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UREAPLASMA UREALYTICUM AND *MYCOPLASMA HOMINIS* IN PREGNANCY AND OBSTETRIC OUTCOME

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SUMMARY

The roles of the genital mycoplasmas *Ureaplasma urealyticum* and *Mycoplasma hominis* in infection-mediated preterm birth, and preterm prelabour rupture of the membranes (PPROM) are reviewed. Studies of vaginal flora during pregnancy and in labour provide conflicting data concerning the possible association between the presence of these microorganisms in the vagina and poor obstetric outcome. However studies of the chorioamnion have clearly established the link between these microorganisms, preterm labour and PPROM. These studies of the chorioamnion show *U. urealyticum* is the most common microorganism found in the chorioamnion and is associated with intrauterine infection, chorioamnionitis, very low birthweight and membrane rupture.

U. urealyticum occurs in increased concentrations in women with bacterial vaginosis but in a prospective study of vaginal flora at 24 weeks gestation there was an additive attributable risk for preterm birth for *U. urealyticum* greater than that due to bacterial vaginosis. However preventative strategies should focus on prevention of bacterial vaginosis.

M. hominis has a lower prevalence in normal vaginal flora (5-10%) than *U. urealyticum* (24-82%), but also occurs in increased concentrations in bacterial vaginosis. Vaginal *M. hominis* in pregnancy is associated with preterm birth, generally in the presence of bacterial vaginosis. It is also a cause of post-partum and post-abortal infection.

Host factors play an important role in determining pregnancy outcome in women with *U. urealyticum* and *M. hominis* ascending infection. Host response studies are required to identify subgroups of women who are at a higher risk of adverse pregnancy outcome, thus enabling strategies for prevention of infection-mediated preterm labour to be formulated. Screening and treatment for *U. urealyticum* and *M. hominis* in pregnancy is not recommended, as their role in preterm birth is linked with the bacterial vaginosis micro-ecology.

INTRODUCTION

Ureaplasma urealyticum and *Mycoplasma hominis* have been associated with infections in pregnancy and labour for more than twenty years, yet their role in infection-mediated preterm birth is still somewhat controversial. Recent

studies have provided further understanding of their pathological role in adverse pregnancy outcome. Preterm labour and delivery has remained constant at 7-10% in the western world, despite significant advances in obstetric medicine, and the aetiology is still not fully understood. With improved neonatal care 85% of very low birthweight babies now survive, however a number have considerable residual morbidity. Evidence is continuing to accumulate that subclinical intrauterine infection is present in a significant proportion of women in preterm labour. Ascending vaginal microorganisms, including the genital mycoplasmas, may cause inflammation and weakening of the membranes, and subsequent invasion of the amniotic fluid. The presence of subclinical infection is associated with increased production of various cytokines in the amniotic fluid, preceding labour. Better understanding of the pathogenesis and aetiology of the genital mycoplasmas in pregnancy and labour may enable strategies for prevention of mycoplasma-associated preterm birth and preterm prelabour rupture of membranes (PPROM) to be implemented.

This is not an exhaustive review of the literature but a brief review to consider evidence for the role of *U. urealyticum* and *M. hominis* in intrauterine infection and preterm birth, and vaginal colonisation as a risk factor.

REVIEW AND DISCUSSION

Mycoplasmas are the smallest free-living, self-replicating organisms known. Twelve species occur in humans, three in the genital tract, *U. urealyticum*, *M. hominis* and *M. genitalium*. *U. urealyticum* and *M. hominis*, known as genital mycoplasmas, colonise the vagina as part of the normal vaginal flora. *M. hominis* and *U. urealyticum* occur in the vagina of 5 to 10 percent and 24 to 82 percent of women of reproductive age respectively (*Carey* et al., 1991; *Cassell* et al., 1993; *McDonald* et al., 1992). There is a close relationship between hormonal status and the occurrence of genital mycoplasmas. *U. urealyticum* prevalence increases with younger age, lower socio-economic status, multiple sexual partners, black ethnicity, and oral contraceptive use (*Eschenbach* 1993). One of the first reports of amniotic fluid infection with genital mycoplasmas was published in 1983 by *Cassell* and co-authors who reported amniotic fluid infection at 16 to 20 weeks' gestation with *M. hominis* and *U. urealyticum* without rupture of the foetal membranes. Since then there have been a number of reports of clinically silent, chronic intrauterine infection with *U. urealyticum* and intact membranes, often during the mid-trimester. The nature and timing of this chronic intrauterine infection appears to be different to that caused by virulent organisms such as group B Streptococcus and *Escherichia coli*, which provoke an intense and clinically apparent chorioamnionitis and rapid onset of labour.

