OBSTETRICS

Vaginal *Ureaplasma parvum* serovars and spontaneous preterm birth

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BACKGROUND: *Ureaplasma species* (*spp*) are the bacteria most often isolated from the amniotic cavity of women with preterm labor or preterm premature rupture of membranes; thus, the link between intrauterine *Ureaplasma spp* infection and adverse pregnancy outcome clearly is established. However, because vaginal *Ureaplasma spp* colonization is very common in pregnant women, the reason that these microorganisms cause ascending infections in some cases but remain asymptomatic in most pregnancies is not clear. Previous studies suggested an association between vaginal colonization with *Ureaplasma parvum* as opposed to *U urealyticum* and preterm delivery. However, because of the high frequency of vaginal *Ureaplasma spp* colonization during pregnancy, additional risk factors are needed to select a group of women who might benefit from treatment.

OBJECTIVE: To further identify pregnant women who are at increased risk for preterm delivery, the aim of the present study was to investigate *U parvum* serovar—specific pathogenicity in a large clinical cohort.

STUDY DESIGN: We serotyped 1316 samples that were positive for *U parvum* using a high-resolution melt polymerase chain reaction assay, and results were correlated with pregnancy outcome.

RESULTS: Within *U parvum* positive samples, serovar 3 was the most common isolate (43.3%), followed by serovar 6 (31.4%) and serovar 1 (25.2%). There was a significantly increased risk for spontaneous preterm birth at very low (<32 weeks gestation; $P<.005$) and extremely low (<28 weeks gestation; $P<.005$) gestational age in the group with vaginal *U parvum* serovar 3 colonization compared with the control group of pregnant women who tested negative for vaginal *Ureaplasma spp* colonization. This association was found for neither serovar 1 nor serovar 6. The combination of vaginal *U parvum* serovar 3 colonization and diagnosis of bacterial vaginosis in early pregnancy or a history of preterm birth further increased the risk for adverse pregnancy outcome.

CONCLUSION: Colonization with *U parvum* serovar 3, but not serovar 1 or serovar 6, in early pregnancy is associated with preterm delivery at very and extremely low gestational age. The combination of *U parvum* serovar 3 colonization and a history of preterm birth or bacterial vaginosis further increases the risk for spontaneous preterm birth at low gestational age and may define a target group for therapeutic intervention studies.

Key words: bacterial vaginosis, colonization, pathogenicity, pregnancy, preterm birth, *Ureaplasma parvum* serovar

Ureaplasma spp are the most commonly isolated bacteria from the amniotic cavity of pregnant women and are associated with adverse pregnancy outcome and neonatal morbidity.[1–7] They can be detected in the umbilical cord blood in approximately 20% of spontaneous preterm births (SPB) and indicate fetal involvement.[8] Although vaginal *Ureaplasma spp* colonization is very frequent in pregnant women, the reason that these microorganisms cause ascending infections in some cases but remain asymptomatic in most pregnancies is still unclear. Given the high prevalence of vaginal *Ureaplasma spp* colonization and the fact that most colonized women give birth at term without any complications,[9,10] additional risk factors for SPB are needed to select a group of women who might benefit from treatment.[11]

The taxonomy for human *ureaplasmas* discriminates between 2 biovars, which refers to a phenotypic and genetically differentiable group of strains within a species, and 14 serovars, termed variants of a subspecies, which are mainly discriminated by antigenic properties. Serovars 1, 3, 6, and 14 are classified as *Ureaplasma parvum* and 2, 4, 5, 7–13 as *U urealyticum*, of which *U parvum* is detected more frequently within the amniotic fluid.[12,13] Several studies have investigated the hypothesis of *Ureaplasma* biovar- or serovar-specific pathogenicity.[14–16] In our previous study, women who tested positive for vaginal *U parvum*, but not *U urealyticum*, colonization in the first trimester of pregnancy had a significantly increased risk for SPB.[11] This is in accordance with the literature that provides evidence that *U parvum* is the most common organism to invade the amniotic cavity and potentially cause infection and obstetric complications and that *U parvum* rather than *U urealyticum* might be associated with adverse pregnancy outcome.[15,17,18] Moreover, several authors attempted to correlate particular *Ureaplasma* serovars with specific clinical outcomes.[16,17,19–21] However, neither human nor animal data have consistently proved *Ureaplasma* subspecies' specific pathogenicity, virulence factors, or severity of infection so far.[22]

Until recently, *U parvum* serovar characterization has been time-consuming and expensive; hence, it has not been used in clinical routine. A high-resolution melt polymerase chain reaction (PCR) assay published by Payne et al[23] now allows the differentiation of the 4 *U parvum* serovars in 1 step. To

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AJOG at a Glance

Why was this study conducted?

