. evade host immune recognition, allowing chronic asymptomatic infections to develop (59).

More recently, we have demonstrated for the first time that *Ureaplasma* clinical isolates from human placentae were also able to vary the size of their *mba*/MBA (E. L. Sweeney, S. Meawad, S. G. Kallapur, C. A. Chougnet, T. Gisslen, S. Stephenson, A. H. Jobe, and C. L. Knox, unpublished data). Clinical isolates that varied the size of their *mba*/MBA were associated with a reduced incidence of histological chorioamnionitis and significantly lower levels of the cord blood cytokines G-CSF and IL-8. In contrast, *Ureaplasma* spp. isolated from placentae that demonstrated no *mba*/MBA size variation were associated with severe histological chorioamnionitis and elevated cord blood cytokines. Further *in vitro* investigations using recombinant MBA (rMBA) proteins of differing sizes (i.e., different numbers of tandem repeat units) and human macrophage cell lines demonstrated immune responses that varied depending on the size of the rMBA. These results were confirmed by Western blot analysis; the expression of nuclear factor kappa B fragment p65 (an activator of transcription) varied when stimulated with the different-size rMBA proteins (Sweeney et al., unpublished). Combined, these results confirm the ability of *Ureaplasma* spp. to vary their surface-exposed MBAs *in vivo* and confirm that this variation is associated with the modulation of the host immune response both *in vivo* and *in vitro*.

Other studies have also demonstrated that *mba*/MBA can undergo phase (on/off-switching) variation. Three studies have identified that selective antibody pressure directed against the MBA can result in the generation of MBA-negative variants (*Ureaplasma* isolates that do not express their MBA protein) in serial passage experiments (59, 251, 254). In these studies, MBA-negative *Ureaplasma* isolates were detected following two to three serial passages in culture medium containing MBA-specific antibodies (59, 251). More recently, phase variation of the MBA occurred in the absence of any selective (antibody) pressures (255), indicating that this antigen is capable of rapid phase variation. Zimmerman et al. (254) hypothesized that the expression of the *mba* gene (locus UU375) is alternated with expression of an adjacent locus (UU376), which encodes a *Ureaplasma*-specific conserved hypothetical protein. Utilizing polyclonal rabbit antisera generated against the conserved (N-terminal, nonrepetitive) regions of MBA and UU376, these authors identified that antibody treatment led to the emergence of escape variants, which expressed the protein that had not been the target of selective pressure. Following this, it was hypothesized that DNA inversion events—presumably occurring at short inversion sequences—were responsible for the switching-on/off expression of these genes (254). Zimmerman and colleagues further investigated the role of DNA inversion sites within the *Ureaplasma* genome and demonstrated experimentally that the *mba* paralogues UU171 and UU172 and the orthologue UU144 were also involved in site-specific DNA inversion/recombination (256). Furthermore, it was shown that the XerC tyrosine recombinase gene of *U. parvum* is the

most likely mediator of these DNA inversion events (257). Subsequent experimental investigation into the ability of XerC to process the recombination event proved successful, indicating that this tyrosine recombinase is able to induce DNA inversion events (258), representing the first evidence of a mechanism which may govern antigenic phase variation in *Ureaplasma* spp.

In a separate series of investigations, whole-genome sequencing was carried out on *Ureaplasma* ATCC strains and a range of clinical isolates and revealed the presence of multiple additional tandem repeat domains within the *mba* locus of all *Ureaplasma* isolates tested (145). Remarkably, it was shown that *mba* was part of a large gene superfamily, comprising 183 genes in *U. parvum* and *U. urealyticum* and 22 gene subfamilies. This study also identified the presence of putative recombination sites surrounding tandem repeating domains, consistent with the theory that *Ureaplasma* spp. may undergo significant antigenic phase and size variation, dependent on which sequences within the genome are expressed. While there is convincing molecular evidence that the *mba* gene is part of a complex phase-variable system, it should be noted that, to the best of our knowledge, MBA-negative *Ureaplasma* variants have not been isolated from human clinical material or experimental animal studies. Rather, there is significant evidence of MBA size variation *in vivo*.