Placental Studies

The recovery of microorganisms from the chorioamnion (i.e. swabs taken aseptically from between the chorionic and the amnionic membranes) is associated with chorioamnionitis (*Hillier* et al., 1988). Microorganisms obtained from this site represent a true infection, not just vaginal contamination (*Eschenbach*, 1993). *U. urealyticum* was isolated from the chorioamnion of 38-66% of women with histological chorioamnionitis and from 13-17% without histological chorioamnionitis (*Hillier* et al., 1988; *Kundsin* et al.,

1984; *Embree* et al., 1980). A variety of microorganisms may be recovered from the chorioamnion including *U. urealyticum* and *M. hominis* but *U. urealyticum* is the most frequent isolate. The recovery of *U. urealyticum* from amniotic fluid was also associated with chorioamnionitis in four of five recent studies (*Gray* et al., 1992; *Horowitz* et al., 1995; *Montuclard* et al., 1996; *Kerki-Nisula* et al., 1997; *Yoon* et al., 1998).

A retrospective four year review of 122 autopsy and placental cultures from spontaneous, unexplained miscarriages and stillbirths between 16 to 26 weeks found that *U. urealyticum* and group B Streptococcus were the most common isolates (*McDonald* and *Chambers*, unpublished results). *U. urealyticum* was more common in the placenta than foetal tissue. In cases with histological chorioamnionitis, *U. urealyticum* was found in 24 percent compared with 8 percent in cases with no such evidence. *U. urealyticum* was also more common in women with ruptured membranes, however it is impossible to know whether this was post rupture invasion of the chorioamnion or whether *U. urealyticum* played a part in weakening and eventual rupture of the membrane. The association of *U. urealyticum* with PPROM in women in the last month of pregnancy in a prospective study of 577 pregnancies (*Jacqui* and *Sedallian*, 1992) and the prospective study of *McDonald* et al. (1992) detailed below, provides further support for this view.

Studies on the relationship between the isolation of genital mycoplasmas from the chorioamnion and low birthweight show that the lower the birthweight the higher the recovery of *U. urealyticum* from the placenta (*Embree* et al., 1980; *Kundsin* et al., 1984). Of six studies looking at isolation of *U. urealyticum* from the placenta and preterm birth, three supported an association between *U. urealyticum* and preterm birth (*Hillier* et al., 1988; *Kundsin* et al., 1984; *Embree* et al., 1980), and three did not (*Naessens* et al., 1989; *Zlatnik* et al., 1990; *Hillier* et al., 1991). The answer to this apparent discrepancy may lie in differing population subgroups in the various studies. In a recent study *U. urealyticum* from the chorioamnion was associated with preterm birth before the 29th week of gestation and with increasing duration of time between rupture of membranes and delivery (*Kundsin* et al., 1996).

Studies of Vaginal Flora During Labour

In several studies vaginal colonisation with *U. urealyticum* and *M. hominis* at time of labour has been associated with preterm birth. Ureaplasmas were isolated from 86% of women who gave birth at less than 34 weeks gestation compared with 46% of a similar gestation who were not in preterm labour, and heavy colonisation with *M. hominis* was detected in 18% compared with 0%, respectively (*Lamont* et al., 1987) (Tables 1 and 2). A recent study by *Abele-Horn* et al. (1997) also showed a significantly higher rate of preterm birth, PPROM and chorioamnionitis in women with *U. urealyticum* in labour. The finding that an association existed between heavy colonisation with *M. hominis* but not the presence of *M. hominis*, and preterm birth (*Lamont* et al., 1987) supports the hypothesis that it is the concentration of *M. hominis* in the vagina that is important rather than just the presence of this organism. However other studies showed no associations between either *U. urealyticum* (*Martius* et al., 1988; *McDonald* et al., 1991) or *M. hominis* (*McDonald* et al., 1991) in labour.

