Association between preterm birth and genital colonization with *Mycoplasma* – literature review

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Abstract

Preterm labor is responsible for most cases of neonatal death. In most of these cases, the causes have not been established, although several risk factors have been incriminated. Mycoplasma hominis is a common vaginal organism that is associated with bacterial vaginosis. The bacterium is considered inoffensive for the most part in nonpregnant women, but it can cause intraamniotic infections which are associated with inflammation, preterm premature rupture of membranes, and preterm birth. The role of these genital tract habitants in their infection during pregnancy and their capacity to invade placental and fetal tissue are discussed. In particular, the role of some of these organisms in prematurity may be mechanistically associated to their ability to induce inflammatory cytokines, thus triggering pathways causing to premature labor. A review of this intensifying research of mycoplasmas in relation to pregnancy lead to several questions and findings that will be important to examine in future research.

Keywords: *preterm birth, genital mycoplasmas, microbial invasion of the amniotic cavity*

Rezumat

Naşterea prematură este responsabilă pentru majoritatea cazurilor de deces neonatal. În cele mai multe dintre aceste cazuri, cauzele nu au fost stabilite, deși mai mulți factori de risc sunt incriminați. Mycoplasma hominis este un organism asociat vaginozei bacteriene. Infecția cu Mycoplasma este considerată inofensivă în cea mai mare parte la femeile care nu sunt gravide, dar poate provoca infecții intraamniotice, care sunt asociate cu inflamația, ruptura prematură a membranelor și naşterea prematură. Rolul acestor bacterii ale tractului genital în infecția din timpul sarcinii și capacitatea lor de a invada țesutul placentar și fetal sunt încă discutate. Rolul unora dintre aceste organisme în prematuritate poate fi mecanic și asociat cu capacitatea lor de a produce citokine inflamatorii, inițiind astfel evenimente finalizate cu travaliul prematur. O analiză critică a literaturii privind implicarea micoplasmelor în naşterea prematură conduce la câteva constatări importante și la necesitatea elaborării unor cercetări viitoare.

Cuvinte-cheie: *naştere prematură, micoplasme genitale, invazie microbiană a cavității amniotice*

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Introduction

Preterm birth is a major public health issue worldwide and a main cause of infant mortality, as well as a serious neonatal morbidity.

Approximately 15 million babies (1 in 10) are born prematurely each year around the world. Prematurity is the leading cause of death among newborns, accounting for 1 million deaths per year, and it is the second leading cause of death in children under the age of 5 years old[1]. Lower genital tract colonization of the cervix and vagina by microorganisms is common and found in 70% of women[2]. *Mycoplasma hominis* is a common vaginal inhabitant which is associated with bacterial vaginosis[3,4]. The bacterium is considered harmless for the most part in nonpregnant women, but can cause intraamniotic infections, which are associated with inflammation, preterm premature rupture of membranes, and preterm birth[5,6]. Genital mycoplasmas are the most frequently isolated organisms from placental membranes and amniotic fluid in chorioamnionitis. Mycoplasmas belong to the class *Mollicutes*, the smallest free-living organisms. Mycoplasmas are distinguished phenotypically from other bacteria by their minute size and total lack of a cell wall (they are limited by a plasma membrane only), rendering them resistant to β-lactam antibiotics[7,8].

Mycoplasma hominis and *Ureaplasma urealyticum* have been isolated from placental membranes in 47% and 30% of causes of confirmed chorioamnionitis, respectively[9]. The mechanism by which lower genital tract colonization leads to adverse pregnancy outcomes is poorly understood. The inflammatory process associated with genital mycoplasmas colonization would be

evidenced by placental histologic chorioamnionitis in the absence of bacterial infection, meaning that genital mycoplasmas colonization is associated with histologic chorioamnionitis independent of placental infection with other bacteria[10].

Materials and method

The objective of this study is to analyze the literature related to the implication of *Mycoplasma* infection in premature birth. The following databases have been reviewed: Pubmed, Medline and Cochrane, using as keywords: preterm birth, genital mycoplasmas, microbial invasion of the amniotic cavity.

