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* spp. 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 recent 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.

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

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 **(Box 1)**. 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 sub-clinical and are not diagnosed until retrospective analysis of the placenta (6) **(Box 2)**. 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 intraamniotic infection or inflammation rather than chorioamnionitis with preterm labor/delivery. However, intraamniotic 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 to '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 'intraamniotic infection' according to their traditional definitions, as described above.

Clinical chorioamnionitis and histological chorioamnionitis affect 1-4% and 23.6% of term births (37-42 weeks of gestation) respectively (5, 11, 12). However, it has been well established that the frequency (13-15) and severity (15, 16) of chorioamnionitis is inversely related to gestational age at the time of delivery. In a study of 7505 placentae from singleton pregnancies, Russell (13) reported that the frequency of chorioamnionitis in patients who delivered between 21-24 weeks of gestation was 94.4% (17/18 patients). More recently, Stoll *et al.* (14) 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 compared to those who had induction of labor at term or delivered *via* Caesarean section in the absence of labor (17, 18). Furthermore, the frequency of histological chorioamnionitis increases in patients with prolonged duration of labor (19) and premature rupture of membranes (20). 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 (20-23).

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 (24). In addition, complications arising from preterm birth are a leading

cause of death in children under the age of 5, second only to pneumonia (25). Microbiological studies have demonstrated that intrauterine infection may be responsible for 25-40% of preterm births (26); however, this is likely to be underreported due to difficulties in detecting fastidious microorganisms using conventional culture methods. Histological chorioamnionitis complicates 40-70% 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) (Figure 1). Maternal CRH plasma levels increase throughout the duration of pregnancy and peak at term (27). 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 (28). PG production is enhanced by the concomitant downregulation of prostaglandin dehydrogenase (PGDH) within the chorion (29) and the production of prostaglandin-endoperoxide synthase-2 (PGS2, formerly cyclooxygenase-2) (30). Both CRH and PGE2 stimulate the release of matrix metalloproteases (31, 32) (MMPs; 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 leads to the upregulation of contraction-associated proteins and myometrial activation (28). Progesterone withdrawal coupled with increased estrogen production is also a key feature of parturition and further promotes uterine contractility (33-35).

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 (Figure 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 (36). Similar results were reported in a separate studies by Moço *et al.* (37) and Kim *et al.* (38), suggesting that the upregulation of TLRs plays an important role in the pathogenesis of chorioamnionitis.

Bacterial endotoxins, such as lipopolysaccharide (LPS) (39), and live microorganisms (40) 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 (38). *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 (41). These findings are consistent with human studies and animal models of chorioamnionitis/intrauterine infection, which demonstrate an increase in interleukin (IL)-1β and IL-6 (42, 43), IL-8 (36) tumor necrosis factor (TNF)-α (44), monocyte chemotactic proteins (45) and granulocyte colony-stimulating factor (G-CSF) (46) in preterm fetal membranes, amniotic fluid and/or cord blood. These inflammatory cytokines and chemokines stimulate PG production (47, 48), neutrophil infiltration and the release of MMPs (49), thus leading to cervical ripening and weakening/rupture of the fetal membranes. Indeed, the levels of MMPs (50) and PGs (40, 51) 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 (52, 53), gastrointestinal tract (54, 55) and skin (56) 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 (57-59), lymphoid tissues (60-62), and distant organs such as the brain (63, 64). 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 (65, 66), and increased fetal plasma white blood cell counts (67). FIRS is associated with multi-organ injury and is associated with severe neonatal morbidity and mortality (66). The fetal immune response to chorioamnionitis has been reviewed in detail elsewhere (68, 69).

In human studies, chorioamnionitis has been associated with neonatal death (11, 70), early-onset neonatal sepsis (70-72), intrauterine growth restriction (73), poor neonatal growth (74), neurologic impairment/injury (75, 76), intraventricular hemorrhage (70), bronchopulmonary dysplasia (77-79), patent ductus arteriosus (70, 73, 77, 80), retinopathy of prematurity (73, 81, 82), cardiovascular abnormalities (83, 84), necrotizing enterocolitis (85, 86), and dermatitis (87). However, low gestational age is often a significant contributing factor (88-90) 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 18-22 months corrected age compared to infants exposed to no chorioamnionitis or histological chorioamnionitis alone (91). When adjusting for gestational age, other studies have confirmed that chorioamnionitis is an independent risk

193 factor for early-onset neonatal sepsis (92, 93), bronchopulmonary dysplasia (79), adverse
194 neurodevelopmental outcome at 3 years (94) and necrotizing enterocolitis (92). Interestingly,
195 the severity of chorioamnionitis has been shown to correlate with an increased frequency of
196 chronic lung disease and necrotizing enterocolitis (95), but has an inverse relationship with
197 the development of respiratory distress syndrome (96).

198 **HOST DEFENSES AND PATHWAYS OF MICROBIAL INVASION OF THE**
199 **CHORIOAMNION AND AMNIOTIC FLUID**

200 Traditionally, the normal intrauterine environment is considered to be a sterile site with the
201 chorioamnion representing the major physical and immunological barrier to the developing
202 fetus. The chorioamnion expresses TLRs, which detect pathogen associated molecular
203 patterns and signal to coordinate cellular immune responses. The chorioamnion also secretes
204 numerous natural antimicrobial peptides and defensins to protect against microbial invasion
205 (97). *In vitro*, human chorion and amnion from healthy pregnancies that delivered at term
206 inhibited the growth of a wide range of pathogenic bacteria, including group B *Streptococcus*,
207 group A *Streptococcus*, *Staphylococcus aureus* and *S. saprophyticus* (98). Parthasarathy *et al*.
208 also reported that human fetal membranes possess strong antimicrobial effects against
209 *Escherichia coli*, *Shigella* spp., and the fungal pathogens *Aspergillus niger* and *A. nidulans*
210 (99). Nonetheless, a wide range of microbes are capable of invading the fetal membranes and
211 amniotic cavity, and causing chorioamnionitis. Specific routes by which microorganisms are
212 thought to access the upper genital tract during pregnancy include: (i) retrograde spread from
213 the peritoneal cavity (*via* the Fallopian tubes); (ii) hematogenous dissemination *via* the
214 placenta and maternal blood supply; (iii) iatrogenic contamination at the time of invasive
215 medical procedures (such as chorionic villus sampling or amniocentesis) and (iv) ascending
216 invasive infections from the lower genital tract (26). While other studies have suggested that
217 bacteria (specifically, *Ureaplasma* spp.) may also gain access to the upper genital tract

attached to spermatozoa (100, 101), 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 (102).

Recent deep sequencing studies have demonstrated that the placental parenchyma harbors a unique microbiome comprising non-pathogenic bacteria from the *Firmicutes*, *Tenericutes*, *Proteobacteria*, and *Fusobacteria* phyla, with distinct similarities to the adult oral microbiota (103). 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*, *A. johnsonii* and *Lactobacillus crispatus* (104). 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 (103) and normal immune development of the fetus (105).

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 (*U. parvum* and *U. urealyticum*), *Fusobacterium* spp., *Streptococcus* spp., and less frequently, *Gardnerella* spp., *Mycoplasma* spp. and *Bacteroides* spp. (1, 46, 104, 106-108). Other studies have

identified that the sexually transmitted pathogens *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, along with the uropathogen *E.coli* and yeast *Candida*, are also infrequently associated with chorioamnionitis (106, 109-112). Viral etiologies of chorioamnionitis include: adenovirus, cytomegalovirus, enterovirus and, less frequently, respiratory syncytial virus and Epstein-Barr virus (113-116). 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, 46, 107, 117, 118), funisitis (104, 119, 120) and preterm birth (1, 121).

THE HUMAN *UREAPLASMA* SPP.

The human *Ureaplasma* spp. were first discovered in 1954 in agar cultures of urethral exudates from male patients with non-gonococcal urethritis (122). Due to their small colony size (5 – 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' (122). However, *Ureaplasma* can be distinguished from *Mycoplasma* spp. (123) by the presence of a urease enzyme, which hydrolyses urea to produce 95% of their energy requirements. The hydrolysis of urea produces ammonia, which leads to an increase in proton electrochemical potential and *de novo* ATP synthesis (124). 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 (125, 126). Due to this distinctive urease activity, the *Ureaplasma* spp. were reclassified into their own genus within the *Mycoplasmataceae* family in 1974 (123). 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, *Ureaplasma* are pleomorphic and individual organisms can range

in size from 100 nm to 1 μm (127). 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, 7-13) (128). *U. parvum* possesses a smaller genome (0.75 – 0.78 Mbp) than *U. urealyticum* (0.84 – 0.95 Mbp) (129) and these two species can also be distinguished based on restriction fragment length polymorphisms, DNA-DNA hybridization, multi-locus sequence typing and sequences of 16S rRNA, multiple banded antigen (*mba*) and urease genes (130-135). Whilst 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 (136, 137), immunoperoxidase tests (138), enzyme-linked immunosorbent assays (139, 140) and colony indirect epi-immunofluorescence (141), 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-based typing methods based on sequencing of the upstream region of the *mba* (135), conventional PCR of the *mba* (142-144) and random amplified polymorphic DNA PCR (142) have also been described. However, these methods do not fully discriminate all 14 *Ureaplasma* serovars. In addition, the *mba* was recently shown to be part of a phase variable gene super-family (129), suggesting its use as a diagnostic target may be limited.

290 Following the release of full genome sequences of *Ureaplasma* American Type Culture
291 Collection (ATCC) strains, Xiao *et al*. designed 14 separate mono-plex real-time PCR assays,
292 which successfully typed all 14 ATCC type strains without cross-reactivity between serovars
293 (145). However, when these real-time PCRs were used to type clinical human *Ureaplasma*
294 isolates, 6% of isolates failed to amplify and could not be typed according to any of the
295 known 14 serovars (146). Whole-genome shotgun sequencing of a selection of these isolates
296 revealed that the gene targets for real-time PCR were completely absent or had been
297 significantly modified, such that one of the primers was unable to bind. Even more intriguing
298 was that following filtering and sub-culture of single *Ureaplasma* colonies isolated from
299 samples thought to contain mixtures of multiple serovars, several isolates continued to
300 express loci from more than one serovar. DNA sequencing revealed that these isolates were
301 in fact 'hybrids' or genetic mosaics that carried multiple serovar markers. Screening of 271
302 clinical samples initially believed to contain multiple serovar mixtures demonstrated that 75
303 (28%) were hybrids, which carried markers of up to 4 different serovars (146). These data, in
304 combination with recent comparative genome sequencing studies, demonstrate that there is
305 extensive evidence of horizontal gene transfer in *Ureaplasma* spp., suggesting that typing
306 these microorganisms into defined serovar groups may be of limited value for diagnostic
307 purposes (146) and that *Ureaplasma* exist as quasi-species (129). On the other hand, it is
308 possible that there are more stable gene targets that have yet to be identified, which could be
309 utilized for the discrimination of *Ureaplasma* serovars or pathogenic versus commensal
310 subtypes. Large scale comparative genome sequencing studies are required to clarify this
311 issue.

312 **_Ureaplasma_ spp. are commensals of the female lower genital tract**

313 *Ureaplasma* can be isolated from the mucosal surfaces of the vagina or cervix from 40-80%
314 of sexually active females (147). *U. parvum* is isolated more frequently from the lower

genital tract of females than *U. urealyticum* (142, 143, 148-150), and serovar 3 is the most common serovar isolated from females in the United States and Australia (100, 142, 147). *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) (107, 151, 152), age (most prevalent in the 14 – 25 year age group and carriage declines with increasing age) (149, 151), the number of recent sexual partners (107, 152), the use of non-barrier contraceptives (107), level of education (151), age of first sexual intercourse (107) and intrauterine devices (151, 153). *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 or without symptoms of genital infection (149, 150). However, others have reported that *Ureaplasma* spp. can cause lower urogenital tract infections, such as symptomatic vaginitis (154, 155), cervicitis (156), bacterial vaginosis (157), pelvic infections (158, 159) and urinary tract infections (160-162).

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 (163-168). A prospective study of 2471 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 - 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 783/1891 women (41.1%) who delivered at term. Similarly, Kataoka *et al.* 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 (169-174).

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 (175). Furthermore, *Ureaplasma* spp. have been isolated from the endometrium and Fallopian tubes of non-pregnant women in the absence of clinical symptoms or abnormal pathology (176, 177). While it was historically thought that the *Ureaplasma* spp. were of 'low virulence' and that their presence in the upper genital tract may 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 non-pregnant women suggests that these microorganisms may infect the embryo at the time of implantation (147). Moreover, they are capable of inducing chorioamnionitis, which can adversely affect the health of the pregnancy and neonate. Herein, 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 (178). 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. to 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 (see 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 - 17 weeks of gestation by PCR and detected *Ureaplasma* spp. in 29/254 (11.4%) of subjects (121). 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 this study who were infected/colonized with *Ureaplasma* that went on to deliver at term with no apparent adverse outcomes. Similarly, Horowitz *et al*. detected intraamniotic *Ureaplasma* infections in six pregnant women (2.8%) but only three (50%) of these women experienced preterm birth (179). 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 (180, 181), while in others there may only be moderate

389 inflammation (182), or there may be no correlation between infection with *Ureaplasma* spp.
390 and inflammation (183) (Figure 2).

391 Although it remains unclear why some women infected with *Ureaplasma* spp. experience
392 adverse pregnancy outcomes, while others do not, some researchers have attributed these
393 differences in sequelae to the virulence of the infecting serovar (184), the bacterial load
394 present (185, 186), or genetic background/ethnicity (187, 188). However, these findings are
395 not always consistent, with a recent study by our group demonstrating no correlation between
396 the numbers of *Ureaplasma* present within placentae, the species/serovar present, or the
397 ethnicity of women infected with *Ureaplasma*, and the incidence or severity of histological
398 chorioamnionitis (46). Furthermore, animal model studies in which *Ureaplasma* spp.
399 infections have been established with the same strain and dose of *U. parvum* resulted in
400 divergent inflammatory responses within the chorioamnion (43, 189, 190) and within other
401 genital tract tissues (191), suggesting that the development or magnitude of host immune
402 responses may contribute to the severity of chorioamnionitis. Indeed, we have demonstrated
403 that the human *Ureaplasma* spp. can undergo immune evasive behavior *in vivo* by varying
404 the expression of their surface exposed antigens, and that the severity of chorioamnionitis is
405 inversely related to the number of antigenically distinct subtypes detected within amniotic
406 fluid (reviewed in detail below). Therefore, we hypothesize that the ability of some
407 *Ureaplasma* strains to 'hide' from the immune system may be an important predictor of
408 outcomes, and may potentially explain why some women do not develop chorioamnionitis
409 despite high bacterial loads within the amniotic fluid and chorioamnion.

410 Table 1 summarizes human studies, which have investigated the role of *Ureaplasma* spp. in
411 chorioamnionitis. These studies showed that the rates of *Ureaplasma*-associated
412 inflammation within the chorioamnion may vary between 0 – 100%, further highlighting the
413 diversity of histological chorioamnionitis and why it is so difficult to confirm the role of

414 these microorganisms as causative agents of chorioamnionitis. Additionally, the pathogenic
415 role of *Ureaplasma* spp. is often unclear as the majority of these infections are clinically
416 silent. *Ureaplasma* infections of the chorioamnion can persist asymptomatically for up to two
417 months in humans (192) and *Ureaplasma* infected placentae cannot be distinguished
418 macroscopically from normal placentae (although there may be histological evidence of
419 chorioamnionitis that is detected following delivery). Due to the predominantly
420 asymptomatic nature of *Ureaplasma* infections, coupled with the fastidious growth
421 requirements of these microorganisms, pregnant women are not routinely screened for
422 *Ureaplasma* spp. and therefore these tiny bacteria are not always suspected (and are,
423 therefore, likely to be under-reported) as causative agents of chorioamnionitis.

424 One of the major reasons as to why the role of *Ureaplasma* spp. in chorioamnionitis has
425 remained unconfirmed is due to the polymicrobial nature of chorioamnionitis (5, 193). The
426 majority of studies investigating chorioamnionitis focus specifically on very preterm (< 28
427 weeks) and early preterm (28 – 32 weeks) pregnancies and these studies have demonstrated
428 that up to 67% of amniotic fluid or placental samples with chorioamnionitis contained at least
429 two detectable microorganisms (often *Ureaplasma* spp. and another microorganism) (Table
430 1). Because of this, researchers have not been able to confidently claim that *Ureaplasma* spp.
431 are true etiological agents of chorioamnionitis. However, a recent study by our research
432 group demonstrated that infections within late preterm (32 – 36 weeks) and term (≥ 37
433 weeks) placentae typically harbored only a single microorganism (90.5%) and that the
434 presence of *Ureaplasma* alone was significantly associated with histological
435 chorioamnionitis, at any gestational age (46). Further investigations confirmed the finding
436 that placental infections with *Ureaplasma* spp. are strongly associated with chorioamnionitis,
437 using whole genome shotgun sequencing of late preterm and term placentae (104). Similarly,
438 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, when compared to placentae infected with *Ureaplasma* spp. alone (194). Taken together, these recent data suggest that not only are *Ureaplasma* spp. likely to be a key etiological agent of chorioamnionitis in the absence of other microorganisms, but these reports 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 non-human primate model, Novy *et al*. (195) inoculated 10^7 colony forming units of *U. parvum* serovar 1 into the amniotic fluid of pregnant Rhesus macaques at day (d) 132 - 147 of gestation (term = 155 – 172 d) *via* an indwelling catheter. Intraamniotic *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 pre-inoculation baseline values. A progressive increase in uterine activity was also observed following *U. parvum* intraamniotic inoculation and the mean inoculation-to-labor onset period was significantly reduced in *U. parvum* infected animals, compared to those inoculated with sterile media 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 (195). Similarly, intraamniotic injection of *U. parvum* serovar 1 into the amniotic cavity of pregnant baboons at day 122 - 123 of gestation (term is 185 d) resulted in

elevated levels of amniotic fluid IL-6 and IL-8 at the time of preterm delivery (125 d), and histological evidence of acute chorioamnionitis (196). In contrast, more recent studies in Rhesus macaques demonstrated that despite the presence of high numbers ($3.9x10^7$ CFU/mL) of *U. parvum* serovar 1 within the amniotic fluid, no chorioamnionitis was detected after acute durations (3 d and 7 d) of infection (197).