Table 1: Evidence linking vaginal *M. hominis* in pregnancy to obstetric outcome

Reference	Year	Gestation	n	Outcome OR/RR (95%)
Braun et al.	1971	First visit	485	n.s.
Ross et al.	1981	Three visits	162	n.s.
Upadhyaya et al.	1983	First visit	135	n.s.
Harrison et al.	1986	First visit	3,224	n.s.
Lamont et al.	1987	Labour- Heavy colonisation		PTB<34 weeks, p<0.05
Sweet et al.	1987	First visit	3,293	n.s.
Polk et al.	1989	22-30 weeks	801	PTB, RR 2 (90% CI 1.42-2.93)
McGregor et al.	1990	24 weeks	202	PTB, RR 5.1 (1.45-17.9)
Carey et al.	1991	22-26 weeks	4,934	n.s.
McDonald et al.	1992	24 weeks	786	n.s.
Jacqui, Sedallian.	1992	Last month	577	PPE, p<0.05
Divers & Lilford	1993	Meta-analysis		PTB, p<0.05
Germain et al.	1995	23-26 weeks	13,914	IUGR, RR 1.16 (1.04-1.29)
Hillier et al.	1995	23-26 weeks	10,397	BV + *M. hominis* RR 1.6 (1.1-2.3)

Studies of Vaginal Flora During Pregnancy

Given the indications that in at least some women ascending genital tract infection with *U. urealyticum* or *M. hominis* may be a cause of preterm labour and PPROM, the question which must be answered is: "Are women with vaginal colonisation with these organisms during pregnancy at higher risk of adverse pregnancy outcome?" In order to answer this question several studies of vaginal flora in early and mid-pregnancy have investigated possible associations between the microorganisms found in vaginal flora and adverse pregnancy outcome. The mycoplasma findings for some of these studies are listed in Tables 1, and 2.

Following our study of vaginal flora in preterm and term labour, we undertook a prospective study of vaginal flora at 24 weeks gestation to investigate whether the carriage of any particular organism during mid trimester placed a woman at higher risk of preterm birth (*McDonald* et al., 1992). As the causes of preterm birth are multi-factorial it is essential that multiple logistic regression analysis be performed to take account of demographic and obstetric variables known to place a woman at increased risk of preterm birth. Also several studies have only focused on one or a few microorganisms and not taken into account the possible association of other organisms. 786 women were cultured for aerobes, anaerobes and mycoplasmas using three high vaginal swabs at approximately 24 weeks gestation. *U. urealyticum* and heavy growth of *Gardnerella vaginalis* were the only two microorganisms found to be associated with preterm birth. Of importance in this study was the finding that *U. urealyticum* was also associated with a three-fold increased risk of PPROM. This confirmed the study of *Minkoff* et al. (1984) who found that *U. urealyticum* but not *M. hominis* was associated with preterm labour. However *Polk* et al. (1989) and *McGregor* et al (1990) found *M. hominis,* but not *U. urealyticum,* was associated with in-

Table 2: Evidence linking vaginal *U. urealyticum* in pregnancy to obstetric outcome

Reference	Year	Gestation	n	Outcome OR/RR (95%)
Braun et al.	1971	First visit	485	n.s.
Ross et al.	1981	Three visits	162	n.s.
Upadhyaya et al.	1983	First visit	135	n.s.
Minkoff et al.	1984	First visit	220	PTL, RR 1.33 ($p<0.05$)
Harrison et al.	1986	First visit	1587	n.s.
Lamont et al.	1987	Labour		PTB<34 weeks, $p<0.05$
McGregor et al.	1990	24 weeks	202	n.s.
Carey et al.	1991	22-26 weeks	4,934	n.s.
McDonald et al.	1992	24 weeks	786	PTB, OR 1.7 (1.1-2.6) PPROM, OR 3.2 (1.7-6.1)
Jacqui, Sedallian.	1992	Last month	577	PPROM, $p<0.05$
Joste et al.	1994	First trimester abortion	63	Early abortion, $p<0.05$
Chua et al.	1994	13-34 weeks	312	n.s.
Germain et al.	1995	23-26 weeks	13,914	IUGR, RR 1.2 (1.05-1.38)
Abele-Horn	1997	Labour	253	PTB, $p<0.001$ PPROM, $p<0.001$

creased risk of preterm birth. Four other prospective studies in pregnancy showed no significant associations with adverse pregnancy outcome (*Braun* et al., 1971; *Ross* et al., 1981; *Upadhyaya* et al., 1983; *Harrison* et al., 1986). The incidence of *M. hominis* and *U. urealyticum* varied from 5 to 47 % and 44 to 79% respectively in these studies which may account for the disparity in results.