Phospholipases A and C

The pathogenesis of phospholipases results from the production of membrane-destabilizing compounds and degradation of the host cell membrane phospholipids (259). Endogenous phospholipase A_1, A_2, and C activity has been previously identified in *U. parvum* serovar 3 and *U. urealyticum* serovars 4 and 8 (260–262). These phospholipases demonstrated higher activity in *Ureaplasma* during their exponential growth phase, suggesting that the *Ureaplasma* phospholipases were membrane bound and were not being secreted (261). It was further identified that phospholipase A_2 activity was 3-fold higher in *U. urealyticum* serovar 8 than in *U. urealyticum* serovar 4 and *U. parvum* serovar 3 (260). However, subsequent whole-genome sequencing of *U. parvum* serovar 3 could not identify any genes of significant similarity to any known sequences of phospholipase A_1, A_2, or C (245). These findings indicated that *Ureaplasma* may encode phospholipases that are evolutionarily distinct from other phospholipase genes or that these phospholipases may not exist within *Ureaplasma* spp. Interestingly, more recent studies by the same research group revealed that whole-genome sequencing of the 14 *Ureaplasma* serovars and four *Ureaplasma* clinical isolates was again unable to detect any phospholipase A_1, A_2, or C genes; however, a phospholipase D domain-containing protein was identified in all *Ureaplasma* spp. (145). These researchers further investigated the presence/activity of these enzymes experimentally and were unable to detect any significant phospholipase C or D activity in *U. parvum* serovar 3 and *U. urealyticum* serovar 8 (145). Further investigations into the presence and activity of phospholipases within *Ureaplasma* spp. are required to elucidate if these enzymes are potential virulence factors of these organisms.

Immunoglobulin A (IgA) Protease

One of the primary defense mechanisms of the mammalian immune system is the production of IgA at mucosal sites (263), and the ability of an organism to degrade IgA antibodies allows the microorganism to evade this host defense mechanism. Robertson et al. published the first evidence of an IgA protease in *U. urealyticum* that was capable of cleaving IgA_1 (264). While it was subsequently determined that all 14 *Ureaplasma* serovars possess an IgA protease with proteolytic activity against IgA_1 (but no proteolytic activity against IgA_2, IgG, or IgM antibodies) (265, 266), more recent evidence has questioned the presence of an IgA protease in *Ureaplasma* spp. Initial genome sequencing studies of *U. parvum* serovar 3 were unable to identify any genes with similarity to known IgA proteases (245), and more recent whole-genome analyses were unable to identify any IgA protease genes within the 14 *Ureaplasma* serovars, nor were they found to be present in any of the *Ureaplasma* clinical isolates tested

(145). Recently, an IgG binding protein and IgG serine protease were identified within *Mycoplasma mycoides* subsp. *capri*. This study provided evidence that both *U. parvum* and *U. urealyticum* contain genes that encode an IgG binding protein and an IgG serine protease within their genomes (267). Based on these recent findings, further studies are warranted to determine if these IgG binding/IgG protease genes are active in cleaving IgG and therefore may be a previously unrecognized virulence factor of the human *Ureaplasma* spp.

Urease

The ability of *Ureaplasma* spp. to hydrolyze urea was first identified in 1966, and the production of ATP via this mechanism appears to be unique within *Ureaplasma* (141, 268). The urease enzyme is a key virulence factor of many ureolytic bacteria, and the ureaplasmal urease gene cluster has a genetic organization similar to that of *E. coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Klebsiella aerogenes* (269). The urease complex constitutes a major component of the ureaplasmal cytoplasm (270), and Takebe et al. demonstrated that the urease of *U. urealyticum* serovar 8 was responsible for urolithiasis in humans (271). The *Ureaplasma* urease has a significantly higher specific activity than other bacterial ureases (272) and was responsible for lethal toxicity in mice following intravenous injection (273). Interestingly, the *Ureaplasma* spp. are some of the few bacterial species which encode a urease enzyme but lack the ability to assimilate ammonia into glutamine or glutamate (274), potentially explaining the very high intracellular ammonia concentration of these microorganisms (140).