Whilst non-human primate models exhibit the closest resemblance to humans with respect to gestational length, uterine anatomy and parturition, experimental intrauterine infection causes preterm delivery (195, 198) and therefore it is only possible to study acute chorioamnionitis in these models. In contrast, sheep do not experience inflammation-induced preterm birth, as intraamniotic infection/inflammation does not cause significant activation of the fetal HPA axis, cortisol production and subsequent progesterone withdrawal, which is required for the initiation of labor in many species (199-201). 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 (201) 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 d (term is 150 d) can chronically colonize the amniotic fluid and fetus (43, 189, 199, 202). Following an intraamniotic injection of 2×10^4 CFU of *U. parvum* serovar 6 at 55 d of gestation, temporal analysis demonstrated that the peak of amniotic fluid infection occurred between 87 d and 101 d of gestation, and that the number of CFU/mL remained high (approximately 10^7 CFU/mL) until the time of surgical delivery at 140 d (43). These data demonstrate that *Ureaplasma* can chronically colonize the amniotic fluid for at least 85 d 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 intraamniotic infection (189, 199, 202-204), and was also isolated from the umbilical cord and other fetal tissues including cerebrospinal fluid, gut, kidney, liver and spleen (189). These findings are consistent with human studies that have reported that *Ureaplasma* spp. may systemically infect the fetus, leading to neonatal morbidity and mortality (205-212).

Both chronic and acute intrauterine *Ureaplasma* infections were capable of causing histological chorioamnionitis in pregnant sheep (43, 189, 190, 202, 203). Intraamniotic *U. parvum* infection was also associated with increased expression of IL-1β, IL-6 and IL-8 mRNA within the chorioamnion (43, 203) and an influx of neutrophils, monocytes/macrophages and lymphocytes (43, 189, 202), compared to media controls. Generally, the severity of chorioamnionitis correlated with increased duration of intraamniotic *Ureaplasma* exposure (190); however, variability in the severity of inflammation was a notable feature of these sheep studies (189, 190), 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 to the normal tissue architecture) to no histological evidence of chorioamnionitis (189). 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* (189).

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 (43), derived from placental isolates, that had caused severe histological chorioamnionitis (virulent-derived strain) or no chorioamnionitis (avirulent-derived strain) in a previous ovine

514 study (189). Regardless of the inoculating clonal strain, moderate to severe chorioamnionitis
515 was observed in experimentally infected animals and there were no differences in the
516 chorioamnion expression of TLR-1, TLR-2, TLR-6, IL-1β, IL-6, IL-8, IL-10 and TNF-α
517 between animals infected with the avirulent-derived strain or virulent-derived strain.
518 Similarly, there were no differences in the numbers of *U. parvum* isolated from the amniotic
519 fluid, chorioamnion, cord or fetal lung at 140 d (43). In the same study, we demonstrated that
520 only a sub-population of infected ewes from each group generated a serum IgG response to
521 intrauterine *U. parvum* infection. When cytokine expression was compared between animals
522 with/without anti-*Ureaplasma* serum IgG, the expression of IL-1β, IL-6 and IL-8 was
523 significantly increased in the chorioamnion of anti-*Ureaplasma* IgG^+ animals. In addition,
524 maternal anti-*Ureaplasma* serum IgG was associated with a significant increase in
525 meconium-stained amniotic fluid (43). These findings are also consistent with human studies
526 that have demonstrated that patients with anti-*Ureaplasma* antibodies are at a higher risk for
527 adverse pregnancy and neonatal outcomes compared to those who do not develop a humoral
528 immune response (213, 214). Taken together, this suggests that *Ureaplasma* strains are not
529 likely to be inherently virulent/avirulent, but that the host response to infection may affect the
530 pathogenesis of chorioamnionitis.

531 **The immune response to *Ureaplasma* chorioamnionitis: harmful or helpful?**

532 Studies in BALB/c and C57Bl/6 mice have provided unique insights into the potentially
533 harmful immune responses that may occur during *Ureaplasma* chorioamnionitis. BALB/c
534 mice typically display a Th1/M1-dominant immune profile, whereas the immune profile of
535 C57Bl/6 mice is consistent with a Th2/M2 bias (187). These differences have enabled
536 researchers to examine the immunopathogenic role of a skewed Th1/M1 or Th2/M2 response
537 in *Ureaplasma* chorioamnionitis. In a model of experimental intrauterine infection, von
538 Chamier *et al.* injected 10^7 CFU of *U. parvum* into the uterine horns of pregnant BALB/c and

539 C57Bl/6 mice at 14 d (187). Examination of the fetal membranes at 72 hours post-infection
540 demonstrated that C57Bl/6 mice exhibited mild-moderate chorioamnionitis, whereas BALB/c
541 mice displayed severe necrotizing chorioamnionitis and extensive neutrophil infiltration.
542 These differences could not be attributed to differences in bacterial load; however, the
543 placental expression of cytokines and calgranulins was markedly different between the strains
544 (187). In a separate study, it was demonstrated that intrauterine *U. parvum* infection increased
545 the expression of TLR2 and CD14 on neutrophils in BALB/c but not C57Bl/6 mice (40).
546 TLR/CD14-mediated signaling triggered by bacterial lipoproteins has been shown to extend
547 the survival of apoptotic neutrophils in infected tissues, thereby increasing the duration of
548 inflammation (215). It is therefore possible that TLR2/CD14 signaling plays a role in the
549 extensive neutrophil infiltration and severe chorioamnionitis observed in BALB/c mice.
550 Interestingly, increased levels of soluble CD14 are also observed in the amniotic fluid of
551 women with intrauterine *Ureaplasma* infection (216), suggesting that CD14 signaling may be
552 an important area for future research. Combined, these studies demonstrate highlight that the
553 host immune response may be a key factor that modulates the pathogenesis of acute
554 *Ureaplasma* chorioamnionitis. Further studies using genetically modified/knock-out mouse
555 lines may significantly improve our understanding of protective versus pathogenic immune
556 responses to intrauterine *Ureaplasma* infection.

557 **Immune effects of *Ureaplasma* spp. on the fetus**

558 Animal model studies from our research group have investigated the fetal immune responses
559 to *U. parvum* exposure during gestation. In a series of experiments in pregnant sheep, it was
560 demonstrated that chronic (69 d), but not acute (7 d), *in utero* infections with *U. parvum*
561 suppressed innate immune responses in fetal sheep. Fetuses were challenged with *E. coli* LPS
562 2 days prior to delivery, and the fetuses that were chronically exposed to intraamniotic
563 *Ureaplasma* spp. demonstrated significant decreases in pro- and anti-inflammatory cytokine

564 expression, as well as fewer CD3+ T lymphocytes and myeloperoxidase+ cells within the
565 fetal lung when compared to the fetuses that were intraamniotically exposed to sterile culture
566 media (vehicle). Blood monocytes obtained from these same animals also had a significantly
567 decreased response to LPS *in vitro* (105), demonstrating that fetal exposure to *U. parvum in*
568 *utero* can markedly alter the neonatal immune responses following delivery. Similarly,
569 chronic exposure to *U. parvum* alone (with no LPS challenge) was sufficient to augment the
570 presence of transforming growth factor (TGF)-β within the fetal lung, which may also
571 contribute to the development of lung pathologies, such as bronchopulmonary dysplasia
572 (217).

573 In both Rhesus macaque and sheep models, intraamniotic *U. parvum* infections decreased the
574 populations of CD4+FOXP3+ regulatory T cells (Tregs) in the preterm fetus, in both the
575 thymus and periphery (197, 218). Furthermore, an interferon-γ response was seen in Tregs
576 exposed to *U. parvum* during gestation, and this response was absent in Tregs of fetuses
577 exposed to control (media) intraamniotic injections. Since it is well established that Tregs are
578 potent anti-inflammatory T-cells (219), these results suggest the existence of a subset of
579 Tregs that can develop a Th1 phenotype early in life, and that this response may be increased
580 in the presence of inflammation (e.g. chorioamnionitis).

581 **MANIPULATION OF HOST CELLS BY *UREAPLASMA* SPP.**

582 Compared to other *Mycoplasma* spp., the cytadherence of *Ureaplasma* has not been
583 investigated in detail. *In vitro* studies have demonstrated that *Ureaplasma* spp. are adherent
584 to erythrocytes (220), placental endothelial cells (221) and human epithelial cells (222);
585 however, the adhesion mechanisms are unknown. Pretreatment of HeLa cells and
586 erythrocytes with neuraminidase significantly reduced ureaplasmal adherence (222),
587 suggesting that *Ureaplasma* may bind to receptors containing sialic acid. In contrast, the

588 adhesion of *Ureaplasma* to spermatozoa is thought to be mediated by
589 sulfogalactoglycerolipid, which is expressed by the mammalian male germ cell membrane
590 (223).

591 The human *Ureaplasma* spp. have been shown to alter/manipulate host cells in several ways.
592 Allam *et al*. reported that *U. parvum* significantly increased filamin A phosphorylation at
593 serine ^{2152} in human benign prostate cells, and altered its intracellular distribution (224).
594 Filamin A is an actin-binding protein that regulates the cytoskeleton and is involved in
595 antimicrobial signaling pathways (225). Further investigation into the upstream and
596 downstream signaling events may therefore reveal novel insights into *Ureaplasma*-host
597 interactions. In endothelial cells isolated from normal and preeclamptic placentae, *U.*
598 *urealyticum* significantly reduced cell viability, altered the expression of heat shock protein
599 70 and significantly increased the intracellular concentration of calcium and iron. It was
600 suggested that these events occurred as part of the cellular stress response to infection and
601 may indicate that cells are progressing towards apoptosis (221). Additional studies have
602 demonstrated that *U. urealyticum* induces apoptosis in other cell types, including human lung
603 epithelial cells (A549) and THP-1-derived macrophages (226). *Ureaplasma*-infected cells
604 demonstrated an altered morphology, underwent DNA fragmentation and translocation of
605 phosphatidylserine to the outside surface of the cell (as determined by Annexin V staining
606 and flow cytometry) (226). *Ureaplasma* spp. further manipulate host cells by suppressing
607 innate host defense pathways. A recent study demonstrated that *Ureaplasma* infection
608 decreased the expression of antimicrobial peptide genes in THP-1 cells *in vitro*, in association
609 with a significant decrease in histone H3K9 acetylation (227). These findings suggest that
610 *Ureaplasma* may downregulate antimicrobial/host defense genes *via* epigenetic modifications
611 (227), which may be an important factor contributing to the ability of these microorganisms
612 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.

The multiple banded antigen

The multiple banded antigen (MBA) was first described by Watson *et al.* (1990) 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. (228). 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 (229-231). 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 (232, 233). The C-terminal region of the MBA that has been shown to alter, both by switching on/off of the gene (antigenic phase variation)

637 and more commonly to vary in size (antigenic size variation) (43, 189, 232-235). *U.*
638 *urealyticum* serovar 13 is the only *Ureaplasma* serovar that does not contain any tandem
639 repeat units in the C-terminal variable domain of the *mba* (129).

640 While some studies demonstrated differences in the size of the MBA protein (giving rise to
641 the name of the protein itself as the multiple banded antigen) (235, 236), the first study to
642 characterize MBA size variation demonstrated that differences in the size of the MBA protein
643 directly correlated with the number of tandem repeating units within the *mba* gene (133, 237).
644 More recently, Knox *et al.* identified *mba*/MBA size variation *in vivo* using an ovine model
645 (189). Pregnant ewes were chronically infected for 69 d with a non-clonal *U. parvum* isolate
646 and the size of the *mba*/MBA was assessed. This study demonstrated that the number of
647 *mba*/MBA size variants was inversely correlated with the severity of inflammation within the
648 chorioamnion: when > 9 *mba*/MBA size variants were identified, there was little or no
649 chorioamnionitis; however, when < 5 *mba*/MBA size variants were identified, there was
650 severe histological chorioamnionitis (189). Other ovine studies have identified that variation
651 in the size of the *mba*/MBA was not seen after three days of intraamniotic infection, while
652 some slight variation was seen after seven days of infection (190) and significant *mba*/MBA
653 size variation was seen after 69 days of chronic intraamniotic *U. parvum* infection (43, 189,
654 190). Dando *et al.* (2012) also demonstrated the ability of *Ureaplasma* spp. to vary their
655 *mba*/MBA throughout the course of gestation and suggested that size variation of the
656 *mba*/MBA (presumably by slipped-strand mispairing) may be a mechanism by which
657 *Ureaplasma* spp. may evade host immune recognition, allowing chronic asymptomatic
658 infections to develop (43).

659 More recently, we have demonstrated for the first time that *Ureaplasma* spp. clinical isolates
660 from human placentae were also able to vary the size of their *mba*/MBA (Sweeney *et al*.,
661 manuscript in preparation). 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 had severe histological chorioamnionitis and elevated cord blood cytokines. Further *in vitro* investigations using recombinant MBA (rMBAs) proteins of differing sizes (i.e. different numbers of tandem repeat units) and human macrophage cells lines demonstrated immune responses that varied depending on the size of the rMBA. These results were confirmed by western blot; the expression of nuclear factor-kappa B fragment p65 (an activator of transcription) varied when stimulated with the different sized rMBA proteins (Sweeney *et al*., manuscript in preparation). Combined, these results confirm the ability of *Ureaplasma* spp. to vary their surface-exposed MBA *in vivo*, and 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 the *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 (43, 234, 238). In these studies, MBA-negative *Ureaplasma* isolates were detected following two to three serial passages in culture medium containing MBA-specific antibodies (43, 234). More recently, phase variation of the MBA occurred in the absence of any selective (antibody) pressures (239), indicating that this antigen is capable of rapid phase variation. Zimmerman *et al.* (2009) hypothesized that the expression of the MBA (locus UU375) is alternated with expression of an adjacent locus (UU376), which encodes an *Ureaplasma*-specific conserved hypothetical protein. Utilizing polyclonal rabbit antisera generated against the conserved (N-terminal, non-repetitive) regions of the 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 of expression of these genes (238). Zimmerman and colleagues further investigated the role of DNA-inversion sites within the *Ureaplasma* genome, and demonstrated experimentally that the *mba* paralogues UU171, UU172 and the orthologue UU144 were also involved in site-specific DNA inversion/recombination (240). Furthermore, it was shown that the XerC tyrosine recombinase gene of *U. parvum* is the most likely mediator of these DNA inversion events (241). Subsequent experimental investigation into the ability of the XerC to process the recombination event proved successful, indicating that this tyrosine recombinase is able to induce DNA inversion events (242), 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* spp. 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 (129). Remarkably, it was shown that the *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. Whilst there is convincing molecular evidence that the *mba* 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*.

Phospholipase A and C

711 The pathogenesis of phospholipases results from the production of membrane-destabilizing
712 compounds and degradation of the host cell membrane phospholipids (243). Endogenous
713 phospholipase A_1, A_2 and C activity has been previously identified in *U. parvum* serovar 3
714 and *U. urealyticum* serovars 4 and 8 (244-246). These phospholipases demonstrated higher
715 activity in *Ureaplasma* in their exponential growth phase; suggesting that the *Ureaplasma*
716 spp. phospholipases were membrane bound and were not being secreted (245). It was further
717 identified that phospholipase A_2 activity was three-fold higher in *U. urealyticum* serovar 8,
718 when compared to *U. urealyticum* serovar 4 and *U. parvum* serovar 3 (244). However,
719 subsequent whole genome sequencing of *U. parvum* serovar 3 could not identify any genes of
720 significant similarity to any known sequences of phospholipase A_1, A_2 or C (228). These
721 findings indicated that *Ureaplasma* may encode phospholipases that are evolutionarily
722 distinct from other phospholipase genes, or that these phospholipases may not exist within
723 *Ureaplasma* spp. Interestingly, more recent studies by the same research group revealed that
724 whole genome sequencing of the 14 *Ureaplasma* spp. serovars and four *Ureaplasma* spp.
725 clinical isolates were again unable to detect any phospholipase A_1, A_2 or C genes; however, a
726 phospholipase D domain containing protein was identified in all *Ureaplasma* spp. (129).
727 These researchers further investigated the presence/activity of these enzymes experimentally
728 and were unable to detect any significant phospholipase C or D activity in *U. parvum* serovar
729 3 and *U. urealyticum* serovar 8 (129). Further investigation into the presence and activity of
730 phospholipases within *Ureaplasma* spp. are required to elucidate if these enzymes are
731 potential virulence factors of these organisms.