In the Vaginosis in Pregnancy Trial 10,397 women were studied at 23 to 26 weeks gestation (*Hillier* et al., 1995). Among women with bacterial vaginosis the highest risk of preterm birth was found in those with both bacterial vaginosis and *M. hominis.* In addition 4,934 women were evaluated for carriage of *U. urealyticum.* After multivariate analysis no association was found with preterm birth or PPROM (*Carey* et al., 1991). However women with other pathogens as well as *U. urealyticum,* such as group B streptococcus and *Chlamydia trachomatis*, were excluded from analysis.

In the study of *McDonald* et al. (1992), the separate risk for preterm birth attributable to *G. vaginalis* was 9% while 24% may be explained by colonisation with *U. urealyticum.* The joint risk attributable to *G. vaginalis* and *U. urealyticum*, after allowing for the effects of previous preterm delivery and multiple pregnancy, was 26%. The *G. vaginalis* and *U. urealyticum* attributable risks were not independent, as 7% of women were colonised with both organisms but there was an independent attributable risk for carriage of *U. urealyticum* over and above the joint attributable risk. This indicates that there is an additional component of risk for preterm birth over and above the risk from bacterial vaginosis.

A meta-analysis (*Divers* and *Lilford*, 1993) of studies available at that time found an overall significantly increased risk of preterm birth with *M. hominis* colonisation during pregnancy. In addition, since 1993 the results of the VIP study showed an increased risk of preterm birth if *M. hominis* was present

in women with bacterial vaginosis. In summary there is considerable evidence indicating a role for high concentrations of *M. hominis* in the vagina in early to mid pregnancy, in infection-mediated preterm birth. This appears to be in conjunction with bacterial vaginosis.

The role of *U. urealyticum* in pregnancy is less well defined. Despite the negative findings of the VIP study, five other studies since 1989 have shown associations with adverse pregnancy outcome (*McDonald* et al., 1992; *Jacqui* and *Sedallian*, 1992; *Joste* et al., 1994; *Germain* et al., 1995; *Abele-Horn* et al., 1997), and this is also reflected in the findings of the placental studies. It may be there are one or more subgroups of pregnant women at risk for ascending infection with the genital mycoplasmas. In severe infections a serological response to *U. urealyticum* can sometimes be measured and it may be that women who lack antibodies to these organisms are at higher risk of ascending infection. Alternatively, certain clinico-pathological conditions may place a woman at risk. If only a proportion of women are at risk of infection, this may explain why a number of prospective studies of vaginal flora in pregnancy do not show an association between *U. urealyticum* in pregnancy and adverse pregnancy outcome.

PATHOGENESIS

U. urealyticum:

U. urealyticum is unique among the mycoplasmas in its ability to metabolise urea through the enzyme urease. Cells of *U. urealyticum* attach to erythrocytes and other eucaryotic cells, and produce a haemolysin which lyses erythrocytes. Like other bacteria associated with preterm birth, *U. urealyticum* is known to produce phospholipase A_2, an enzyme which frees bound arachidonic acid from foetal membrane cells, resulting in a cascade of prostaglandin synthesis that leads to uterine contractions. IgA protease activity has been demonstrated for *U. urealyticum*, and this is thought to be an important virulence factor of mucosal pathogens. The influence of hormones upon vaginal colonisation was demonstrated in studies in female mice in which treatment with oestradiol enabled colonisation to occur (*Furr* and *Taylor-Robinson*, 1989). It has been shown that *U. urealyticum* by itself can produce chorioamnionitis, although many chorioamniotic infections occur mixed with other organisms, especially bacterial vaginosis organisms, indicating a symbiosis. *U. urealyticum* is thought to be a cause of chronic lung disease in the very low birthweight neonate (*Cassell* et al., 1993). It has also been associated with non-gonococcal urethritis, pelvic inflammatory disease, infertility, septic arthritis and urinary stone formation. Two biovars, parvo and T960 have been determined in pregnant women. Although biovar parvo was more common, T960 was dominant in women with preterm birth and miscarriage (*Abele-Horn*, 1997). Fourteen serotypes of *U. urealyticum* are known but only some are involved in disease.