Our recent studies suggest that *Ureaplasma* infection, and a subsequent increase in ammonia due to urease metabolism, can alter the pH of amniotic fluid and fetal lung fluid in an ovine model (206). This study also identified that the increased pH within the fetal lung was associated with lung damage, even in the absence of inflammatory responses, and provides the first evidence that increased pH *in vivo* may be due to *Ureaplasma* infections. Other studies have demonstrated that *Ureaplasma* infections can result in hyperammonemia (275). Clinical reports describe that patients who underwent lung transplantation and subsequently developed hyperammonemia (abnormally high levels of ammonia within the blood) were found to be infected with *Ureaplasma* spp. within their blood or bronchoalveolar lavage fluid. When these patients received antibiotic treatment to eradicate the *Ureaplasma* spp., their syndromes resolved, and only one relapse was identified, in a patient colonized with an antimicrobial-resistant *Ureaplasma* strain (275). Taken together, these findings suggest that the activity of the *Ureaplasma* urease enzyme can result in an alkaline environment in both fetal and adult lungs and also within amniotic fluid.

HORIZONTAL GENE TRANSFER (HGT) AND THE ABILITY OF *UREAPLASMA* SPP. TO RAPIDLY ADAPT TO HOST MICROENVIRONMENTS

HGT is an important mechanism used by microorganisms to acquire genetic material. Although *Ureaplasma* spp. maintain minimal genomes that have undergone significant degenerative evolution (245), recent evidence has identified that HGT is likely to occur within these microorganisms and may be an important determinant of virulence. As previously discussed, the identification of genetic hybrids (162) suggests that the *Ureaplasma* spp. may be genetically promiscuous. Comparative genome sequencing studies have provided further evidence of this and identified integrase-recombinase genes, transposases, and phage-related proteins in *Ureaplasma* genomes (145), which are highly indicative of HGT events. Interestingly, *U. urealyticum* genomes generally contained a higher number of these genes, suggesting that this species may be more capable of acquiring genes horizontally than *U. parvum* (145).

Early attempts to define the phylogeny of *Mycoplasma* suggested that *Mycoplasma* spp. with the smallest genomes have high mutation rates and undergo rapid evolution (276, 277). Dando et al. provided evidence of the ability of the human *Ureaplasma* spp. to rapidly adapt to their microenvironment in a sheep model of intrauterine infection (278). Following injection of a nonclonal *U. parvum* serovar 3 isolate into the amniotic

fluid of pregnant sheep at 55 days, significant genetic variability within the 23S rRNA gene was detected between *U. parvum* isolated from the amniotic fluid and chorioamnion at the time of preterm surgical delivery (125 days). While *U. parvum* isolated from amniotic fluid showed 100% 23S rRNA domain V sequence homology to the original strain injected, highly polymorphic sequences (containing only 64 to 82% sequence homology to the inoculating strain) were detected within *Ureaplasma* isolates from the chorioamnion. Furthermore, chorioamnion *Ureaplasma* isolates demonstrated the presence of macrolide resistance genes, which were not evident in amniotic fluid isolates. While this study did not investigate the presence of potential genetic transfer elements flanking these variable gene sequences, these data support the concept that *Ureaplasma* spp. may undergo significant HGT *in vivo*. Furthermore, this study suggests that different anatomical sites (amniotic fluid versus chorioamnion) may select for different *Ureaplasma* subtypes within nonclonal populations and thus influence the sociomicrobiological structure of the bacterial population (278). Taken together, there is increasing evidence that *Ureaplasma* spp. undergo significant genetic variation, allowing them to diversify their populations, and this is likely to contribute to the overall pathogenicity of the *Ureaplasma* spp.