732 **Immunoglobulin (Ig) A protease**

733 One of the primary defense mechanisms of the mammalian immune system is the production
734 of IgA at mucosal sites (247) and the ability of an organism to degrade IgA antibodies allows
735 the microorganism to evade this host defense mechanism. Robertson *et al.* published the first

736 evidence of an IgA protease in *U. urealyticum* that was capable of cleaving IgA_1 (248). While
737 it was subsequently determined that all 14 *Ureaplasma* serovars possess an IgA protease with
738 proteolytic activity against IgA_1 (but no proteolytic activity against IgA_2, IgG or IgM
739 antibodies) (249, 250), more recent evidence has questioned the presence of an IgA protease
740 in *Ureaplasma* spp. Initial genome sequencing studies of *U. parvum* serovar 3 were unable to
741 identify any genes with similarity to known IgA proteases (228) and more recent whole
742 genome analyses were unable to identify any IgA protease genes within the 14 *Ureaplasma*
743 serovars, nor was it found to be present in any of the *Ureaplasma* spp. clinical isolates tested
744 (129). Recently, an IgG binding protein and IgG serine protease were identified within
745 *Mycoplasma mycoides* subspecies *capri.* This study provided evidence that both *U. parvum*
746 and *U. urealyticum* contain genes that encode an IgG binding protein and an IgG serine
747 protease within their genomes (251). Based on these recent findings, further studies are
748 warranted to determine if these IgG binding/IgG protease genes are active in cleaving IgG
749 and therefore may be a previously unrecognized virulence factor of the human *Ureaplasma*
750 spp.

751 **Urease**

752 The ability of *Ureaplasma* spp. to hydrolyze urea was first identified in 1966, and the
753 production of adenosine triphosphate (ATP) *via* this mechanism appears to be unique within
754 *Ureaplasma* (125, 252). The urease enzyme is a key virulence factor of many ureolytic
755 bacteria, and the ureaplasmal urease gene cluster has a similar genetic organization to that of
756 *E. coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *K. aerogenes* (253). The urease
757 complex constitutes a major component of the ureaplasmal cytoplasm (254) and Takebe *et al*.
758 demonstrated that the urease of *U. urealyticum* serovar 8 was responsible for urolithiasis in
759 humans (255). The *Ureaplasma* spp. urease has a significantly higher specific activity
760 compared to other bacterial ureases (256) and was responsible for lethal toxicity in mice

following intravenous injection (257). Interestingly, the *Ureaplasma* spp. are one of few bacteria which encode a urease enzyme but lack the ability to assimilate ammonia into glutamine or glutamate (258), potentially explaining the very high intracellular ammonia concentration of these microorganisms (124).

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 (190). 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* spp. infections can result in hyperammonemia (259). Clinical reports of 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 (259). 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 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 (228), recent evidence has identified that HGT is likely to occur within these microorganisms and may be an important determinant of virulence. As

785 previously discussed, the identification of genetic hybrids (146) suggests that the *Ureaplasma*
786 spp. may be genetically promiscuous. Comparative genome sequencing studies have provided
787 further evidence of this and identified integrase-recombinase genes, transposases and phage
788 related proteins in *Ureaplasma* spp. genomes (129), which are highly indicative of HGT
789 events. Interestingly, *U. urealyticum* genomes generally contained a higher number of these
790 genes, suggesting that this species may be more capable of acquiring genes horizontally than
791 *U. parvum* (129).

792 Early attempts to define the phylogeny of *Mycoplasma* suggested that *Mycoplasma* spp. with
793 the smallest genomes have high mutation rates and undergo rapid evolution (260, 261).
794 Dando *et al.* provided evidence of the ability of the human *Ureaplasma* spp. to rapidly adapt
795 to their microenvironment in a sheep model of intrauterine infection (262). Following
796 injection of a non-clonal *U. parvum* serovar 3 isolate into the amniotic fluid of pregnant
797 sheep at 55 d, significant genetic variability within the 23S ribosomal (r) RNA gene was
798 detected between *U. parvum* isolated from the amniotic fluid and chorioamnion at the time of
799 preterm surgical delivery (125 d). While *U. parvum* isolated from amniotic fluid showed
800 100% 23S rRNA domain V sequence homology to the original strain injected, highly
801 polymorphic sequences (containing only 64 – 82% sequence homology to the inoculating
802 strain) were detected within *Ureaplasma* isolates from the chorioamnion. Furthermore,
803 chorioamnion *Ureaplasma* isolates demonstrated the presence of macrolide resistance genes,
804 which were not evident in amniotic fluid isolates. Whilst this study did not investigate the
805 presence of potential genetic transfer elements flanking these variable gene sequences, these
806 data support the concept that *Ureaplasma* spp. may undergo significant HGT *in vivo*.
807 Furthermore, this study suggests that different anatomical sites (amniotic fluid versus
808 chorioamnion) may select for different *Ureaplasma* subtypes within non-clonal populations
809 and thus influence the socio-microbiological structure of the bacterial population (262).

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 *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. Whilst antibiotics are recommended for women with preterm pre-labor rupture of membranes (263) 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/mid gestation. It has been suggested that the administration of appropriate antibiotics before 22 weeks of gestation (or before inflammation and maternal-fetal damage occurs) could significantly decrease the incidence of preterm birth (264). 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 (265). However, due to the concern of antibiotic resistance, widespread antimicrobial treatment is not recommended unless there is evidence of intraamniotic 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.

834 An additional complicating factor for the treatment of *Ureaplasma* chorioamnionitis includes
835 the often polymicrobial nature of this disease, which suggests that more than one
836 antimicrobial agent may be required to successfully eradicate infection. Furthermore,
837 treatment options for pregnant women are limited due to potential teratogenic and harmful
838 effects associated with the use of some antimicrobials during pregnancy. Even fewer options
839 are available for the treatment of intrauterine *Ureaplasma* infections, as these
840 microorganisms are inherently resistant to beta-lactam and glycopeptide antibiotics (due to
841 their lack of a cell wall), as well as trimethoprim and sulphonamides (as *Ureaplasma* spp. do
842 not synthesize folic acid) (266). Antimicrobials that are potentially active against *Ureaplasma*
843 include the tetracyclines, fluoroquinolones and macrolides; however, resistance to these
844 antimicrobial classes has also been well described (267-271).

845 Erythromycin, a 14-membered lactone ring macrolide, is the most common antibiotic used
846 for the treatment of neonatal *Ureaplasma* infections and is routinely used in clinical
847 obstetrics. Large randomized controls and meta-analyses have demonstrated that
848 erythromycin administration for preterm pre-labor rupture of membranes can reduce the risk
849 of chorioamnionitis and neonatal morbidity, and delay preterm birth (272-274). However, it
850 is less clear if maternal erythromycin can eradicate existing human intrauterine infections due
851 to conflicting reports within the literature (275-277). In pregnant sheep, maternal
852 intramuscular erythromycin treatment (30 mg/kg/d for 4 days) failed to eradicate an
853 erythromycin susceptible strain of *U. parvum* from the amniotic fluid, chorioamnion and fetal
854 lung (202), presumably due to poor transplacental passage (202, 278-280). In a follow-up
855 study, it was again demonstrated that intraamniotic *Ureaplasma* infection was not eradicated
856 following: (i) single intraamniotic and repeated maternal intramuscular erythromycin, or (ii)
857 single maternal intramuscular and repeated intraamniotic erythromycin injections (281).

These data suggest that erythromycin may not be beneficial for the treatment of intrauterine *Ureaplasma* infections.

Azithromycin is 15-membered semisynthetic macrolide with superior tissue penetration, a prolonged half-life and broader antimicrobial coverage than erythromycin (282). Azithromycin is well tolerated during pregnancy and achieves peak concentrations of 151 ± 46 ng/mL within human amniotic fluid and 2130 ± 340 ng/mL within human placentae at 6 hours post-injection, before rapidly declining (282). In pregnant sheep, a single intraamniotic injection of azithromycin achieved therapeutic concentrations that were sustained for 48 hours; however there was poor maternal-fetal transfer (280). Despite this, a single maternal intravenous azithromycin injection or a single maternal intravenous azithromycin injection combined with an intraamniotic azithromycin injection completely eradicated an established *U. parvum* infection from the amniotic fluid, chorioamnion and fetal lung in pregnant sheep (283). Similarly, studies in Rhesus macaques demonstrated that maternal intravenous azithromycin (25 mg/kg/d for 10 d) administered 6-8 d after intraamniotic *U. parvum* inoculation successfully eradicated *Ureaplasma* from the amniotic fluid (284, 285). It should be noted that in both of these sheep (283) and monkey (285) 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 amniotic fluid (286). 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 hours (286).

In vitro, solithromycin has potent activity against human clinical *Ureaplasma* isolates (287, 288), in addition to a range of other important pathogens (289-293). Both maternal intravenous solithromycin and maternal intravenous solithromycin combined with intraamniotic solithromycin effectively eradicated *U. parvum* from the amniotic cavity of pregnant sheep, but similar to azithromycin, failed to reduce inflammation of the chorioamnion and fetal lung (283). These findings suggest that solithromycin may not accumulate in high enough concentrations to exert anti-inflammatory effects and that co-administration 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 are able to 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 NF-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

908 in *Ureaplasma* spp. This may also assist in understanding the progression of disease during
909 *Ureaplasma* infections and provide unique insights into the host-microbe interactions that
910 occur *in vivo*. Furthermore, the development of genetic tools to create isogenic deletion
911 mutants would enable researchers to assign definitive roles to proposed ureaplasmal virulence
912 factors.

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

References

1. **Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA.** 1988. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N Engl J Med **319:**972-978.
2. **Romero R, Miranda J, Kusanovic JP, Chaiworapongsa T, Chaemsaithong P, Martinez A, Gotsch F, Dong Z, Ahmed AI, Shaman M, Lannaman K, Yoon BH, Hassan SS, Kim CJ, Korzeniewski SJ, Yeo L, Kim YM.** 2015. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. J Perinat Med **43:**19-36.
3. **Romero R, Chaemsaithong P, Docheva N, Korzeniewski SJ, Kusanovic JP, Yoon BH, Kim JS, Chaiyasit N, Ahmed AI, Qureshi F, Jacques SM, Kim CJ, Hassan SS, Chaiworapongsa T, Yeo L, Kim YM.** 2016. Clinical chorioamnionitis at term VI: acute chorioamnionitis and funisitis according to the presence or absence of microorganisms and inflammation in the amniotic cavity. J Perinat Med **44:**33-51.
4. **Roberts DJ, Celi AC, Riley LE, Onderdonk AB, Boyd TK, Johnson LC, Lieberman E.** 2012. Acute histologic chorioamnionitis at term: nearly always noninfectious. PLoS One **7:**e31819.
5. **Tita AT, Andrews WW.** 2010. Diagnosis and management of clinical chorioamnionitis. Clin Perinatol **37:**339-354.
6. **Horvath B, Lakatos F, Toth C, Bodecs T, Bodis J.** 2014. Silent chorioamnionitis and associated pregnancy outcomes: a review of clinical data gathered over a 16-year period. J Perinat Med **42:**441-447.
7. **Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C.** 2003. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol **6:**435-448.

8. **Kim CJ, Romero R, Kusanovic JP, Yoo W, Dong Z, Topping V, Gotsch F, Yoon BH, Chi JG, Kim JS.** 2010. The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. Mod Pathol **23:**1000-1011.

9. **Kim CJ, Romero R, Chaemsaithong P, Chaiyasit N, Yoon BH, Kim YM.** 2015. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. Am J Obstet Gynecol **213:**S29-52.

10. **Higgins RD, Saade G, Polin RA, Grobman WA, Buhimschi IA, Watterberg K, Silver RM, Raju TN.** 2016. Evaluation and Management of Women and Newborns With a Maternal Diagnosis of Chorioamnionitis: Summary of a Workshop. Obstet Gynecol **127:**426-436.

11. **Malloy MH.** 2014. Chorioamnionitis: epidemiology of newborn management and outcome United States 2008. J Perinatol **34:**611-615.

12. **Mi Lee S, Romero R, Lee KA, Jin Yang H, Joon Oh K, Park CW, Yoon BH.** 2011. The frequency and risk factors of funisitis and histologic chorioamnionitis in pregnant women at term who delivered after the spontaneous onset of labor. J Matern Fetal Neonatal Med **24:**37-42.

13. **Russell P.** 1979. Inflammatory lesions of the human placenta: clinical significance of acute chorioamnionitis. American Journal of Diagnostic Gynecology and Obstetrics **2:**705-737.

14. **Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, Hale EC, Newman NS, Schibler K, Carlo WA, Kennedy KA, Poindexter BB, Finer NN, Ehrenkranz RA, Duara S, Sanchez PJ, O'Shea TM, Goldberg RN, Van Meurs KP, Faix RG, Phelps DL, Frantz ID, 3rd, Watterberg KL, Saha S, Das A,**

Higgins RD. 2010. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics **126:**443-456.

15. **Mueller-Heubach E, Rubinstein DN, Schwarz SS.** 1990. Histologic chorioamnionitis and preterm delivery in different patient populations. Obstet Gynecol **75:**622-626.

16. **Torricelli M, Voltolini C, Toti P, Vellucci FL, Conti N, Cannoni A, Moncini I, Occhini R, Severi FM, Petraglia F.** 2014. Histologic chorioamnionitis: different histologic features at different gestational ages. J Matern Fetal Neonatal Med **27:**910-913.

17. **Seong HS, Lee SE, Kang JH, Romero R, Yoon BH.** 2008. The frequency of microbial invasion of the amniotic cavity and histologic chorioamnionitis in women at term with intact membranes in the presence or absence of labor. Am J Obstet Gynecol **199:**375.e371-375.

18. **Park HS, Romero R, Lee SM, Park CW, Jun JK, Yoon BH.** 2010. Histologic chorioamnionitis is more common after spontaneous labor than after induced labor at term. Placenta **31:**792-795.

19. **Laughon SK, Berghella V, Reddy UM, Sundaram R, Lu Z, Hoffman MK.** 2014. Neonatal and maternal outcomes with prolonged second stage of labor. Obstet Gynecol **124:**57-67.

20. **Rickert VI, Wiemann CM, Hankins GD, McKee JM, Berenson AB.** 1998. Prevalence and risk factors of chorioamnionitis among adolescents. Obstet Gynecol **92:**254-257.

21. **Seaward PG, Hannah ME, Myhr TL, Farine D, Ohlsson A, Wang EE, Haque K, Weston JA, Hewson SA, Ohel G, Hodnett ED.** 1997. International Multicentre Term Prelabor Rupture of Membranes Study: evaluation of predictors of clinical

chorioamnionitis and postpartum fever in patients with prelabor rupture of membranes at term. Am J Obstet Gynecol **177:**1024-1029.

22. **Denney JM, Culhane JF.** 2009. Bacterial vaginosis: a problematic infection from both a perinatal and neonatal perspective. Semin Fetal Neonatal Med **14:**200-203.

23. **Anderson BL, Simhan HN, Simons KM, Wiesenfeld HC.** 2007. Untreated asymptomatic group B streptococcal bacteriuria early in pregnancy and chorioamnionitis at delivery. Am J Obstet Gynecol **196:**524.e521-525.

24. **Howson CP, Kinney MV, McDougall L, Lawn JE.** 2013. Born too soon: preterm birth matters. Reprod Health **10 Suppl 1:**S1.

25. **Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M, Mathers C, Black RE.** 2012. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet **379:**2151-2161.

26. **Goldenberg RL, Hauth JC, Andrews WW.** 2000. Intrauterine infection and preterm delivery. N Engl J Med **342:**1500-1507.

27. **Goland RS, Wardlaw SL, Blum M, Tropper PJ, Stark RI.** 1988. Biologically active corticotropin-releasing hormone in maternal and fetal plasma during pregnancy. Am J Obstet Gynecol **159:**884-890.

28. **Smith R.** 2007. Parturition. N Engl J Med **356:**271-283.

29. **Johnson RF, Mitchell CM, Clifton V, Zakar T.** 2004. Regulation of 15-hydroxyprostaglandin dehydrogenase (PGDH) gene activity, messenger ribonucleic acid processing, and protein abundance in the human chorion in late gestation and labor. J Clin Endocrinol Metab **89:**5639-5648.

30. **Phillips RJ, Al-Zamil H, Hunt LP, Fortier MA, Lopez Bernal A.** 2011. Genes for prostaglandin synthesis, transport and inactivation are differentially expressed in

1028 human uterine tissues, and the prostaglandin F synthase AKR1B1 is induced in
1029 myometrial cells by inflammatory cytokines. Mol Hum Reprod **17:**1-13.
1030 31. **Li W, Challis JR.** 2005. Corticotropin-releasing hormone and urocortin induce
1031 secretion of matrix metalloproteinase-9 (MMP-9) without change in tissue inhibitors
1032 of MMP-1 by cultured cells from human placenta and fetal membranes. J Clin
1033 Endocrinol Metab **90:**6569-6574.
1034 32. **Ulug U, Goldman S, Ben-Shlomo I, Shalev E.** 2001. Matrix metalloproteinase
1035 (MMP)-2 and MMP-9 and their inhibitor, TIMP-1, in human term decidua and fetal
1036 membranes: the effect of prostaglandin F(2alpha) and indomethacin. Mol Hum
1037 Reprod **7:**1187-1193.
1038 33. **Xie N, Liu L, Li Y, Yu C, Lam S, Shynlova O, Gleave M, Challis JR, Lye S, Dong**
1039 **X.** 2012. Expression and function of myometrial PSF suggest a role in progesterone
1040 withdrawal and the initiation of labor. Mol Endocrinol **26:**1370-1379.
1041 34. **Nadeem L, Shynlova O, Matysiak-Zablocki E, Mesiano S, Dong X, Lye S.** 2016.
1042 Molecular evidence of functional progesterone withdrawal in human myometrium.
1043 Nat Commun **7:**11565.
1044 35. **Maiti K, Paul JW, Read M, Chan EC, Riley SC, Nahar P, Smith R.** 2011. G-1-
1045 activated membrane estrogen receptors mediate increased contractility of the human
1046 myometrium. Endocrinology **152:**2448-2455.
1047 36. **Waring GJ, Robson SC, Bulmer JN, Tyson-Capper AJ.** 2015. Inflammatory
1048 signalling in fetal membranes: increased expression levels of TLR 1 in the presence of
1049 preterm histological chorioamnionitis. PLoS One **10:**e0124298.
1050 37. **Moco NP, Martin LF, Pereira AC, Polettini J, Peracoli JC, Coelho KI, da Silva**
1051 **MG.** 2013. Gene expression and protein localization of TLR-1, -2, -4 and -6 in

amniochorion membranes of pregnancies complicated by histologic chorioamnionitis. Eur J Obstet Gynecol Reprod Biol **171:**12-17.