Results

A cohort study that implicated >10,000 pregnant women established that women with bacterial vaginosis had an increased risk for preterm birth; among women with bacterial vaginosis, those who were colonized with *Mycoplasma hominis* had a considerable risk increase[11]. Intraamniotic infections can cause inflammation, which triggers spontaneous preterm birth[12]. One of three preterm births is associated with microbial invasion of the amniotic cavity[13,14,15]. It has been reported that women exposed to microbial invasion of the amniotic cavity have a significantly earlier gestational age at admission and delivery, a shorter latency to delivery, and a worse neonatal outcome compared with women who weren't exposed to microbial invasion[16,17]. Intraamniotic infection is common in births that occur at <32 weeks of gestation. This conclusion is significant because morbidity and death increase with decreasing gestational age at delivery. *Mycoplasma hominis* is frequently isolated from infected fetal membranes and amniotic fluid, and *Ureaplasma* is isolated from umbilical cord blood in approximately 20% of very preterm newborn infants (<32 weeks of gestation)[18].

Under normal conditions, the amniotic cavity is sterile for microorganisms, using cultivation and molecular microbiologic techniques, based on the detection of the 16S rRNAgene[19].

The pathogenesis and the cause of preterm birth infectious are poorly understood. Ascending microbial invasion from the lower genital tract appears to be the most frequent pathway for intraamniotic infection[20].

The rupture of membranes is not obligatory for bacteria to reach the amniotic cavity; there is experimental evidence that bacteria can traverse intact membranes[21]. Most of these infections are subclinical and occur in the absence of clinical chorioamnionitis[22,23]. Therefore, most of these infections are undetected unless the amniotic fluid is analyzed. The most frequent microorganisms detect in the amniotic cavity are genital mycoplasmas[24,25].

Although some studies consider that there is a stage in which the bacteria are diffusely located in the choriodecidual layer, other studies, using FISH with a bacterial 16S rRNA probe, indicate that there is no extensive implication of the chorion-decidua in cases with microbial invasion of the amniotic cavity[26,27].

Microbial invasion of the amniotic cavity induces a strong local inflammatory response, and this is accompanied by a considerable increase in the concentrations of proinflammatory cytokines such as IL-1[28,29], IL-6[30], IL-8[31,32] and TNF-α[33].

The primary cells and tissues responsible for the intraamniotic inflammatory response include fetal skin, cells comprising the chorioamniotic membranes, and the umbilical cord. The amnion and chorion-decidua respond to bacterial products by increasing the expression of IL-1β[34] and TNF-α[35]. Amnion cells also synthesize IL-8[36].

In another study, the detection of *M. hominis* was associated with elevated intraamniotic concentrations of IL-4[37].

The most accurate evidence that intraamniotic infection is associated with acute chorioamnionitis is obtained from studies in which a transabdominal amniocentesis was performed in patients with preterm labor and intact membranes, and the placenta was examined within 48 hours of the procedure. Placentas with acute chorioamnionitis and acute funisitis were from mothers who had intraamniotic infection proven by culture in 71.1% and 78.7% of cases, respectively. The prevalence of microbial invasion of the amniotic cavity was 38%. The negative predictive values of acute chorioamnionitis and funisitis for intraamniotic infection were 87% and 82%, respectively[38].

Recently, it was reported a new type of intraamniotic inflammation termed "sterile inflammation". Interestingly, sterile intraamniotic inflammation is associated with acute histological chorioamnionitis (40-60% of cases)[39].

Roberts et el. reported, using cultivation and molecular microbiologic techniques, that only 4% of patients with acute histologic chorioamnionitis at term had microorganisms in the placenta[40].

The frequency with which microorganisms invade the human fetus is difficult to establish. Studies in which amniocentesis and cordocentesis have been performed in patients with preterm premature rupture of membranes indicate that 30% of patients with microbial invasion of the amniotic cavity have positive fetal blood cultures for microorganisms[41,42]. Similar conclusions have been reported when cultures for genital mycoplasmas have been performed in umbilical cord blood at the time of birth[43,44].