TREATMENT OF *UREAPLASMA* CHORIOAMNIONITIS AND THERAPEUTIC CONSIDERATIONS

The major difficulty in treating chorioamnionitis is that a large proportion of cases are clinically asymptomatic and therefore are not diagnosed until retrospective analysis of the placenta and fetal membranes. This is particularly problematic for the human *Ureaplasma* spp., which can cause chronic, asymptomatic intrauterine infections that modulate the host immune response to prevent significant pathological events but are still associated with adverse outcomes. While antibiotics are recommended for women with preterm prelabor rupture of membranes (279) to prevent ascending invasive infections from the lower genital tract, the timing of administration may be too late to have beneficial effects against chronic *Ureaplasma* infections that were established in early/midgestation. It has been suggested that the administration of appropriate antibiotics before 22 weeks of gestation (or before inflammation and maternal-fetal damage occur) could significantly decrease the incidence of preterm birth (280). This is supported by a meta-analysis which demonstrated that the administration of macrolides and clindamycin during the second trimester of pregnancy was associated with a reduced risk of preterm delivery (281). However, due to concern about antibiotic resistance, widespread antimicrobial treatment is not recommended unless there is evidence of intra-amniotic infection. Culture and/or PCR detection of *Ureaplasma* spp. within amniotic fluid remains the gold standard for diagnosis; however, amniocentesis is an invasive procedure that is not routinely performed, and it is likely that high numbers of *Ureaplasma* infections during pregnancy remain undetected and therefore untreated.

An additional complicating factor for the treatment of *Ureaplasma* chorioamnionitis includes the often polymicrobial nature of this disease, which suggests that more than one antimicrobial agent may be required to successfully eradicate infection. Furthermore, treatment options for pregnant women are limited due to potential teratogenic and harmful effects associated with the use of some antimicrobials during pregnancy. Even fewer options are available for the treatment of intrauterine *Ureaplasma* infections, as these microorganisms are inherently resistant to beta-lactam and glycopeptide antibiotics (due to their lack of a cell wall), as well as trimethoprim and sulfonamides (as *Ureaplasma* spp. do not synthesize folic acid) (282). Antimicrobials that are potentially active against *Ureaplasma* include the tetracyclines, fluoroquinolones, and macrolides; however, resistance to these antimicrobial classes has also been well described (283–287).

Erythromycin, a 14-membered lactone ring macrolide, is the most common antibiotic used for the treatment of neonatal *Ureaplasma* infections and is routinely used in clinical obstetrics. Large randomized controls and meta-analyses have demonstrated

that erythromycin administration for preterm prelabor rupture of membranes can reduce the risk of chorioamnionitis and neonatal morbidity and delay preterm birth (288–290). However, it is less clear if maternal erythromycin can eradicate existing human intrauterine infections due to conflicting reports within the literature (291–293). In pregnant sheep, maternal intramuscular erythromycin treatment (30 mg/kg of body weight/day for 4 days) failed to eradicate an erythromycin-susceptible strain of *U. parvum* from the amniotic fluid, chorioamnion, and fetal lung (218), presumably due to poor transplacental passage (218, 294–296). In a follow-up study, it was again demonstrated that intra-amniotic *Ureaplasma* infection was not eradicated following (i) single intra-amniotic and repeated maternal intramuscular erythromycin or (ii) single maternal intramuscular and repeated intra-amniotic erythromycin injections (297). These data suggest that erythromycin may not be beneficial for the treatment of intrauterine *Ureaplasma* infections.