38. **Kim YM, Romero R, Chaiworapongsa T, Kim GJ, Kim MR, Kuivaniemi H, Tromp G, Espinoza J, Bujold E, Abrahams VM, Mor G.** 2004. Toll-like receptor-2 and -4 in the chorioamniotic membranes in spontaneous labor at term and in preterm parturition that are associated with chorioamnionitis. Am J Obstet Gynecol **191:**1346-1355.

39. **Holmlund U, Cebers G, Dahlfors AR, Sandstedt B, Bremme K, Ekstrom ES, Scheynius A.** 2002. Expression and regulation of the pattern recognition receptors Toll-like receptor-2 and Toll-like receptor-4 in the human placenta. Immunology **107:**145-151.

40. **Allam AB, von Chamier M, Brown MB, Reyes L.** 2014. Immune profiling of BALB/C and C57BL/6 mice reveals a correlation between *Ureaplasma parvum*-Induced fetal inflammatory response syndrome-like pathology and increased placental expression of TLR2 and CD14. Am J Reprod Immunol **71:**241-251.

41. **Gillaux C, Mehats C, Vaiman D, Cabrol D, Breuiller-Fouche M.** 2011. Functional screening of TLRs in human amniotic epithelial cells. J Immunol **187:**2766-2774.

42. **Marconi C, de Andrade Ramos BR, Peracoli JC, Donders GG, da Silva MG.** 2011. Amniotic fluid interleukin-1 beta and interleukin-6, but not interleukin-8 correlate with microbial invasion of the amniotic cavity in preterm labor. Am J Reprod Immunol **65:**549-556.

43. **Dando SJ, Nitsos I, Kallapur SG, Newnham JP, Polglase GR, Pillow JJ, Jobe AH, Timms P, Knox CL.** 2012. The role of the multiple banded antigen of *Ureaplasma parvum* in intra-amniotic infection: major virulence factor or decoy? PLoS One **7:**e29856.

44. **Yoon BH, Romero R, Park JS, Chang JW, Kim YA, Kim JC, Kim KS.** 1998. Microbial invasion of the amniotic cavity with *Ureaplasma urealyticum* is associated with a robust host response in fetal, amniotic, and maternal compartments. Am J Obstet Gynecol **179:**1254-1260.

45. **Holst RM, Laurini R, Jacobsson B, Samuelsson E, Savman K, Doverhag C, Wennerholm UB, Hagberg H.** 2007. Expression of cytokines and chemokines in cervical and amniotic fluid: relationship to histological chorioamnionitis. J Matern Fetal Neonatal Med **20:**885-893.

46. **Sweeney EL, Kallapur SG, Gisslen T, Lambers DS, Chougnet CA, Stephenson SA, Jobe AH, Knox CL.** 2016. Placental infection with *Ureaplasma* species is associated with histologic chorioamnionitis and adverse outcomes in moderately preterm and late-preterm infants. J Infect Dis **213:**1340-1347.

47. **Romero R, Mazor M, Wu YK, Avila C, Oyarzun E, Mitchell MD.** 1989. Bacterial endotoxin and tumor necrosis factor stimulate prostaglandin production by human decidua. Prostaglandins Leukot Essent Fatty Acids **37:**183-186.

48. **Norwitz ER, Lopez Bernal A, Starkey PM.** 1992. Tumor necrosis factor-alpha selectively stimulates prostaglandin F2 alpha production by macrophages in human term decidua. Am J Obstet Gynecol **167:**815-820.

49. **Sadowsky DW, Adams KM, Gravett MG, Witkin SS, Novy MJ.** 2006. Preterm labor is induced by intraamniotic infusions of interleukin-1beta and tumor necrosis factor-alpha but not by interleukin-6 or interleukin-8 in a nonhuman primate model. Am J Obstet Gynecol **195:**1578-1589.

50. **Maymon E, Romero R, Pacora P, Gomez R, Athayde N, Edwin S, Yoon BH.** 2000. Human neutrophil collagenase (matrix metalloproteinase 8) in parturition,

premature rupture of the membranes, and intrauterine infection. Am J Obstet Gynecol **183:**94-99.

51. **Park JY, Romero R, Lee J, Chaemsaithong P, Chaiyasit N, Yoon BH.** 2016. An elevated amniotic fluid prostaglandin F2alpha concentration is associated with intra-amniotic inflammation/infection, and clinical and histologic chorioamnionitis, as well as impending preterm delivery in patients with preterm labor and intact membranes. J Matern Fetal Neonatal Med **29:**2563-2572.

52. **Schmidt AF, Kannan PS, Kemp MW, Kramer BW, Newnham JP, Jobe AH, Kallapur SG.** 2014. Intra-amniotic LPS modulates expression of antimicrobial peptides in the fetal sheep lung. Pediatr Res **76:**441-447.

53. **Collins JJ, Kuypers E, Nitsos I, Jane Pillow J, Polglase GR, Kemp MW, Newnham JP, Cleutjens JP, Frints SG, Kallapur SG, Jobe AH, Kramer BW.** 2012. LPS-induced chorioamnionitis and antenatal corticosteroids modulate Shh signaling in the ovine fetal lung. Am J Physiol Lung Cell Mol Physiol **303:**L778-787.

54. **Wolfs TG, Kramer BW, Thuijls G, Kemp MW, Saito M, Willems MG, Senthamarai-Kannan P, Newnham JP, Jobe AH, Kallapur SG.** 2014. Chorioamnionitis-induced fetal gut injury is mediated by direct gut exposure of inflammatory mediators or by lung inflammation. Am J Physiol Gastrointest Liver Physiol **306:**G382-393.

55. **Nikiforou M, Kemp MW, van Gorp RH, Saito M, Newnham JP, Reynaert NL, Janssen LE, Jobe AH, Kallapur SG, Kramer BW, Wolfs TG.** 2016. Selective IL-1alpha exposure to the fetal gut, lung, and chorioamnion/skin causes intestinal inflammatory and developmental changes in fetal sheep. Lab Invest **96:**69-80.

56. **Zhang L, Saito M, Jobe A, Kallapur SG, Newnham JP, Cox T, Kramer B, Yang H, Kemp MW.** 2012. Intra-amniotic administration of *E. coli* lipopolysaccharides causes sustained inflammation of the fetal skin in sheep. Reprod Sci **19:**1181-1189.

57. **Howman RA, Charles AK, Jacques A, Doherty DA, Simmer K, Strunk T, Richmond PC, Cole CH, Burgner DP.** 2012. Inflammatory and haematological markers in the maternal, umbilical cord and infant circulation in histological chorioamnionitis. PLoS One **7:**e51836.

58. **Zanardo V, Peruzzetto C, Trevisanuto D, Cavallin F, Vedovato S, Straface G, Chiarelli S.** 2012. Relationship between the neonatal white blood cell count and histologic chorioamnionitis in preterm newborns. J Matern Fetal Neonatal Med **25:**2769-2772.

59. **Rueda CM, Wells CB, Gisslen T, Jobe AH, Kallapur SG, Chougnet CA.** 2015. Effect of chorioamnionitis on regulatory T cells in moderate/late preterm neonates. Hum Immunol **76:**65-73.

60. **Melville JM, Bischof RJ, Meeusen EN, Westover AJ, Moss TJ.** 2012. Changes in fetal thymic immune cell populations in a sheep model of intrauterine inflammation. Reprod Sci **19:**740-747.

61. **Kuypers E, Willems MG, Jellema RK, Kemp MW, Newnham JP, Delhaas T, Kallapur SG, Jobe AH, Wolfs TG, Kramer BW.** 2015. Responses of the spleen to intraamniotic lipopolysaccharide exposure in fetal sheep. Pediatr Res **77:**29-35.

62. **Collins JJ, Kallapur SG, Knox CL, Nitsos I, Polglase GR, Pillow JJ, Kuypers E, Newnham JP, Jobe AH, Kramer BW.** 2010. Inflammation in fetal sheep from intra-amniotic injection of *Ureaplasma parvum*. Am J Physiol Lung Cell Mol Physiol **299:**L852-860.

63. **Strackx E, Sparnaaij MA, Vlassaks E, Jellema R, Kuypers E, Vles JS, Kramer BW, Gavilanes AW.** 2015. Lipopolysaccharide-induced chorioamnionitis causes acute inflammatory changes in the ovine central nervous system. CNS Neurol Disord Drug Targets **14:**77-84.

64. **Kuypers E, Jellema RK, Ophelders DR, Dudink J, Nikiforou M, Wolfs TG, Nitsos I, Pillow JJ, Polglase GR, Kemp MW, Saito M, Newnham JP, Jobe AH, Kallapur SG, Kramer BW.** 2013. Effects of intra-amniotic lipopolysaccharide and maternal betamethasone on brain inflammation in fetal sheep. PLoS One **8:**e81644.

65. **Madsen-Bouterse SA, Romero R, Tarca AL, Kusanovic JP, Espinoza J, Kim CJ, Kim JS, Edwin SS, Gomez R, Draghici S.** 2010. The transcriptome of the fetal inflammatory response syndrome. Am J Reprod Immunol **63:**73-92.

66. **Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM.** 1998. The fetal inflammatory response syndrome. Am J Obstet Gynecol **179:**194-202.

67. **Romero R, Savasan ZA, Chaiworapongsa T, Berry SM, Kusanovic JP, Hassan SS, Yoon BH, Edwin S, Mazor M.** 2012. Hematologic profile of the fetus with systemic inflammatory response syndrome. J Perinat Med **40:**19-32.

68. **Kallapur SG, Presicce P, Rueda CM, Jobe AH, Chougnet CA.** 2014. Fetal immune response to chorioamnionitis. Semin Reprod Med **32:**56-67.

69. **Gantert M, Been JV, Gavilanes AW, Garnier Y, Zimmermann LJ, Kramer BW.** 2010. Chorioamnionitis: a multiorgan disease of the fetus? J Perinatol **30 Suppl:**S21-30.

70. **Arayici S, Kadioglu Simsek G, Oncel MY, Eras Z, Canpolat FE, Oguz SS, Uras N, Zergeroglu S, Dilmen U.** 2014. The effect of histological chorioamnionitis on the short-term outcome of preterm infants </=32 weeks: a single-center study. J Matern Fetal Neonatal Med **27:**1129-1133.

71. **Arora P, Bagga R, Kalra J, Kumar P, Radhika S, Gautam V.** 2015. Mean gestation at delivery and histological chorioamnionitis correlates with early-onset neonatal sepsis following expectant management in pPROM. J Obstet Gynaecol **35:**235-240.

72. **Stimac M, Juretic E, Vukelic V, Matasic NP, Kos M, Babic D.** 2014. Effect of chorioamnionitis on mortality, early onset neonatal sepsis and bronchopulmonary dysplasia in preterm neonates with birth weight of < 1,500 grams. Coll Antropol **38:**167-171.

73. **Moscuzza F, Belcari F, Nardini V, Bartoli A, Domenici C, Cuttano A, Ghirri P, Boldrini A.** 2011. Correlation between placental histopathology and fetal/neonatal outcome: chorioamnionitis and funisitis are associated to intraventricular haemorrage and retinopathy of prematurity in preterm newborns. Gynecol Endocrinol **27:**319-323.

74. **Mestan K, Yu Y, Matoba N, Cerda S, Demmin B, Pearson C, Ortiz K, Wang X.** 2010. Placental inflammatory response is associated with poor neonatal growth: preterm birth cohort study. Pediatrics **125:**e891-898.

75. **Lu HY, Zhang Q, Wang QX, Lu JY.** 2016. Contribution of histologic chorioamnionitis and fetal inflammatory response syndrome to increased risk of brain injury in infants with preterm premature rupture of membranes. Pediatr Neurol doi:10.1016/j.pediatrneurol.2016.05.001.

76. **Korzeniewski SJ, Romero R, Cortez J, Pappas A, Schwartz AG, Kim CJ, Kim JS, Kim YM, Yoon BH, Chaiworapongsa T, Hassan SS.** 2014. A "multi-hit" model of neonatal white matter injury: cumulative contributions of chronic placental inflammation, acute fetal inflammation and postnatal inflammatory events. J Perinat Med **42:**731-743.

77. **Perrone S, Toti P, Toti MS, Badii S, Becucci E, Gatti MG, Marzocchi B, Picardi A, Buonocore G.** 2012. Perinatal outcome and placental histological characteristics: a single-center study. J Matern Fetal Neonatal Med **25 Suppl 1:**110-113.

78. **Eriksson L, Haglund B, Odlind V, Altman M, Kieler H.** 2014. Prenatal inflammatory risk factors for development of bronchopulmonary dysplasia. Pediatr Pulmonol **49:**665-672.

79. **Hartling L, Liang Y, Lacaze-Masmonteil T.** 2012. Chorioamnionitis as a risk factor for bronchopulmonary dysplasia: a systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed **97:**F8-f17.

80. **Park HW, Choi YS, Kim KS, Kim SN.** 2015. Chorioamnionitis and patent ductus arteriosus: a systematic review and meta-analysis. PLoS One **10:**e0138114.

81. **Chen ML, Allred EN, Hecht JL, Onderdonk A, VanderVeen D, Wallace DK, Leviton A, Dammann O.** 2011. Placenta microbiology and histology and the risk for severe retinopathy of prematurity. Invest Ophthalmol Vis Sci **52:**7052-7058.

82. **Woo SJ, Park KH, Jung HJ, Kim S, Choe G, Ahn J, Park KH.** 2012. Effects of maternal and placental inflammation on retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol **250:**915-923.

83. **Romero R, Espinoza J, Goncalves LF, Gomez R, Medina L, Silva M, Chaiworapongsa T, Yoon BH, Ghezzi F, Lee W, Treadwell M, Berry SM, Maymon E, Mazor M, DeVore G.** 2004. Fetal cardiac dysfunction in preterm premature rupture of membranes. J Matern Fetal Neonatal Med **16:**146-157.

84. **Yanowitz TD, Jordan JA, Gilmour CH, Towbin R, Bowen A, Roberts JM, Brozanski BS.** 2002. Hemodynamic disturbances in premature infants born after chorioamnionitis: association with cord blood cytokine concentrations. Pediatr Res **51:**310-316.

85. **Moore SW, Arnold M, Wright C.** 2013. Necrotizing enterocolitis and the placenta - a key etiological link. J Pediatr Surg **48:**359-362.

86. **Been JV, Lievense S, Zimmermann LJ, Kramer BW, Wolfs TG.** 2013. Chorioamnionitis as a risk factor for necrotizing enterocolitis: a systematic review and meta-analysis. J Pediatr **162:**236-242.e232.

87. **Kim YM, Romero R, Chaiworapongsa T, Espinoza J, Mor G, Kim CJ.** 2006. Dermatitis as a component of the fetal inflammatory response syndrome is associated with activation of Toll-like receptors in epidermal keratinocytes. Histopathology **49:**506-514.

88. **Lee Y, Kim HJ, Choi SJ, Oh SY, Kim JS, Roh CR, Kim JH.** 2015. Is there a stepwise increase in neonatal morbidities according to histological stage (or grade) of acute chorioamnionitis and funisitis?: effect of gestational age at delivery. J Perinat Med **43:**259-267.

89. **Hendson L, Russell L, Robertson CM, Liang Y, Chen Y, Abdalla A, Lacaze-Masmonteil T.** 2011. Neonatal and neurodevelopmental outcomes of very low birth weight infants with histologic chorioamnionitis. J Pediatr **158:**397-402.

90. **Gisslen T, Alvarez M, Wells C, Soo MT, Lambers DS, Knox CL, Meinzen-Derr JK, Chougnet CA, Jobe AH, Kallapur SG.** 2016. Fetal inflammation associated with minimal acute morbidity in moderate/late preterm infants. Arch Dis Child Fetal Neonatal Ed.

91. **Pappas A, Kendrick DE, Shankaran S, Stoll BJ, Bell EF, Laptook AR, Walsh MC, Das A, Hale EC, Newman NS, Higgins RD.** 2014. Chorioamnionitis and early childhood outcomes among extremely low-gestational-age neonates. JAMA Pediatr **168:**137-147.

92. **Garcia-Munoz Rodrigo F, Galan Henriquez G, Figueras Aloy J, Garcia-Alix Perez A.** 2014. Outcomes of very-low-birth-weight infants exposed to maternal clinical chorioamnionitis: a multicentre study. Neonatology **106:**229-234.