A study conducted in 2015 sequenced genomes of two amniotic fluid isolates and a placental isolate of *Mycoplasma hominis* from pregnancies that resulted in preterm births and compared them with the previously sequenced genome of the type strain PG21. Genes that were isolated from amniotic fluid and placenta were identified, then the microbial load and the presence of these genes were determined in another set of subjects from which amniotic fluid samples were collected and were positive for *Mycoplasma hominis*. There were identified two genes that encode surface-located membrane proteins (Lmp1 and Lmp-like) in

the sequenced amniotic fluid/placental isolates that were truncated severely in PG21. It has been identified a gene in *M. hominis* that was significantly associated with colonization or infection of the upper reproductive tract during pregnancy and with premature birth. The presence of the gene was associated with bacterial burden in the amniotic fluid, suggesting that it could play a role in survival. This observation is clinically relevant because the mycoplasmal bacterial burden in amniotic fluid correlates with histological chorioamnionitis[45]. At present, little information exists about the pathogenesis of microbial invasion of the amniotic cavity, but in order for a bacterium in the vagina to invade the amniotic cavity, it would probably must colonize the vagina, ascend through the cervix to the uterine cavity, colonize the uterine cavity, and evade immune defenses and potentially other antagonistic bacterial species. In order to cause amnionitis, it would also need to pass the fetal membranes or placenta, and survive and grow in a nutrient-poor, iron-limited amniotic fluid. Probably, this induces premature labor, and it would then have to elicit a maternal or fetal inflammatory response. Randis et al. investigated recently the role of group B *Streptococcus* β-hemolysin/cytolysin in the pathogenesis of ascending group B streptococcal infection using a mouse model[46]. It was found that the toxins had a role in vaginal colonization and in inducing inflammation, host tissue injure and preterm birth or fetal death, but they were not required for ascending infection. They found a gene that was associated with bacterial burden in amniotic fluid and with preterm birth, but it was not required for amnionitis. It was not significantly associated with the presence of bacterial load of *M. hominis* in the vagina, suggesting that it does not play a role in survival in the vagina. Therefore, it may be more likely that the product of this gene is involved in bacterial survival or growth on placenta and in the amniotic cavity rather than being required for ascension or invasion. Of note, they also found that the two isolates from amniotic fluid are more similar to each other than they are to the placental isolate, suggesting that these two strains may have a higher genetic potential for traversing fetal membranes or surviving in amniotic fluid. There were detected differences in genes encoding surface-associated lipoproteins that have been previously implicated in adherence to host cells and colonization. They noted that upon liquid culture, the amniotic fluid/placental strains appeared dispersed, whereas PG21 appeared flocculated and adhered to the plastic culture vessel, suggesting a variation on the bacterial surface. The variation in the sequence and structure of surface lipoproteins likely concur to the avidity of the bacteria for host cells and tissues, and therefore it may be associated with disease outcome. There are found considerable variation between genes. The genes encoding an Lmp-like protein and Lmp1 are more similar among the amniotic fluid/placental strains than they are to homologs in PG21. These lipoproteins could contribute to adherence to host cells in some way that promotes ascension from the lower to the upper genitourinary tract or colonization of the uterine cavity[47].

Conclusions

The evidence regarding genital mycoplasmas in initiating preterm labor and delivery is becoming more convincing.

It was identified a gene in *Mycoplasma hominis* which was significantly associated with colonization and infection of the upper reproductive tract during pregnancy and with preterm birth.

Acute chorioamnionitis and acute funisitis are acute inflammatory lesions with important short- and long-term clinical significance. Substantial progress has been made in the understanding of the mechanisms responsible for maternal and fetal inflammation in the context of infection.

The causes of sterile intraamniotic inflammation are unknown, and there are important clinical and scientific challenges. ■

Conflict of interests: The authors declare no conflict of interests.

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