M. hominis:

M. hominis organisms can adhere to many eucaryotic cells. Like *U. urealyticum*, colonisation of the genital tract of mice by *M. hominis* is dependent on oestradiol, but not progesterone, hormone treatment, (*Furr* and *Taylor-Robinson*, 1989). High concentrations of *M. hominis* occur in some women with bacterial vaginosis. Heavy *M. hominis* colonisation, generally in the presence of bacterial vaginosis, is associated with preterm birth. In addition to

genital tract infections, post partum and post abortal fever, *M. hominis* has been found in a number of blood, joint, wound, central nervous system and respiratory infections, generally in debilitated, immunosuppressed or neonatal patients. *M. hominis* is a potent stimulator of neutrophil chemoattractant cytokines in alveolar type II cells.

PREVENTION STRATEGIES AND *U. UREALYTICUM* ERYTHROMYCIN TREATMENT TRIALS

If there is an association between vaginal carriage of the genital mycoplasmas during pregnancy and adverse pregnancy outcome, then formulation of strategies for prevention of preterm birth may be possible. However, eradication of *U. urealyticum* vaginal colonisation is very difficult to achieve. Treatment with oral erythromycin at 29 weeks' gestation was not successful in eliminating *U. urealyticum* from the vagina in a randomised, double-blind trial to prevent preterm delivery (*Eschenbach* et al., 1991). After four weeks of erythromycin or placebo, recovery of *U. urealyticum* from the vagina was no different (79% vs. 84%). This is not surprising as erythromycin would not be expected to be effective at the low pH of the vaginal mucosa. Also it is very difficult to eradicate any organism present as mucosal normal flora, as these organisms are usually not in an actively replicating stage. Finally the gestation of treatment in this study was too late to prevent ascending infection. If treatment is to have any chance of preventing ascending infection it should be instituted early in pregnancy before organisms colonise the endometrium.

TREATMENT IN PRETERM LABOUR

A number of studies have treated women in preterm labour with erythromycin in an attempt to prevent delivery. Whilst some have shown an increase in time to delivery of several days when compared with controls, we do not know how effective erythromycin is in eradicating *U. urealyticum* from the placenta, and we know that erythromycin does not penetrate the amniotic sac (*Cassell* et al., 1993). Therefore other preventative strategies must be sought to prevent ascending infection in susceptible women.

HOST FACTORS AND IDENTIFICATION OF SUBGROUPS AT RISK

Why do some women develop ascending infection and others do not? The current urgent requirement is the identification of which particular women are at risk of ascending infection. If a specific subgroup of women can be identified, strategies can then be focused upon this group of women rather than all pregnant women, with a correspondingly much greater chance of success. A recent study analysed a range of demographic and other variables but failed to identify any particular population subgroup at higher risk of adverse pregnancy outcome when *U. urealyticum* colonisation was present (*Eschenbach* et

al., 1991). Other studies have indicated lack of specific protective antibody may be a risk factor, e.g. for group B Streptococcus.

Current studies on cytokine production during pregnancy seek to identify women at risk and women in whom an infectious process has already begun. Other studies on the immune response aim to identify women at risk before infection has commenced, i.e., those who are more susceptible to infection. However the aetiology of preterm birth is multi-factorial and we would expect a number of host factors may be involved.

CONCLUSIONS

In conclusion we know that intrauterine infection with *U. urealyticum* and *M. hominis* can occur via ascending genital tract infection and this is associated with chorioamnionitis. We also know that infection of the chorioamnion with these microorganisms is associated with preterm birth, spontaneous abortion, miscarriage and PPROM. There is evidence that *M. hominis* vaginal colonisation, generally in the presence of bacterial vaginosis, increases the risk of infection-mediated preterm birth. There is an additional attributable risk for preterm birth for vaginal *U. urealyticum* in addition to the joint attributable risk for bacterial vaginosis, although there does not appear to be a significant association between vaginal *U. urealyticum* and preterm birth.

Host factors are equally as important as microbial factors in determining pregnancy outcome, yet these have not been as well studied to date. Further studies need to be undertaken, especially studies of the local immune response and host response factors.

Finally screening and treatment for *U. urealyticum* and *M. hominis* in pregnancy is not recommended, as their role in preterm birth is linked with the bacterial vaginosis micro-ecology. If screening for vaginal infections is being considered in women at risk of preterm birth, then screening and treatment for bacterial vaginosis may be undertaken, as eradication of bacterial vaginosis should also reduce the risk of *U. urealyticum* and *M. hominis* ascending infection.

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