93. **Botet F, Figueras J, Carbonell-Estrany X, Arca G, The Castrillo Study G.** 2010. Effect of maternal clinical chorioamnionitis on neonatal morbidity in very-low birthweight infants: a case-control study. J Perinat Med **38:**269-273.

94. **Soraisham AS, Trevenen C, Wood S, Singhal N, Sauve R.** 2013. Histological chorioamnionitis and neurodevelopmental outcome in preterm infants. J Perinatol **33:**70-75.

95. **Yamada N, Sato Y, Moriguchi-Goto S, Yamashita A, Kodama Y, Sameshima H, Asada Y.** 2015. Histological severity of fetal inflammation is useful in predicting neonatal outcome. Placenta **36:**1490-1493.

96. **Sato M, Nishimaki S, Yokota S, Seki K, Horiguchi H, An H, Ishida F, Fujita S, Ao K, Yatake H.** 2011. Severity of chorioamnionitis and neonatal outcome. J Obstet Gynaecol Res **37:**1313-1319.

97. **King AE, Kelly RW, Sallenave JM, Bocking AD, Challis JR.** 2007. Innate immune defences in the human uterus during pregnancy. Placenta **28:**1099-1106.

98. **Kjaergaard N, Hein M, Hyttel L, Helmig RB, Schonheyder HC, Uldbjerg N, Madsen H.** 2001. Antibacterial properties of human amnion and chorion *in vitro*. Eur J Obstet Gynecol Reprod Biol **94:**224-229.

99. **Parthasarathy M, Sasikala R, Gunasekaran P, Raja J.** 2014. Antimicrobial activity of human amniotic and chorionic membranes. Journal of Academia and Industrial Research **2:**545-547.

100. **Knox CL, Allan JA, Allan JM, Edirisinghe WR, Stenzel D, Lawrence FA, Purdie DM, Timms P.** 2003. *Ureaplasma parvum* and *Ureaplasma urealyticum* are detected

in semen after washing before assisted reproductive technology procedures. Fertil Steril **80:**921-929.

101. **Quinn PA, Li HC, Th'ng C, Dunn M, Butany J.** 1993. Serological response to *Ureaplasma urealyticum* in the neonate. Clin Infect Dis **17 Suppl 1:**S136-143.

102. **Kim MJ, Romero R, Gervasi MT, Kim JS, Yoo W, Lee DC, Mittal P, Erez O, Kusanovic JP, Hassan SS, Kim CJ.** 2009. Widespread microbial invasion of the chorioamniotic membranes is a consequence and not a cause of intra-amniotic infection. Lab Invest **89:**924-936.

103. **Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J.** 2014. The placenta harbors a unique microbiome. Sci Transl Med **6:**237ra265.

104. **Prince AL, Ma J, Kannan PS, Alvarez M, Gisslen T, Harris RA, Sweeney EL, Knox CL, Lambers DS, Jobe AH, Chougnet CA, Kallapur SG, Aagaard KM.** 2016. The placental membrane microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis. Am J Obstet Gynecol **214:**627.e621-627.e616.

105. **Kallapur SG, Kramer BW, Knox CL, Berry CA, Collins JJ, Kemp MW, Nitsos I, Polglase GR, Robinson J, Hillman NH, Newnham JP, Chougnet C, Jobe AH.** 2011. Chronic fetal exposure to *Ureaplasma parvum* suppresses innate immune responses in sheep. J Immunol **187:**2688-2695.

106. **DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, Gotsch F, Kim CJ, Erez O, Edwin S, Relman DA.** 2008. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PLoS One **3:**e3056.

107. **Knox CL, Cave DG, Farrell DJ, Eastment HT, Timms P.** 1997. The role of *Ureaplasma urealyticum* in adverse pregnancy outcome. Aust N Z J Obstet Gynaecol **37:**45-51.

108. **Kundsin RB, Leviton A, Allred EN, Poulin SA.** 1996. *Ureaplasma urealyticum* infection of the placenta in pregnancies that ended prematurely. Obstet Gynecol **87:**122-127.

109. **Iwatani S, Mizobuchi M, Sofue T, Tanaka S, Sakai H, Yoshimoto S, Nakao H.** 2014. Neonatal leukemoid reaction associated with *Candida albicans* chorioamnionitis. Pediatr Int **56:**277-279.

110. **Ljubin-Sternak S, Mestrovic T.** 2014. *Chlamydia trachomatis* and genital mycoplasmas: pathogens with an impact on human reproductive health. J Pathog **2014:**183167.

111. **Montelongo EM, Blue NR, Lee RH.** 2015. Placenta accreta in a woman with *Escherichia coli* chorioamnionitis with intact membranes. Case Rep Obstet Gynecol **2015:**121864.

112. **Rours GI, de Krijger RR, Ott A, Willemse HF, de Groot R, Zimmermann LJ, Kornelisse RF, Verbrugh HA, Verkooijen RP.** 2011. *Chlamydia trachomatis* and placental inflammation in early preterm delivery. Eur J Epidemiol **26:**421-428.

113. **Wenstrom KD, Andrews WW, Bowles NE, Towbin JA, Hauth JC, Goldenberg RL.** 1998. Intrauterine viral infection at the time of second trimester genetic amniocentesis. Obstet Gynecol **92:**420-424.

114. **Miller JL, Harman C, Weiner C, Baschat AA.** 2009. Perinatal outcomes after second trimester detection of amniotic fluid viral genome in asymptomatic patients. J Perinat Med **37:**140-143.

115. **Reddy UM, Baschat AA, Zlatnik MG, Towbin JA, Harman CR, Weiner CP.** 2005. Detection of viral deoxyribonucleic acid in amniotic fluid: association with fetal malformation and pregnancy abnormalities. Fetal Diagn Ther **20:**203-207.

116. **Baschat AA, Towbin J, Bowles NE, Harman CR, Weiner CP.** 2003. Prevalence of viral DNA in amniotic fluid of low-risk pregnancies in the second trimester. J Matern Fetal Neonatal Med **13:**381-384.

117. **Cox C, Saxena N, Watt AP, Gannon C, McKenna JP, Fairley DJ, Sweet D, Shields MD, S LC, Coyle PV.** 2016. The common vaginal commensal bacterium *Ureaplasma parvum* is associated with chorioamnionitis in extreme preterm labor. J Matern Fetal Neonatal Med**:**1-6.

118. **Namba F, Hasegawa T, Nakayama M, Hamanaka T, Yamashita T, Nakahira K, Kimoto A, Nozaki M, Nishihara M, Mimura K, Yamada M, Kitajima H, Suehara N, Yanagihara I.** 2010. Placental features of chorioamnionitis colonized with *Ureaplasma* species in preterm delivery. Pediatr Res **67:**166-172.

119. **Egawa T, Morioka I, Morisawa T, Yokoyama N, Nakao H, Ohashi M, Matsuo M.** 2007. *Ureaplasma urealyticum* and *Mycoplasma hominis* presence in umbilical cord is associated with pathogenesis of funisitis. Kobe J Med Sci **53:**241-249.

120. **Park CW, Yoon BH, Park JS, Jun JK.** 2013. A fetal and an intra-amniotic inflammatory response is more severe in preterm labor than in preterm PROM in the context of funisitis: unexpected observation in human gestations. PLoS One **8:**e62521.

121. **Gerber S, Vial Y, Hohlfeld P, Witkin SS.** 2003. Detection of *Ureaplasma urealyticum* in second-trimester amniotic fluid by polymerase chain reaction correlates with subsequent preterm labor and delivery. J Infect Dis **187:**518-521.

122. **Shepard MC.** 1954. The recovery of pleuropneumonia-like organisms from Negro men with and without nongonococcal urethritis. Am J Syph Gonorrhea Vener Dis **38:**113-124.

123. **Shepard MC, Lunceford CD, Ford DK, Purcell RH, Taylor-Robinson D, Razin S, Black FT.** 1974. *Ureaplasma urealyticum* gen. nov., sp. nov.: proposed nomenclature for the human T (T-strain) mycoplasmas. Int J Styst Bacteriol **24:**160-171.

124. **Smith DG, Russell WC, Ingledew WJ, Thirkell D.** 1993. Hydrolysis of urea by *Ureaplasma urealyticum* generates a transmembrane potential with resultant ATP synthesis. J Bacteriol **175:**3253-3258.

125. **Shepard MC, Lunceford CD.** 1976. Differential agar medium (A7) for identification of *Ureaplasma urealyticum* (human T mycoplasmas) in primary cultures of clinical material. J Clin Microbiol **3:**613-625.

126. **Tully JG.** 1983. Cloning and filtration techniques for mycoplasmas, p 173-177. *In* Razin S (ed), Methods in Mycoplasmology, vol 1. Academic Press, Inc., New York.

127. **Shepard MC, Masover GK.** 1979. Special features of the ureaplasmas, p 452-494. *In* Barile MF, Razin S (ed), The Mycoplasmas, vol 1. Academic Press, Inc., New York.

128. **Robertson JA, Stemke GW, Davis JW, Jr., Harasawa R, Thirkell D, Kong F, Shepard MC, Ford DK.** 2002. Proposal of *Ureaplasma parvum* sp. nov. and emended description of *Ureaplasma urealyticum* (Shepard *et al*. 1974) Robertson *et al*. 2001. Int J Syst Evol Microbiol **52:**587-597.

129. **Paralanov V, Lu J, Duffy LB, Crabb DM, Shrivastava S, Methe BA, Inman J, Yooseph S, Xiao L, Cassell GH, Waites KB, Glass JI.** 2012. Comparative genome analysis of 19 *Ureaplasma urealyticum* and *Ureaplasma parvum* strains. BMC Microbiol **12:**88.

130. **Christiansen C, Black FT, Freundt EA.** 1981. Hybridization experiments with deoxyribonucleic acid from *Ureaplasma urealyticum* serovars I to VIII Int J Syst Bacteriol **31:**259-262.

131. **Razin S, Harasawa R, Barile MF.** 1983. Cleavage patterns of the mycoplasma chromosome, obtained by using restriction endonucleases, as indicators of genetic relatedness among strains Int J Syst Bacteriol **33:**201-206.

132. **Kong F, James G, Ma Z, Gordon S, Bin W, Gilbert GL.** 1999. Phylogenetic analysis of *Ureaplasma urealyticum* - support for the establishment of a new species, *Ureaplasma parvum*. Int J Syst Bacteriol **49 Pt 4:**1879-1889.

133. **Teng LJ, Zheng X, Glass JI, Watson HL, Tsai J, Cassell GH.** 1994. *Ureaplasma urealyticum* biovar specificity and diversity are encoded in multiple-banded antigen gene. J Clin Microbiol **32:**1464-1469.

134. **Zhang J, Kong Y, Feng Y, Huang J, Song T, Ruan Z, Song J, Jiang Y, Yu Y, Xie X.** 2014. Development of a multilocus sequence typing scheme for *Ureaplasma*. Eur J Clin Microbiol Infect Dis **33:**537-544.

135. **Knox CL, Giffard P, Timms P.** 1998. The phylogeny of *Ureaplasma urealyticum* based on the *mba* gene fragment. Int J Syst Bacteriol **48 Pt 4:**1323-1331.

136. **Black FT.** 1973. Modifications of the growth inhibition test and its application to human T-mycoplasmas. Appl Microbiol **25:**528-533.

137. **Shepard MC, Lunceford CD.** 1978. Serological typing of *Ureaplasma urealyticum* isolates from urethritis patients by an agar growth inhibition method. J Clin Microbiol **8:**566-574.

138. **Quinn PA, Arshoff LU, Li HC.** 1981. Serotyping of *Ureaplasma urealyticum* by immunoperoxidase assay. J Clin Microbiol **13:**670-676.

139. **Turunen H, Leinikki P, Jansson E.** 1982. Serological characterisation of *Ureaplasma urealyticum* strains by enzyme-linked immunosorbent assay (ELISA). J Clin Pathol **35:**439-443.

140. **Echahidi F, Muyldermans G, Lauwers S, Naessens A.** 2001. Development of an enzyme-linked immunosorbent assay for serotyping *Ureaplasma urealyticum* strains using monoclonal antibodies. Clin Diagn Lab Immunol **8:**52-57.

141. **Stemke GW, Robertson JA.** 1981. Modified colony indirect epifluorescence test for serotyping *Ureaplasma urealyticum* and an adaptation to detect common antigenic specificity. J Clin Microbiol **14:**582-584.

142. **Knox CL, Timms P.** 1998. Comparison of PCR, nested PCR, and random amplified polymorphic DNA PCR for detection and typing of *Ureaplasma urealyticum* in specimens from pregnant women. J Clin Microbiol **36:**3032-3039.

143. **Kong F, Ma Z, James G, Gordon S, Gilbert GL.** 2000. Species identification and subtyping of *Ureaplasma parvum* and *Ureaplasma urealyticum* using PCR-based assays. J Clin Microbiol **38:**1175-1179.

144. **Kong F, Ma Z, James G, Gordon S, Gilbert GL.** 2000. Molecular genotyping of human *Ureaplasma* species based on multiple-banded antigen (MBA) gene sequences. Int J Syst Evol Microbiol **50 Pt 5:**1921-1929.

145. **Xiao L, Glass JI, Paralanov V, Yooseph S, Cassell GH, Duffy LB, Waites KB.** 2010. Detection and characterization of human *Ureaplasma* species and serovars by real-time PCR. J Clin Microbiol **48:**2715-2723.

146. **Xiao L, Paralanov V, Glass JI, Duffy LB, Robertson JA, Cassell GH, Chen Y, Waites KB.** 2011. Extensive horizontal gene transfer in ureaplasmas from humans questions the utility of serotyping for diagnostic purposes. J Clin Microbiol **49:**2818-2826.

147. **Cassell GH, Waites KB, Watson HL, Crouse DT, Harasawa R.** 1993. *Ureaplasma urealyticum* intrauterine infection: role in prematurity and disease in newborns. Clin Microbiol Rev **6:**69-87.

148. **Vancutsem E, Faron G, Foulon W, Naessens A.** 2015. Genital tract colonization with *Ureaplasma* spp. and its association with abnormal vaginal flora. J Med Microbiol **64:**654-656.

149. **Marovt M, Kese D, Kotar T, Kmet N, Miljkovic J, Soba B, Maticic M.** 2015. *Ureaplasma parvum* and *Ureaplasma urealyticum* detected with the same frequency among women with and without symptoms of urogenital tract infection. Eur J Clin Microbiol Infect Dis **34:**1237-1245.

150. **Hunjak B, Sabol I, Vojnovic G, Fistonic I, Erceg AB, Persic Z, Grce M.** 2014. *Ureaplasma urealyticum* and *Ureaplasma parvum* in women of reproductive age. Arch Gynecol Obstet **289:**407-412.

151. **Tibaldi C, Cappello N, Latino MA, Masuelli G, Marini S, Benedetto C.** 2009. Vaginal and endocervical microorganisms in symptomatic and asymptomatic non-pregnant females: risk factors and rates of occurrence. Clin Microbiol Infect **15:**670-679.

152. **McCormack WM.** 1986. *Ureaplasma urealyticum*: ecologic niche and epidemiologic considerations. Pediatr Infect Dis **5:**S232-233.

153. **Kaliterna V, Kucisec-Tepes N, Pejkovic L, Zavorovic S, Petrovic S, Barisic Z.** 2011. An intrauterine device as a possible cause of change in the microbial flora of the female genital system. J Obstet Gynaecol Res **37:**1035-1040.

154. **De Francesco MA, Negrini R, Pinsi G, Peroni L, Manca N.** 2009. Detection of *Ureaplasma* biovars and polymerase chain reaction-based subtyping of *Ureaplasma*

parvum in women with or without symptoms of genital infections. Eur J Clin Microbiol Infect Dis **28:**641-646.

155. **Zdrodowska-Stefanow B, Klosowska WM, Ostaszewska-Puchalska I, Bulhak-Koziol V, Kotowicz B.** 2006. *Ureaplasma urealyticum* and *Mycoplasma hominis* infection in women with urogenital diseases. Adv Med Sci **51:**250-253.

156. **Liu L, Cao G, Zhao Z, Zhao F, Huang Y.** 2014. High bacterial loads of *Ureaplasma* may be associated with non-specific cervicitis. Scand J Infect Dis **46:**637-641.

157. **Povlsen K, Thorsen P, Lind I.** 2001. Relationship of *Ureaplasma urealyticum* biovars to the presence or absence of bacterial vaginosis in pregnant women and to the time of delivery. Eur J Clin Microbiol Infect Dis **20:**65-67.

158. **Plummer DC, Garland SM, Gilbert GL.** 1987. Bacteraemia and pelvic infection in women due to *Ureaplasma urealyticum* and *Mycoplasma hominis*. Med J Aust **146:**135-137.

159. **Kelly VN, Garland SM, Gilbert GL.** 1987. Isolation of genital mycoplasmas from the blood of neonates and women with pelvic infection using conventional SPS-free blood culture media. Pathology **19:**277-280.

160. **Latthe PM, Toozs-Hobson P, Gray J.** 2008. Mycoplasma and ureaplasma colonisation in women with lower urinary tract symptoms. J Obstet Gynaecol **28:**519-521.

161. **Baka S, Kouskouni E, Antonopoulou S, Sioutis D, Papakonstantinou M, Hassiakos D, Logothetis E, Liapis A.** 2009. Prevalence of *Ureaplasma urealyticum* and *Mycoplasma hominis* in women with chronic urinary symptoms. Urology **74:**62-66.