Azithromycin is a 15-membered semisynthetic macrolide with superior tissue penetration, a prolonged half-life, and broader antimicrobial coverage than erythromycin (298). Azithromycin is well tolerated during pregnancy and achieves peak concentrations of 151 $\pm$ 46 ng/ml within human amniotic fluid and 2,130 $\pm$ 340 ng/ml within human placentae at 6 h postinjection, before rapidly declining (298). In pregnant sheep, a single intra-amniotic injection of azithromycin achieved therapeutic concentrations that were sustained for 48 h; however, there was poor maternal-fetal transfer (296). Despite this, a single maternal intravenous azithromycin injection or a single maternal intravenous azithromycin injection combined with an intra-amniotic azithromycin injection completely eradicated an established *U. parvum* infection from the amniotic fluid, chorioamnion, and fetal lung in pregnant sheep (299). Similarly, studies in rhesus macaques demonstrated that maternal intravenous azithromycin (25 mg/kg/day for 10 days) administered 6 to 8 days after intra-amniotic *U. parvum* inoculation successfully eradicated *Ureaplasma* from the amniotic fluid (300, 301). It should be noted that in both of these sheep (299) and monkey (301) studies, histological evidence of chorioamnionitis was still observed at the time of delivery, suggesting that azithromycin treatment alone is not sufficient to reduce/eliminate inflammation within the fetal membranes.

Recent research efforts have evaluated a new, broad-spectrum fluoroketolide, solithromycin, in pregnant sheep and demonstrated that a single maternal dose can deliver therapeutic concentrations to both the fetus and the amniotic fluid (302). The transplacental transfer of solithromycin was significantly higher than that reported for other macrolides, including azithromycin, and a maternal intravenous infusion resulted in sustained therapeutic concentrations within maternal plasma, fetal plasma, and amniotic fluid for $>$12 h (302). *In vitro*, solithromycin has potent activity against human clinical *Ureaplasma* isolates (303, 304), in addition to a range of other important pathogens (305–309). Both maternal intravenous solithromycin and maternal intravenous solithromycin combined with intra-amniotic solithromycin effectively eradicated *U. parvum* from the amniotic cavity of pregnant sheep but, similarly to azithromycin, failed to reduce inflammation of the chorioamnion and fetal lung (299). These findings suggest that solithromycin may not accumulate in high-enough concentrations to exert anti-inflammatory effects and that coadministration of immune modulators should be investigated. To date, solithromycin is the most potent antimicrobial for the treatment of genital mycoplasmas and has several pharmacokinetic advantages over older macrolides, suggesting that it may be useful for the treatment of intrauterine infections. Human studies are required to further examine the effectiveness and safety of solithromycin in pregnancy and chorioamnionitis.

CONCLUDING REMARKS AND FUTURE RESEARCH DIRECTIONS

In conclusion, the findings of both human and animal studies have now demonstrated that infection with *Ureaplasma* spp. alone can cause chorioamnionitis, demonstrating a true causal role for these microorganisms in disease. Furthermore, the ability of *Ureaplasma* spp. to vary the expression and size of their major surface-exposed antigen, the MBA, indicates that these pathogens have evolved specific virulence mechanisms to avoid immune

detection by the host. Despite the lack of genetic manipulation studies, both animal and human research has now shown the involvement of the MBA in modulating the host response to chorioamnionitis, and our most recent study has demonstrated that recombinant MBA proteins of different sizes elicit different immune responses, potentially as a consequence of altered nuclear factor kappa B activation. We predict that this highly variable surface antigen expression facilitates immune evasion, enabling these microorganisms to cause chronic *in utero* infections, and further research is required to elucidate the mechanisms of antigenic variation in *Ureaplasma* spp. This may also assist in understanding the progression of disease during *Ureaplasma* infections and provide unique insights into the host-microbe interactions that occur *in vivo*. Furthermore, the development of genetic tools to create isogenic deletion mutants would enable researchers to assign definitive roles to proposed ureaplasmal virulence factors.

Due to the difficulties associated with identifying and diagnosing *Ureaplasma* infections and chorioamnionitis, additional research should be undertaken to identify biomarkers for the rapid diagnosis of *Ureaplasma* in order to detect subclinical infections and clinically silent chorioamnionitis. Due to the unique metabolism of the *Ureaplasma* spp., "omics" profiling of *Ureaplasma*-infected amniotic fluid may identify unique molecular signatures that could be used for diagnostic purposes, in combination with conventional *Ureaplasma* culture/PCR identification. This is a critical area of research that may lead to the improved identification and treatment of *in utero* inflammation, which will ultimately lead to improved maternal and neonatal outcomes. We also propose that amniotic fluid collected from pregnant women undergoing amniocentesis should be routinely tested for *Ureaplasma* spp., even in the absence of clinical signs/symptoms of chorioamnionitis. Additionally, further studies are required to identify effective and targeted therapies that eradicate intrauterine *Ureaplasma* infections and reduce inflammation. Continued research investigating the pharmacokinetics and anti-*Ureaplasma* activity of new-generation drugs, potentially in combination with immunomodulatory agents, may lead to the development of more effective treatment options for *Ureaplasma* chorioamnionitis.