162. **Gilbert GL, Garland SM, Fairley KF, McDowall DM.** 1986. Bacteriuria due to ureaplasmas and other fastidious organisms during pregnancy: prevalence and significance. Pediatr Infect Dis **5:**S239-243.

163. **Kataoka S, Yamada T, Chou K, Nishida R, Morikawa M, Minami M, Yamada H, Sakuragi N, Minakami H.** 2006. Association between preterm birth and vaginal colonization by mycoplasmas in early pregnancy. J Clin Microbiol **44:**51-55.

164. **Gonzalez Bosquet E, Gene A, Ferrer I, Borras M, Lailla JM.** 2006. Value of endocervical *Ureaplasma* species colonization as a marker of preterm delivery. Gynecol Obstet Invest **61:**119-123.

165. **Kafetzis DA, Skevaki CL, Skouteri V, Gavrili S, Peppa K, Kostalos C, Petrochilou V, Michalas S.** 2004. Maternal genital colonization with *Ureaplasma urealyticum* promotes preterm delivery: association of the respiratory colonization of premature infants with chronic lung disease and increased mortality. Clin Infect Dis **39:**1113-1122.

166. **Abele-Horn M, Scholz M, Wolff C, Kolben M.** 2000. High-density vaginal *Ureaplasma urealyticum* colonization as a risk factor for chorioamnionitis and preterm delivery. Acta Obstet Gynecol Scand **79:**973-978.

167. **Kwak DW, Hwang HS, Kwon JY, Park YW, Kim YH.** 2014. Co-infection with vaginal *Ureaplasma urealyticum* and *Mycoplasma hominis* increases adverse pregnancy outcomes in patients with preterm labor or preterm premature rupture of membranes. J Matern Fetal Neonatal Med **27:**333-337.

168. **DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, Sun CL, Goltsman DS, Wong RJ, Shaw G, Stevenson DK, Holmes SP, Relman DA.** 2015. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci U S A **112:**11060-11065.

169. **Choi SJ, Park SD, Jang IH, Uh Y, Lee A.** 2012. The prevalence of vaginal microorganisms in pregnant women with preterm labor and preterm birth. Ann Lab Med **32:**194-200.

170. **Carey JC, Blackwelder WC, Nugent RP, Matteson MA, Rao AV, Eschenbach DA, Lee ML, Rettig PJ, Regan JA, Geromanos KL, et al.** 1991. Antepartum cultures for *Ureaplasma urealyticum* are not useful in predicting pregnancy outcome. The Vaginal Infections and Prematurity Study Group. Am J Obstet Gynecol **164:**728-733.

171. **Kwak DW, Cho HY, Kwon JY, Park YW, Kim YH.** 2015. Usefulness of maternal serum C-reactive protein with vaginal *Ureaplasma urealyticum* as a marker for prediction of imminent preterm delivery and chorioamnionitis in patients with preterm labor or preterm premature rupture of membranes. J Perinat Med **43:**409-415.

172. **Donders GG, Van Calsteren K, Bellen G, Reybrouck R, Van den Bosch T, Riphagen I, Van Lierde S.** 2009. Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. Bjog **116:**1315-1324.

173. **Vogel I, Thorsen P, Hogan VK, Schieve LA, Jacobsson B, Ferre CD.** 2006. The joint effect of vaginal *Ureaplasma urealyticum* and bacterial vaginosis on adverse pregnancy outcomes. Acta Obstet Gynecol Scand **85:**778-785.

174. **Lee SE, Romero R, Kim EC, Yoon BH.** 2009. A high Nugent score but not a positive culture for genital mycoplasmas is a risk factor for spontaneous preterm birth. J Matern Fetal Neonatal Med **22:**212-217.

175. **Kasprzykowska U, Elias J, Elias M, Maczynska B, Sobieszczanska BM.** 2014. Colonization of the lower urogenital tract with *Ureaplasma parvum* can cause

asymptomatic infection of the upper reproductive system in women: a preliminary study. Arch Gynecol Obstet **289:**1129-1134.

176. **Taylor-Robinson D, Jensen JS, Svenstrup H, Stacey CM.** 2012. Difficulties experienced in defining the microbial cause of pelvic inflammatory disease. Int J STD AIDS **23:**18-24.

177. **Cassell GH, Younger JB, Brown MB, Blackwell RE, Davis JK, Marriott P, Stagno S.** 1983. Microbiologic study of infertile women at the time of diagnostic laparoscopy. Association of *Ureaplasma urealyticum* with a defined subpopulation. N Engl J Med **308:**502-505.

178. **Shurin PA, Alpert S, Bernard Rosner BA, Driscoll SG, Lee YH.** 1975. Chorioamnionitis and colonization of the newborn infant with genital mycoplasmas. N Engl J Med **293:**5-8.

179. **Horowitz S, Mazor M, Romero R, Horowitz J, Glezerman M.** 1995. Infection of the amniotic cavity with *Ureaplasma urealyticum* in the midtrimester of pregnancy. J Reprod Med **40:**375-379.

180. **Holst RM, Mattsby-Baltzer I, Wennerholm UB, Hagberg H, Jacobsson B.** 2005. Interleukin-6 and interleukin-8 in cervical fluid in a population of Swedish women in preterm labor: relationship to microbial invasion of the amniotic fluid, intra-amniotic inflammation, and preterm delivery. Acta Obstet Gynecol Scand **84:**551-557.

181. **Witt A, Berger A, Gruber CJ, Petricevic L, Apfalter P, Husslein P.** 2005. IL-8 concentrations in maternal serum, amniotic fluid and cord blood in relation to different pathogens within the amniotic cavity. J Perinat Med **33:**22-26.

182. **Menon R, Peltier MR, Eckardt J, Fortunato SJ.** 2009. Diversity in cytokine response to bacteria associated with preterm birth by fetal membranes. Am J Obstet Gynecol **201:**306.e301-306.

183. **Perni SC, Vardhana S, Korneeva I, Tuttle SL, Paraskevas LR, Chasen ST, Kalish RB, Witkin SS.** 2004. *Mycoplasma hominis* and *Ureaplasma urealyticum* in midtrimester amniotic fluid: association with amniotic fluid cytokine levels and pregnancy outcome. Am J Obstet Gynecol **191:**1382-1386.

184. **Naessens A, Foulon W, Breynaert J, Lauwers S.** 1988. Serotypes of *Ureaplasma urealyticum* isolated from normal pregnant women and patients with pregnancy complications. J Clin Microbiol **26:**319-322.

185. **Jacobsson B, Aaltonen R, Rantakokko-Jalava K, Morken NH, Alanen A.** 2009. Quantification of *Ureaplasma urealyticum* DNA in the amniotic fluid from patients in PTL and pPROM and its relation to inflammatory cytokine levels. Acta Obstet Gynecol Scand **88:**63-70.

186. **Kasper DC, Mechtler TP, Reischer GH, Witt A, Langgartner M, Pollak A, Herkner KR, Berger A.** 2010. The bacterial load of *Ureaplasma parvum* in amniotic fluid is correlated with an increased intrauterine inflammatory response. Diagn Microbiol Infect Dis **67:**117-121.

187. **von Chamier M, Allam A, Brown MB, Reinhard MK, Reyes L.** 2012. Host genetic background impacts disease outcome during intrauterine infection with *Ureaplasma parvum*. PLoS One **7:**e44047.

188. **Menon R, Williams SM, Fortunato SJ.** 2007. Amniotic fluid interleukin-1beta and interleukin-8 concentrations: racial disparity in preterm birth. Reprod Sci **14:**253-259.

189. **Knox CL, Dando SJ, Nitsos I, Kallapur SG, Jobe AH, Payton D, Moss TJ, Newnham JP.** 2010. The severity of chorioamnionitis in pregnant sheep is associated with *in vivo* variation of the surface-exposed multiple-banded antigen/gene of *Ureaplasma parvum*. Biol Reprod **83:**415-426.

190. **Robinson JW, Dando SJ, Nitsos I, Newnham J, Polglase GR, Kallapur SG, Pillow JJ, Kramer BW, Jobe AH, Payton D, Knox CL.** 2013. *Ureaplasma parvum* serovar 3 multiple banded antigen size variation after chronic intra-amniotic infection/colonization. PLoS One **8:**e62746.

191. **Reyes L, Reinhard M, Brown MB.** 2009. Different inflammatory responses are associated with *Ureaplasma parvum*-induced UTI and urolith formation. BMC Infect Dis **9:**9.

192. **Cassell GH, Davis RO, Waites KB, Brown MB, Marriott PA, Stagno S, Davis JK.** 1983. Isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from amniotic fluid at 16-20 weeks of gestation: potential effect on outcome of pregnancy. Sex Transm Dis **10:**294-302.

193. **Czikk MJ, McCarthy FP, Murphy KE.** 2011. Chorioamnionitis: from pathogenesis to treatment. Clin Microbiol Infect **17:**1304-1311.

194. **Stirling KM, Hussain N, Sanders MM, Campbell W.** 2016. Association between maternal genital mycoplasma colonization and histologic chorioamnionitis in preterm births. J Neonatal Perinatal Med **9:**201-209.

195. **Novy MJ, Duffy L, Axthelm MK, Sadowsky DW, Witkin SS, Gravett MG, Cassell GH, Waites KB.** 2009. *Ureaplasma parvum* or *Mycoplasma hominis* as sole pathogens cause chorioamnionitis, preterm delivery, and fetal pneumonia in rhesus macaques. Reprod Sci **16:**56-70.

196. **Yoder BA, Coalson JJ, Winter VT, Siler-Khodr T, Duffy LB, Cassell GH.** 2003. Effects of antenatal colonization with *Ureaplasma urealyticum* on pulmonary disease in the immature baboon. Pediatr Res **54:**797-807.

197. **Senthamaraikannan P, Presicce P, Rueda CM, Maneenil G, Schmidt AF, Miller LA, Waites KB, Jobe AH, Kallapur SG, Chougnet CA.** 2016. Intra-amniotic

Ureaplasma parvum induced maternal and fetal inflammation and immune responses in rhesus macaque. J Infect Dis doi:10.1093/infdis/jiw408.

198. **Gravett MG, Witkin SS, Haluska GJ, Edwards JL, Cook MJ, Novy MJ.** 1994. An experimental model for intraamniotic infection and preterm labor in rhesus monkeys. Am J Obstet Gynecol **171:**1660-1667.

199. **Moss TJ, Nitsos I, Ikegami M, Jobe AH, Newnham JP.** 2005. Experimental intrauterine ureaplasma infection in sheep. Am J Obstet Gynecol **192:**1179-1186.

200. **Nitsos I, Moss TJ, Cock ML, Harding R, Newnham JP.** 2002. Fetal responses to intra-amniotic endotoxin in sheep. J Soc Gynecol Investig **9:**80-85.

201. **Mitchell BF, Taggart MJ.** 2009. Are animal models relevant to key aspects of human parturition? Am J Physiol Regul Integr Comp Physiol **297:**R525-545.

202. **Dando SJ, Nitsos I, Newnham JP, Jobe AH, Moss TJ, Knox CL.** 2010. Maternal administration of erythromycin fails to eradicate intrauterine ureaplasma infection in an ovine model. Biol Reprod **83:**616-622.

203. **Moss TJ, Knox CL, Kallapur SG, Nitsos I, Theodoropoulos C, Newnham JP, Ikegami M, Jobe AH.** 2008. Experimental amniotic fluid infection in sheep: effects of *Ureaplasma parvum* serovars 3 and 6 on preterm or term fetal sheep. Am J Obstet Gynecol **198:**122.e121-128.

204. **Polglase GR, Dalton RG, Nitsos I, Knox CL, Pillow JJ, Jobe AH, Moss TJ, Newnham JP, Kallapur SG.** 2010. Pulmonary vascular and alveolar development in preterm lambs chronically colonized with *Ureaplasma parvum*. Am J Physiol Lung Cell Mol Physiol **299:**L232-241.

205. **Goldenberg RL, Andrews WW, Goepfert AR, Faye-Petersen O, Cliver SP, Carlo WA, Hauth JC.** 2008. The Alabama Preterm Birth Study: umbilical cord blood

Ureaplasma urealyticum and *Mycoplasma hominis* cultures in very preterm newborn infants. Am J Obstet Gynecol **198:**43.e41-45.

206. **Viscardi RM, Hashmi N, Gross GW, Sun CC, Rodriguez A, Fairchild KD.** 2008. Incidence of invasive ureaplasma in VLBW infants: relationship to severe intraventricular hemorrhage. J Perinatol **28:**759-765.

207. **Colaizy TT, Morris CD, Lapidus J, Sklar RS, Pillers DA.** 2007. Detection of ureaplasma DNA in endotracheal samples is associated with bronchopulmonary dysplasia after adjustment for multiple risk factors. Pediatr Res **61:**578-583.

208. **Kotecha S, Hodge R, Schaber JA, Miralles R, Silverman M, Grant WD.** 2004. Pulmonary *Ureaplasma urealyticum* is associated with the development of acute lung inflammation and chronic lung disease in preterm infants. Pediatr Res **55:**61-68.

209. **Fonseca LT, Silveira RC, Procianoy RS.** 2011. Ureaplasma bacteremia in very low birth weight infants in Brazil. Pediatr Infect Dis J **30:**1052-1055.

210. **Quinn PA, Gillan JE, Markestad T, St John MA, Daneman A, Lie KI, Li HC, Czegledy-Nagy E, Klein A.** 1985. Intrauterine infection with *Ureaplasma urealyticum* as a cause of fatal neonatal pneumonia. Pediatr Infect Dis **4:**538-543.

211. **Madan E, Meyer MP, Amortegui AJ.** 1988. Isolation of genital mycoplasmas and *Chlamydia trachomatis* in stillborn and neonatal autopsy material. Arch Pathol Lab Med **112:**749-751.

212. **Ollikainen J, Hiekkaniemi H, Korppi M, Katila ML, Heinonen K.** 1993. *Ureaplasma urealyticum* cultured from brain tissue of preterm twins who died of intraventricular hemorrhage. Scand J Infect Dis **25:**529-531.

213. **Quinn PA.** 1986. Evidence of an immune response to *Ureaplasma urealyticum* in perinatal morbidity and mortality. Pediatr Infect Dis **5:**S282-287.

214. **Horowitz S, Mazor M, Horowitz J, Porath A, Glezerman M.** 1995. Antibodies to *Ureaplasma urealyticum* in women with intraamniotic infection and adverse pregnancy outcome. Acta Obstet Gynecol Scand **74:**132-136.

215. **Power CP, Wang JH, Manning B, Kell MR, Aherne NJ, Wu QD, Redmond HP.** 2004. Bacterial lipoprotein delays apoptosis in human neutrophils through inhibition of caspase-3 activity: regulatory roles for CD14 and TLR-2. J Immunol **173:**5229-5237.

216. **Espinoza J, Chaiworapongsa T, Romero R, Gomez R, Kim JC, Yoshimatsu J, Edwin S, Rathnasabapathy C, Yoon BH.** 2002. Evidence of participation of soluble CD14 in the host response to microbial invasion of the amniotic cavity and intra-amniotic inflammation in term and preterm gestations. J Matern Fetal Neonatal Med **12:**304-312.

217. **Collins JJ, Kallapur SG, Knox CL, Kemp MW, Kuypers E, Zimmermann LJ, Newnham JP, Jobe AH, Kramer BW.** 2013. Repeated intrauterine exposures to inflammatory stimuli attenuated transforming growth factor-beta signaling in the ovine fetal lung. Neonatology **104:**49-55.

218. **Wolfs TG, Kallapur SG, Knox CL, Thuijls G, Nitsos I, Polglase GR, Collins JJ, Kroon E, Spierings J, Shroyer NF, Newnham JP, Jobe AH, Kramer BW.** 2013. Antenatal ureaplasma infection impairs development of the fetal ovine gut in an IL-1-dependent manner. Mucosal Immunol **6:**547-556.

219. **Josefowicz SZ, Lu LF, Rudensky AY.** 2012. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol **30:**531-564.

220. **Saada AB, Terespolski Y, Adoni A, Kahane I.** 1991. Adherence of *Ureaplasma urealyticum* to human erythrocytes. Infect Immun **59:**467-469.

221. **Padmini E, Uthra V.** 2012. Role of *Ureaplasma urealyticum* in altering the endothelial metal concentration during preeclampsia. Placenta **33:**304-311.

222. **Smith DG, Russell WC, Thirkell D.** 1994. Adherence of *Ureaplasma urealyticum* to human epithelial cells. Microbiology **140 (Pt 10):**2893-2898.

223. **Lingwood CA, Quinn PA, Wilansky S, Nutikka A, Ruhnke HL, Miller RB.** 1990. Common sulfoglycolipid receptor for mycoplasmas involved in animal and human infertility. Biol Reprod **43:**694-697.

224. **Allam AB, Alvarez S, Brown MB, Reyes L.** 2011. *Ureaplasma parvum* infection alters filamin A dynamics in host cells. BMC Infect Dis **11:**101.

225. **Malathi K, Siddiqui MA, Dayal S, Naji M, Ezelle HJ, Zeng C, Zhou A, Hassel BA.** 2014. RNase L interacts with Filamin A to regulate actin dynamics and barrier function for viral entry. MBio **5:**e02012.

226. **Li YH, Chen M, Brauner A, Zheng C, Skov Jensen J, Tullus K.** 2002. *Ureaplasma urealyticum* induces apoptosis in human lung epithelial cells and macrophages. Biol Neonate **82:**166-173.

227. **Xiao L, Crabb DM, Dai Y, Chen Y, Waites KB, Atkinson TP.** 2014. Suppression of antimicrobial peptide expression by *Ureaplasma* species. Infect Immun **82:**1657-1665.

228. **Glass JI, Lefkowitz EJ, Glass JS, Heiner CR, Chen EY, Cassell GH.** 2000. The complete sequence of the mucosal pathogen *Ureaplasma urealyticum*. Nature **407:**757-762.

229. **Triantafilou M, De Glanville B, Aboklaish AF, Spiller OB, Kotecha S, Triantafilou K.** 2013. Synergic activation of toll-like receptor (TLR) 2/6 and 9 in response to *Ureaplasma parvum* & *urealyticum* in human amniotic epithelial cells. PLoS One **8:**e61199.

230. **Shimizu T, Kida Y, Kuwano K.** 2008. *Ureaplasma parvum* lipoproteins, including MB antigen, activate NF-kappa B through TLR1, TLR2 and TLR6. Microbiology **154:**1318-1325.

231. **Uchida K, Nakahira K, Mimura K, Shimizu T, De Seta F, Wakimoto T, Kawai Y, Nomiyama M, Kuwano K, Guaschino S, Yanagihara I.** 2013. Effects of *Ureaplasma parvum* lipoprotein multiple-banded antigen on pregnancy outcome in mice. J Reprod Immunol **100:**118-127.

232. **Zheng X, Teng LJ, Watson HL, Glass JI, Blanchard A, Cassell GH.** 1995. Small repeating units within the *Ureaplasma urealyticum* MB antigen gene encode serovar specificity and are associated with antigen size variation. Infect Immun **63:**891-898.

233. **Zheng X, Lau K, Frazier M, Cassell GH, Watson HL.** 1996. Epitope mapping of the variable repetitive region with the MB antigen of *Ureaplasma urealyticum*. Clin Diagn Lab Immunol **3:**774-778.

234. **Monecke S, Helbig JH, Jacobs E.** 2003. Phase variation of the multiple banded protein in *Ureaplasma urealyticum* and *Ureaplasma parvum*. Int J Med Microbiol **293:**203-211.

235. **Zheng X, Watson HL, Waites KB, Cassell GH.** 1992. Serotype diversity and antigen variation among invasive isolates of *Ureaplasma urealyticum* from neonates. Infect Immun **60:**3472-3474.

236. **Watson HL, Blalock DK, Cassell GH.** 1990. Variable antigens of *Ureaplasma urealyticum* containing both serovar-specific and serovar-cross-reactive epitopes. Infect Immun **58:**3679-3688.

237. **Zheng X, Teng LJ, Glass JI, Blanchard A, Cao Z, Kempf MC, Watson HL, Cassell GH.** 1994. Size variation of a major serotype-specific antigen of *Ureaplasma urealyticum*. Ann N Y Acad Sci **730:**299-301.

238. **Zimmerman CU, Stiedl T, Rosengarten R, Spergser J.** 2009. Alternate phase variation in expression of two major surface membrane proteins (MBA and UU376) of *Ureaplasma parvum* serovar 3. FEMS Microbiol Lett **292:**187-193.

239. **Zimmerman CU, Stiedl T, Spergser J, Rosengarten R.** 2014. A simple agar plate preparation for effective transfer of ureaplasma colonies onto nitrocellulose membranes for colony immunoblotting. J Microbiol Methods **104:**79-81.

240. **Zimmerman CU, Rosengarten R, Spergser J.** 2011. Ureaplasma antigenic variation beyond MBA phase variation: DNA inversions generating chimeric structures and switching in expression of the MBA N-terminal paralogue UU172. Mol Microbiol **79:**663-676.

241. **Zimmerman CU, Rosengarten R, Spergser J.** 2013. Interaction of the putative tyrosine recombinases RipX (UU145), XerC (UU222), and CodV (UU529) of *Ureaplasma parvum* serovar 3 with specific DNA. FEMS Microbiol Lett **340:**55-64.

242. **Zimmerman CU, Herrmann R, Rosengarten R.** 2015. XerC-mediated DNA inversion at the inverted repeats of the UU172-phase-variable element of *Ureaplasma parvum* serovar 3. Microbiol Res **170:**263-269.

243. **Istivan TS, Coloe PJ.** 2006. Phospholipase A in Gram-negative bacteria and its role in pathogenesis. Microbiology **152:**1263-1274.

244. **De Silva NS, Quinn PA.** 1986. Endogenous activity of phospholipases A and C in *Ureaplasma urealyticum*. J Clin Microbiol **23:**354-359.

245. **De Silva NS, Quinn PA.** 1991. Localization of endogenous activity of phospholipases A and C in *Ureaplasma urealyticum*. J Clin Microbiol **29:**1498-1503.

246. **DeSilva NS, Quinn PA.** 1999. Characterization of phospholipase A1, A2, C activity in *Ureaplasma urealyticum* membranes. Mol Cell Biochem **201:**159-167.

247. **Janeway J, C. A, Travers P, Walport M, Schlochik MJ.** 2001. Immunobiology: the immune system in health and disease, 5th ed. Garland Science, New York.

248. **Robertson JA, Stemler ME, Stemke GW.** 1984. Immunoglobulin A protease activity of *Ureaplasma urealyticum*. J Clin Microbiol **19:**255-258.

249. **Kilian M, Brown MB, Brown TA, Freundt EA, Cassell GH.** 1984. Immunoglobulin A1 protease activity in strains of *Ureaplasma urealyticum*. Acta Pathol Microbiol Immunol Scand B **92:**61-64.

250. **Kilian M, Freundt EA.** 1984. Exclusive occurrence of an extracellular protease capable of cleaving the hinge region of human immunoglobulin A1 in strains of *Ureaplasma urealyticum*. Isr J Med Sci **20:**938-941.

251. **Arfi Y, Minder L, Di Primo C, Le Roy A, Ebel C, Coquet L, Claverol S, Vashee S, Jores J, Blanchard A, Sirand-Pugnet P.** 2016. MIB-MIP is a mycoplasma system that captures and cleaves immunoglobulin G. Proc Natl Acad Sci U S A **113:**5406-5411.

252. **Purcell RH, Taylor-Robinson D, Wong D, Chanock RM.** 1966. Color test for the measurement of antibody to T-strain mycoplasmas. J Bacteriol **92:**6-12.

253. **Neyrolles O, Ferris S, Behbahani N, Montagnier L, Blanchard A.** 1996. Organization of *Ureaplasma urealyticum* urease gene cluster and expression in a suppressor strain of *Escherichia coli*. J Bacteriol **178:**2725.

254. **Blanchard A, Razin S, Kenny GE, Barile MF.** 1988. Characteristics of *Ureaplasma urealyticum* urease. J Bacteriol **170:**2692-2697.

255. **Takebe S, Numata A, Kobashi K.** 1984. Stone formation by *Ureaplasma urealyticum* in human urine and its prevention by urease inhibitors. J Clin Microbiol **20:**869-873.

256. **Mobley HL, Island MD, Hausinger RP.** 1995. Molecular biology of microbial ureases. Microbiol Rev **59:**451-480.

257. **Ligon JV, Kenny GE.** 1991. Virulence of ureaplasmal urease for mice. Infect Immun **59:**1170-1171.

258. **Williams LE, Wernegreen JJ.** 2010. Unprecedented loss of ammonia assimilation capability in a urease-encoding bacterial mutualist. BMC Genomics **11:**687.

259. **Bharat A, Cunningham SA, Scott Budinger GR, Kreisel D, DeWet CJ, Gelman AE, Waites K, Crabb D, Xiao L, Bhorade S, Ambalavanan N, Dilling DF, Lowery EM, Astor T, Hachem R, Krupnick AS, DeCamp MM, Ison MG, Patel R.** 2015. Disseminated ureaplasma infection as a cause of fatal hyperammonemia in humans. Sci Transl Med **7:**284re283.

260. **Rogers MJ, Simmons J, Walker RT, Weisburg WG, Woese CR, Tanner RS, Robinson IM, Stahl DA, Olsen G, Leach RH, et al.** 1985. Construction of the mycoplasma evolutionary tree from 5S rRNA sequence data. Proc Natl Acad Sci U S A **82:**1160-1164.

261. **Woese CR, Stackebrandt E, Ludwig W.** 1984. What are mycoplasmas: the relationship of tempo and mode in bacterial evolution. J Mol Evol **21:**305-316.

262. **Dando SJ, Nitsos I, Polglase GR, Newnham JP, Jobe AH, Knox CL.** 2014. *Ureaplasma parvum* undergoes selection in utero resulting in genetically diverse isolates colonizing the chorioamnion of fetal sheep. Biol Reprod **90:**27.

263. **Yudin MH, van Schalkwyk J, Van Eyk N, Boucher M, Castillo E, Cormier B, Gruslin A, Money DM, Murphy K, Ogilvie G, Paquet C, Steenbeek A, Wong T, Gagnon R, Hudon L, Basso M, Bos H, Delisle MF, Farine D, Grabowska K, Menticoglou S, Mundle WR, Murphy-Kaulbeck LC, Ouellet A, Pressey T,**

Roggensack A. 2009. Antibiotic therapy in preterm premature rupture of the membranes. J Obstet Gynaecol Can **31:**863-867, 868-874.

264. **Lamont RF, Jaggat AN.** 2007. Emerging drug therapies for preventing spontaneous preterm labor and preterm birth. Expert Opin Investig Drugs **16:**337-345.

265. **Morency AM, Bujold E.** 2007. The effect of second-trimester antibiotic therapy on the rate of preterm birth. J Obstet Gynaecol Can **29:**35-44.

266. **Waites KB, Katz B, Schelonka RL.** 2005. Mycoplasmas and ureaplasmas as neonatal pathogens. Clin Microbiol Rev **18:**757-789.

267. **Beeton ML, Chalker VJ, Jones LC, Maxwell NC, Spiller OB.** 2016. Antibiotic resistance among clinical ureaplasma isolates recovered from neonates in England and Wales between 2007 and 2013. Antimicrob Agents Chemother **60:**52-56.

268. **Fernandez J, Karau MJ, Cunningham SA, Greenwood-Quaintance KE, Patel R.** 2016. Antimicrobial susceptibility and clonality of clinical ureaplasma isolates in the United States. Antimicrob Agents Chemother **60:**4793-4798.

269. **Song J, Qiao Y, Kong Y, Ruan Z, Huang J, Song T, Zhang J, Xie X.** 2015. Frequent topoisomerase IV mutations associated with fluoroquinolone resistance in *Ureaplasma* species. J Med Microbiol **64:**1315-1320.

270. **Xiao L, Crabb DM, Duffy LB, Paralanov V, Glass JI, Waites KB.** 2012. Chromosomal mutations responsible for fluoroquinolone resistance in *Ureaplasma* species in the United States. Antimicrob Agents Chemother **56:**2780-2783.

271. **Xiao L, Crabb DM, Duffy LB, Paralanov V, Glass JI, Hamilos DL, Waites KB.** 2011. Mutations in ribosomal proteins and ribosomal RNA confer macrolide resistance in human *Ureaplasma* spp. Int J Antimicrob Agents **37:**377-379.

272. **Kenyon SL, Taylor DJ, Tarnow-Mordi W.** 2001. Broad-spectrum antibiotics for preterm, prelabour rupture of fetal membranes: the ORACLE I randomised trial. ORACLE Collaborative Group. Lancet **357:**979-988.

273. **Kenyon S, Boulvain M, Neilson J.** 2001. Antibiotics for preterm premature rupture of membranes. Cochrane Database Syst Rev doi:10.1002/14651858.cd001058**:**Cd001058.

274. **Kenyon S, Boulvain M, Neilson J.** 2004. Antibiotics for preterm rupture of the membranes: a systematic review. Obstet Gynecol **104:**1051-1057.

275. **Gomez R, Romero R, Nien JK, Medina L, Carstens M, Kim YM, Espinoza J, Chaiworapongsa T, Gonzalez R, Iams JD, Rojas I.** 2007. Antibiotic administration to patients with preterm premature rupture of membranes does not eradicate intra-amniotic infection. J Matern Fetal Neonatal Med **20:**167-173.

276. **Mazor M, Chaim W, Horowitz S, Leiberman JR, Glezerman M.** 1993. Successful treatment of preterm labour by eradication of *Ureaplasma urealyticum* with erythromycin. Arch Gynecol Obstet **253:**215-218.

277. **Berg TG, Philpot KL, Welsh MS, Sanger WG, Smith CV.** 1999. Ureaplasma/mycoplasma-infected amniotic fluid: pregnancy outcome in treated and nontreated patients. J Perinatol **19:**275-277.

278. **Heikkinen T, Laine K, Neuvonen PJ, Ekblad U.** 2000. The transplacental transfer of the macrolide antibiotics erythromycin, roxithromycin and azithromycin. Bjog **107:**770-775.

279. **Philipson A, Sabath LD, Charles D.** 1973. Transplacental passage of erythromycin and clindamycin. N Engl J Med **288:**1219-1221.

280. **Keelan JA, Nitsos I, Saito M, Musk GC, Kemp MW, Timmins M, Li S, Yaegashi N, Newnham JP.** 2011. Maternal-amniotic-fetal distribution of macrolide antibiotics

following intravenous, intramuscular, and intraamniotic administration in late pregnant sheep. Am J Obstet Gynecol **204:**546.e510-547.

281. **Kemp MW, Miura Y, Payne MS, Watts R, Megharaj S, Jobe AH, Kallapur SG, Saito M, Spiller OB, Keelan JA, Newnham JP.** 2014. Repeated maternal intramuscular or intraamniotic erythromycin incompletely resolves intrauterine *Ureaplasma parvum* infection in a sheep model of pregnancy. Am J Obstet Gynecol **211:**134.e131-139.

282. **Ramsey PS, Vaules MB, Vasdev GM, Andrews WW, Ramin KD.** 2003. Maternal and transplacental pharmacokinetics of azithromycin. Am J Obstet Gynecol **188:**714-718.

283. **Miura Y, Payne MS, Keelan JA, Noe A, Carter S, Watts R, Spiller OB, Jobe AH, Kallapur SG, Saito M, Stock SJ, Newnham JP, Kemp MW.** 2014. Maternal intravenous treatment with either azithromycin or solithromycin clears *Ureaplasma parvum* from the amniotic fluid in an ovine model of intrauterine infection. Antimicrob Agents Chemother **58:**5413-5420.

284. **Acosta EP, Grigsby PL, Larson KB, James AM, Long MC, Duffy LB, Waites KB, Novy MJ.** 2014. Transplacental transfer of azithromycin and its use for eradicating intra-amniotic ureaplasma infection in a primate model. J Infect Dis **209:**898-904.

285. **Grigsby PL, Novy MJ, Sadowsky DW, Morgan TK, Long M, Acosta E, Duffy LB, Waites KB.** 2012. Maternal azithromycin therapy for ureaplasma intraamniotic infection delays preterm delivery and reduces fetal lung injury in a primate model. Am J Obstet Gynecol **207:**475.e471-475.e414.

286. **Keelan JA, Kemp MW, Payne MS, Johnson D, Stock SJ, Saito M, Fernandes P, Newnham JP.** 2014. Maternal administration of solithromycin, a new, potent, broad-

spectrum fluoroketolide antibiotic, achieves fetal and intra-amniotic antimicrobial protection in a pregnant sheep model. Antimicrob Agents Chemother **58:**447-454.

287. **Furfaro LL, Spiller OB, Keelan JA, Payne MS.** 2015. *In vitro* activity of solithromycin and its metabolites, CEM-214 and N-acetyl-CEM-101, against 100 clinical *Ureaplasma* spp. isolates compared with azithromycin. Int J Antimicrob Agents **46:**319-324.

288. **Waites KB, Crabb DM, Duffy LB.** 2009. Comparative *in vitro* susceptibilities of human mycoplasmas and ureaplasmas to a new investigational ketolide, CEM-101. Antimicrob Agents Chemother **53:**2139-2141.

289. **Farrell DJ, Flamm RK, Sader HS, Jones RN.** 2016. Results from the Solithromycin International Surveillance Program (2014). Antimicrob Agents Chemother **60:**3662-3668.

290. **Putnam SD, Castanheira M, Moet GJ, Farrell DJ, Jones RN.** 2010. CEM-101, a novel fluoroketolide: antimicrobial activity against a diverse collection of Gram-positive and Gram-negative bacteria. Diagn Microbiol Infect Dis **66:**393-401.

291. **Putnam SD, Sader HS, Farrell DJ, Biedenbach DJ, Castanheira M.** 2011. Antimicrobial characterisation of solithromycin (CEM-101), a novel fluoroketolide: activity against staphylococci and enterococci. Int J Antimicrob Agents **37:**39-45.

292. **Golparian D, Fernandes P, Ohnishi M, Jensen JS, Unemo M.** 2012. *In vitro* activity of the new fluoroketolide solithromycin (CEM-101) against a large collection of clinical *Neisseria gonorrhoeae* isolates and international reference strains, including those with high-level antimicrobial resistance: potential treatment option for gonorrhea? Antimicrob Agents Chemother **56:**2739-2742.

293. **Rodgers W, Frazier AD, Champney WS.** 2013. Solithromycin inhibition of protein synthesis and ribosome biogenesis in *Staphylococcus aureus*, *Streptococcus*

pneumoniae, and *Haemophilus influenzae*. Antimicrob Agents Chemother **57:**1632-1637.