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Emma L. Sweeney received her Ph.D. in 2015 from Queensland University of Technology (QUT), Australia. Her project investigated the presence and diversity of microorganisms, particularly the human *Ureaplasma* species, in adverse pregnancy outcomes with a focus on *Ureaplasma* pathogenesis in histological chorioamnionitis. Dr. Sweeney was subsequently appointed a postdoctoral research fellow at QUT, investigating the oral neonatal microbiome and how oral bacterial communities are regulated by reactive oxygen species that are produced when human breast milk and neonatal saliva combine. Dr. Sweeney has worked on the topic of *Ureaplasma* spp. for 6 years and hopes to continue research into the role of these minimalistic pathogens in human and animal infections and the host-microbe interactions that facilitate disease.

Samantha J. Dando received her Ph.D. in microbiology in 2012 from Queensland University of Technology, Australia, where she studied the pathogenesis of intrauterine *Ureaplasma* infections in an experimental ovine model. She has published seminal papers in this field, which have significantly improved our understanding of chronic, intra-amniotic ureaplasma infections. Dr. Dando subsequently undertook postdoctoral research at Griffith University, where she investigated the novel mechanisms by which *Burkholderia pseudomallei* can directly invade the central nervous system via the olfactory and trigeminal nerves within the nasal cavity. In her current position at Monash Biomedicine Discovery Institute, Monash University, Dr. Dando's research focuses on characterizing myeloid cell populations within various subcompartments of the eye and brain and how these cells respond to systemic inflammatory mediators. Dr. Dando also continues to be active in *Ureaplasma* research and is interested in the ability of these microorganisms to undergo antigenic variation and modulate the host immune response.

Suhas G. Kallapur received his bachelor in medicine (M.B.B.S.) and a doctorate in medicine (M.D.) from Bombay University, India. He then completed a residency in pediatrics at Wayne State University, Michigan, USA, followed by a fellowship in neonatal-perinatal medicine at Cincinnati Children's Hospital, Ohio, USA. He is currently a tenured Professor of Pediatrics at Cincinnati Children's Hospital, University of Cincinnati, and is a practicing neonatologist. Dr. Kallapur leads a laboratory, funded by NIH, the March of Dimes, and the Burroughs Wellcome Trust, whose main thrust since 2000 is to understand the pathogenesis of infection- or inflammation-mediated preterm birth. This condition is an important contributor to prematurity, which is a leading cause of infant mortality and morbidity worldwide. *Ureaplasma* species most commonly cause perinatal infections, and Dr. Kallapur has collaborated with coauthors and others to create sheep and rhesus macaque models of intrauterine infection and inflammation.

Christine L. Knox obtained her Ph.D. in 1998 from the Queensland University of Technology (QUT), where she pioneered the study at QUT of the *Ureaplasma* species and their role in adverse pregnancy outcomes. As an Associate Professor, she now leads the Reproductive Health Research Group at QUT and was the principal Ph.D. supervisor for Samantha Dando and Emma Sweeney, the joint first authors of this review. Funding from the National Institute of Health and the National Health and Medical Research Council, Australia, has enabled this group to further investigate the pathogenesis of *Ureaplasma* spp. in pregnant women delivering late preterm and at term and in an ovine model of intra-amniotic infection. Dr. Knox is an appointed member of the International Subcommittee for the Taxonomy of *Mollicutes*, and in 2016 she was the chair of the local organizing committee of the 21st Congress of the International Organization for Mycoplasmology.