294. **Hassanein SM, El-Farrash RA, Hafez HM, Hassanin OM, Abd El Rahman NA.** 2012. Cord blood interleukin-6 and neonatal morbidities among preterm infants with PCR-positive *Ureaplasma urealyticum*. J Matern Fetal Neonatal Med **25:**2106-2110.

295. **Gray DJ, Robinson HB, Malone J, Thomson RB, Jr.** 1992. Adverse outcome in pregnancy following amniotic fluid isolation of *Ureaplasma urealyticum*. Prenat Diagn **12:**111-117.

296. **Yoon BH, Romero R, Moon J, Chaiworapongsa T, Espinoza J, Kim YM, Edwin S, Kim JC, Camacho N, Bujold E, Gomez R.** 2003. Differences in the fetal interleukin-6 response to microbial invasion of the amniotic cavity between term and preterm gestation. J Matern Fetal Neonatal Med **13:**32-38.

297. **Kacerovsky M, Musilova I, Hornychova H, Kutova R, Pliskova L, Kostal M, Jacobsson B.** 2014. Bedside assessment of amniotic fluid interleukin-6 in preterm prelabor rupture of membranes. Am J Obstet Gynecol **211:**385.e381-389.

298. **Romero R, Miranda J, Chaemsaithong P, Chaiworapongsa T, Kusanovic JP, Dong Z, Ahmed AI, Shaman M, Lannaman K, Yoon BH, Hassan SS, Kim CJ, Korzeniewski SJ, Yeo L, Kim YM.** 2015. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med **28:**1394-1409.

299. **Stepan M, Cobo T, Maly J, Navratilova M, Musilova I, Hornychova H, Jacobsson B, Kacerovsky M.** 2016. Neonatal outcomes in subgroups of women with preterm prelabor rupture of membranes before 34 weeks. J Matern Fetal Neonatal Med **29:**2373-2377.

300. **Musilova I, Kutova R, Pliskova L, Stepan M, Menon R, Jacobsson B, Kacerovsky M.** 2015. Intraamniotic inflammation in women with preterm prelabor rupture of membranes. PLoS One **10:**e0133929.

301. **Stepan M, Cobo T, Musilova I, Hornychova H, Jacobsson B, Kacerovsky M.** 2016. Maternal serum c-reactive protein in women with preterm prelabor rupture of membranes. PLoS One **11:**e0150217.

302. **Berger A, Witt A, Haiden N, Kaider A, Klebermasz K, Fuiko R, Langgartner M, Pollak A.** 2009. Intrauterine infection with *Ureaplasma* species is associated with adverse neuromotor outcome at 1 and 2 years adjusted age in preterm infants. J Perinat Med **37:**72-78.

303. **Stein A, Boubli L, Blanc B, Raoult D.** 1994. Genital mycoplasmas in placental infections. Infect Dis Obstet Gynecol **1:**275-281.

304. **Van Marter LJ, Dammann O, Allred EN, Leviton A, Pagano M, Moore M, Martin C.** 2002. Chorioamnionitis, mechanical ventilation, and postnatal sepsis as modulators of chronic lung disease in preterm infants. J Pediatr **140:**171-176.

305. **Miralles R, Hodge R, McParland PC, Field DJ, Bell SC, Taylor DJ, Grant WD, Kotecha S.** 2005. Relationship between antenatal inflammation and antenatal infection identified by detection of microbial genes by polymerase chain reaction. Pediatr Res **57:**570-577.

306. **Olomu IN, Hecht JL, Onderdonk AO, Allred EN, Leviton A.** 2009. Perinatal correlates of *Ureaplasma urealyticum* in placenta parenchyma of singleton pregnancies that end before 28 weeks of gestation. Pediatrics **123:**1329-1336.

307. **Kundsin RB, Driscoll SG, Monson RR, Yeh C, Biano SA, Cochran WD.** 1984. Association of *Ureaplasma urealyticum* in the placenta with perinatal morbidity and mortality. N Engl J Med **310:**941-945.

308. **Benirschke K, Burton, G. J., Baergen, R. N.** 2012. Anatomy and pathology of the placental membranes, p 249-307. *In* Benirschke K, Burton, G. J., Baergen, R. N. (ed), Pathology of the Human Placenta, 6th ed. Springer, Heidleberg.

309. **Calvin SE, Oyen ML.** 2007. Microstructure and mechanics of the chorioamnion membrane with an emphasis on fracture properties. Ann N Y Acad Sci **1101:**166-185.

310. **Bourne GL.** 1966. The anatomy of the human amnion and chorion. Proc R Soc Med **59:**1127-1128.

311. **Niknejad H, Yazdanpanah G, Ahmadiani A.** 2016. Induction of apoptosis, stimulation of cell-cycle arrest and inhibition of angiogenesis make human amnion-derived cells promising sources for cell therapy of cancer. Cell Tissue Res **363:**599-608.

312. **Chaemsaithong P, Romero R, Korzeniewski SJ, Martinez-Varea A, Dong Z, Yoon BH, Hassan SS, Chaiworapongsa T, Yeo L.** 2016. A point of care test for interleukin-6 in amniotic fluid in preterm prelabor rupture of membranes: a step toward the early treatment of acute intra-amniotic inflammation/infection. J Matern Fetal Neonatal Med **29:**360-367.

313. **Romero R, Kadar N, Miranda J, Korzeniewski SJ, Schwartz AG, Chaemsaithong P, Rogers W, Soto E, Gotsch F, Yeo L, Hassan SS, Chaiworapongsa T.** 2014. The diagnostic performance of the Mass Restricted (MR) score in the identification of microbial invasion of the amniotic cavity or intra-amniotic inflammation is not superior to amniotic fluid interleukin-6. J Matern Fetal Neonatal Med **27:**757-769.

314. **Kim KW, Romero R, Park HS, Park CW, Shim SS, Jun JK, Yoon BH.** 2007. A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic

1951 inflammation in women with preterm premature rupture of membranes. Am J Obstet
1952 Gynecol **197:**292.e291-295.
1953 315. **Romero R, Chaemsaithong P, Korzeniewski SJ, Tarca AL, Bhatti G, Xu Z,**
1954 **Kusanovic JP, Dong Z, Docheva N, Martinez-Varea A, Yoon BH, Hassan SS,**
1955 **Chaiworapongsa T, Yeo L.** 2016. Clinical chorioamnionitis at term II: the intra-
1956 amniotic inflammatory response. J Perinat Med **44:**5-22.
1957 316. **Kim SM, Romero R, Park JW, Oh KJ, Jun JK, Yoon BH.** 2015. The relationship
1958 between the intensity of intra-amniotic inflammation and the presence and severity of
1959 acute histologic chorioamnionitis in preterm gestation. J Matern Fetal Neonatal Med
1960 **28:**1500-1509.
1961 317. **Jacobsson B, Holst RM, Andersson B, Hagberg H.** 2005. Monocyte chemotactic
1962 protein-2 and -3 in amniotic fluid: relationship to microbial invasion of the amniotic
1963 cavity, intra-amniotic inflammation and preterm delivery. Acta Obstet Gynecol Scand
1964 **84:**566-571.
1965 318. **Oh KJ, Park KH, Kim SN, Jeong EH, Lee SY, Yoon HY.** 2011. Predictive value of
1966 intra-amniotic and serum markers for inflammatory lesions of preterm placenta.
1967 Placenta **32:**732-736.
1968 319. **Bhat G, Peltier MR, Syed TA, Drobek CO, Saade G, Menon R.** 2013. Fetal
1969 membrane biomarker network diversity and disease functions induced by intra-
1970 amniotic pathogens. Am J Reprod Immunol **69:**124-133.
1971 320. **Gervasi MT, Romero R, Bracalente G, Erez O, Dong Z, Hassan SS, Yeo L, Yoon**
1972 **BH, Chaiworapongsa T.** 2012. Midtrimester amniotic fluid concentrations of
1973 interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity
1974 of intra-amniotic inflammation and associations with spontaneous early (<32 weeks)
1975 and late (>32 weeks) preterm delivery. J Perinat Med **40:**329-343.

321. **Liu Y, Liu Y, Du C, Zhang R, Feng Z, Zhang J.** 2016. Diagnostic value of amniotic fluid inflammatory biomarkers for subclinical chorioamnionitis. Int J Gynaecol Obstet **134:**160-164.

322. **Myntti T, Rahkonen L, Tikkanen M, Patari-Sampo A, Paavonen J, Stefanovic V.** 2016. Amniotic fluid rapid biomarkers are associated with intra-amniotic infection in preterm pregnancies regardless of the membrane status. J Perinatol.

AUTHOR BIOS:

Emma L. Sweeney:

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. Emma 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 breastmilk and neonatal saliva combine. Emma has worked on the topic of *Ureaplasma* spp. for six 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:

Samantha J. Dando received her PhD 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, intraamniotic ureaplasma

infections. Samantha 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, Samantha's research focuses on characterizing myeloid cell populations within various sub-compartments of the eye and brain, and how these cells respond to systemic inflammatory mediators. Samantha 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:

Suhas G. Kallapur received his Bachelor in medicine (MBBS) and a doctorate in medicine (MD) degree from the Bombay University, India. He then completed a residency in Pediatrics at the 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, March of Dimes, and 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 world-wide. *Ureaplasma* species most commonly cause perinatal infections, and Dr. Kallapur has collaborated with co-authors and others to create sheep and Rhesus macaque models of intrauterine infection and inflammation.

Christine L. Knox:

2022 Christine L. Knox obtained her PhD in 1998 from the Queensland University of Technology
2023 (QUT), where she pioneered the study at QUT of the *Ureaplasma* species and their role in
2024 adverse pregnancy outcomes. As an Associate Professor she now leads the Reproductive
2025 Health Research Group at QUT and was the principal PhD supervisor for Samantha Dando
2026 and Emma Sweeney, the joint co-authors of this review. Funding from the National Institute
2027 of Health and the National Health and Medical Research Council, Australia has enabled this
2028 group to further investigate the pathogenesis of *Ureaplasma* spp. in pregnant women
2029 delivering late preterm and at term, and in an ovine model of intraamniotic infection.
2030 Christine Knox is an appointed member of the ´International Subcommittee for the
2031 Taxonomy of *Mollicutes'* and in 2016 she was the Chair of the local organizing committee of
2032 the 21st Congress of the International Organization for Mycoplasmology.

2033

FIGURE LEGENDS

Figure 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 $F2_{\alpha}$, 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; TLRs = Toll-like receptors. The direction of the black arrows represents either an increase or decrease in expression.

Figure 2: Differences in the presence of chorioamnionitis in *Ureaplasma* spp.-infected women.

Hematoxylin and eosin stained chorioamnion tissue demonstrates that some women whose placentae are colonized with *Ureaplasma* spp. have no evidence of chorioamnionitis (panels

2058 A & B), whilst other women have mild/moderate (panels C & D) or severe (panels E & F)
2059 evidence of inflammation (demonstrated by neutrophil influx, arrows) within their
2060 chorioamnion, despite high numbers of *Ureaplasma* spp. present within the tissue. Images are
2061 shown at x200 (A, C, E) and x400 (B, D, F) total magnification; boxed areas in A, C and E
2062 are shown in B, D and F respectively.

Author	Gestational age (GA) in weeks	Specimen	n =	Incidence of *Ureaplasma* spp. infection	Incidence of polymicrobial infections	*Ureaplasma* spp. with chorioamnionitis	*Ureaplasma* spp. without chorioamnionitis	Reference
Viscardi *et al.* (2008)	< 33	S/CSF	313	74/313 (23.6%)	- [a]	30/46 (65.0%)	16/46 (35.0%)	(206)
Hassanein *et al.* (2012)	< 35	CB	30	13/30 (43.3%)	no polymicrobial infections	7/13 (53.8%)	6/13 (46.2%)	(294)
Gray *et al.* (1992)	< 28	AF	2461	8/2461 (0.4%)	- [b]	8/8 (100.0%)	0/8 (0.0%)	(295)
Yoon *et al.* (1998)	≤ 36	AF	120	25/120 (20.8%)	11/120 (9.0%)	5/25 (20.0%)	-	(44)
Yoon *et al.* (2003)	≤ 35	AF	252	23/252 (9.1%)	- [c]	-	-	(296)
Park *et al.* (2013)	< 34	AF	56	35/56 (62.5%)	7/56 (12.5%)	26/47 (55.31%) [#]	0/3 (0.0%)	(120)
Kacerovsky *et al.* (2014)	24 – 36	AF	124	26/124 (21.0%)	5/124 (4.0%) [d]	-	-	(297)
Romero *et al.* (2015)	≤ 35	AF	59	6/24 (25.0%)	10/24 (41.7%)	3/6 (50.0%)	2/6 (33.3%) [#]	(298)
Stepan *et al.* (2016)	24 - 34	AF	122	33/122 (27.0%)	8/122 (6.6%)	29/33 (87.9%)	4/33 (12.1%)	(299)
Musilova *et al.* (2015)	24 – 36	AF	166	40/166 (24.1%)	19/166 (11.4%)	26/40 (65.0%)	14/40 (35.0%)	(300)

Stepan *et al.* (2016)	24 – 36	AF	386	103/386 (26.7%)	32/386 (8.3%)	70/103 (68.0%) [#]	16/103 (15.5%) [#]	(301)
Berger *et al.* (2009)	≤ 33	AF/PL	114	32/114 (28.1%)	- [a]	11/25 (44.0%) [#]	14/25 (66.0%) [#]	(302)
Hillier *et al.* (1988)	< 37	PL	112	32/112 (28.6%)	- [c]	19/29 (65.5%) [#]	10/65 (15.4%) [#]	(1)
Stein *et al.* (1994)	Any GA	PL	182	21/182 (11.5%)	- [e]	11/16[#]	5/16[#]	(303)
Van Marter *et al.* (2002)	< 36	PL	206	58/155 (37.4%)	- [e]	51/77 (66.2%)	7/78 (9.0%)	(304)
Miralles *et al.* (2005)	< 33	PL	14	5/14 (35.7%)	5/14 (35.7%)	4/5 (80.0%)	1/5 (20.0%)	(305)
Egawa *et al.* (2007)	< 32	PL	83	4 (4.8%)	5/83 (6.0%) [b]	4/4 (100.0%)	0/4 (0.0%)	(119)
Olomu *et al.* (2009)	< 28	PL	866	52/866 (6.0%)	21/52 (40.4%)	34/52 (65.4%)	18/52 (34.6%)	(306)
Kasper *et al.* (2010)	< 34	AF	118	32/118 (27.1%)	- [a]	5/19 (26.3%) [#]	14/19 (73.7%) [#]	(186)
Namba *et al.* (2010)	≤ 32	PL	151	63/151 (41.7%)	13/151 (8.6%)	52/63 (82.5%)	11/63 (17.5%)	(118)
Roberts *et al.* (2012)	> 37	PL	195	2/195 (1.0%)	1/195 (0.5%)	0/2 (0.0%)	2/2 (100.0%)	(4)
Kundsin *et al.*	Various	PL	801	156/801 (19.5%)	18/801 (2.2%) [b]	32/53 (60.4%) [#]	21/53 (39.6%)	(307)

(1984)								
Sweeney *et al.* (2016)	> 32	PL	535	42/535 (7.9%)	4/57 (7.0%)	26/38 (68.4%)	12/38 (31.6%)	(46)
Cox *et al.* (2016)	< 37	PL	57	13/57 (22.8%)	-	9/24 (37.5%)	4/33 (12.1%)	(117)

Table 1. The incidence of *Ureaplasma* spp. infection, polymicrobial infections and chorioamnionitis in women delivering preterm, late preterm or at term. The incidence of chorioamnionitis in *Ureaplasma* spp.-infected women is frequently high, indicating that these microbes are associated with chorioamnionitis. AF = amniotic fluid; CB = cord blood; GA = gestational age; PL = placenta; S = serum.

[a] Only *Ureaplasma* spp. were tested for within 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

[#] not all placentae in study were tested

Box 1: 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 10-12 weeks of gestation (308). The avascular chorioamniotic membranes persist until term in healthy pregnancies and perform critical barrier and container functions (309). 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 (310). The amniotic epithelial cells and mesenchymal cells possess stem cell and immunomodulatory properties, and have shown promising results for use in regenerative medicine (311). The chorion comprises four layers: (i) a cellular, fibroblast layer; (ii) a reticular layer; (iii) a pseudo-basement membrane and (iv) a trophoblast layer (310).

Box 2: 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 (IL)-6, IL-8, matrix metalloproteinase (MMP)-8, MMP-9 and monocyte chemotactic proteins within amniotic fluid are positive predictors of intra-amniotic inflammation and/or clinical chorioamnionitis (297, 312-317); however, these markers may have poor positive predictive values for the detection of sub-clinical, histologic chorioamnionitis and may be variably expressed within the amniotic fluid and fetal membranes during chorioamnionitis (318-320). Recently, Liu *et al*. (321) reported that surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) for the detection of human neutrophil defensins (HNP) -1 and HNP-2, calgranulins A and calgranulins C within amniotic fluid was highly accurate for the diagnosis of sub-clinical chorioamnionitis, but further studies with larger patient cohorts are required to validate these findings. Non-inflammatory markers such as amniotic fluid lactate dehydrogenase and glucose were also recently investigated for the detection of histologic chorioamnionitis (322), but the diagnostic accuracy of these assays was low, suggesting that additional amniotic fluid biomarkers should be investigated for the diagnosis of chorioamnionitis.