This study was conducted to investigate the association between first-trimester vaginal colonization with different *Ureaplasma parvum* serovars in pregnant women and spontaneous preterm birth.

Key findings

Vaginal *U parvum* serovar 3 colonization significantly increased the risk for spontaneous preterm birth at <32 ($P<.005$) and <28 weeks ($P<.005$) gestational age, which was not found for serovars 1 and 6. Risk for adverse pregnancy outcome further increased when colonization was accompanied by a history of preterm birth or bacterial vaginosis.

What does this add to what is known?

It has been shown before that vaginal *U parvum* as opposed to *U urealyticum* is associated with an increased risk of spontaneous preterm birth. Results of the present study suggest an *U parvum* serovar−specific pathogenicity, with vaginal *U parvum* serovar 3 as the most relevant serovar in association with spontaneous preterm birth.

further identify a group of pregnant women at increased risk for SPB, the aim of the present study was to investigate *U parvum* serovar-specific pathogenicity in a large clinical cohort.

Material and Methods

Study design and sample collection

A total of 4330 pregnant women who attended routine nuchal translucency screening between 12 and 14 weeks gestational age (GA) were enrolled in a prospective multicenter study that analyzed the association between vaginal *Ureaplasma* biovar colonization in early pregnancy and outcome, as published previously.[11] The study was conducted at the Departments of Obstetrics and Gynaecology of the Medical University Vienna, the Danube Hospital Vienna, and Hospital Rudolfstiftung Vienna, Austria; the first 2 locations are level III centers; the third location is a level II center for perinatal care. Samples that tested positive for *U parvum* and those that tested negative for any *Ureaplasma spp* were included in the current study.

Ethics statement

The study was approved by the ethics committees of the Medical University of Vienna (EK NR 655/2008) and the City of Vienna (EK 09-120-VK). All women gave written informed consent before participation.

Clinical outcomes

Demographic and clinical outcome data were collected from medical records, as previously described.[11] GA was determined based on the ultrasound scan in the first trimester of pregnancy. Potential risk factors for preterm delivery were recorded in the following manner: age, parity, history of preterm birth (PTB), multiple gestation, abnormal vaginal flora (defined as "normal flora," "intermediate flora," and "bacterial vaginosis" (BV) according to a scoring system by Nugent et al[24]) and vaginal candidiasis (spores and hyphae on Gram stain). History of PTB was defined as self-reported previous preterm delivery or pregnancy loss at any gestational week. Primary outcome parameter SPB was defined as spontaneous late pregnancy loss (12−20 weeks gestation) or spontaneous preterm delivery (<37 weeks gestation) per vaginal delivery or cesarean delivery after preterm labor or preterm premature rupture of membranes in single pregnancies.[11]

Differentiation of *Ureaplasma parvum* serovars

High-resolution melt PCR

Melting curve analysis is the use of serovar-specific characteristics of the dissociation of double-stranded DNA during heating that is visualized with fluorophore. Samples that were positive for *U parvum* were serotyped as either serovar 1, 3, 6, or 14 in accordance with a high-resolution melt PCR assay published by Payne et al[23] with the use of MeltDoctor high-resolution melt reagent kit (Life Technologies, Carlsbad, CA). High-resolution melt profiles were analyzed using the Rotor-Gene 6000 software, vs 1.7 (QIAGEN, N.V., Hilden, Germany). All reactions were performed in duplicate and retested in triplicate in case high-resolution melt curves were not identical in duplicates. In addition, all possible combinations of the 4 American Type Culture Collection (ATCC, Manassas, VA) positive standard serovar samples were mixed and analyzed with high-resolution melt PCR to produce standard curves for samples that contained multiple serovars.

Sequencing of high-resolution melt PCR samples

To validate the high-resolution melt assay, 5 samples of each serovar that produced melt curve patterns that were identical with the ATCC positive standard samples were sequenced. Moreover, all samples that produced nonstandard melt curve patterns during high-resolution melt analysis were subject to DNA sequencing as well.

PCR amplicons were generated with the use of the same high-resolution melt assay.[23] PCR reactions were checked for successful amplification on an 1% agarose gel; amplicons were purified with the use of a QIAquick PCR purification kit (QIAGEN) as per manufacturer's instructions and subsequently sent to VBC Biotech (Vienna, Austria) for sequencing. Serovar identity was determined with the NCBI nucleotide Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi), an online database that checks for nucleotide sequence homology.

Statistical analysis

The study population was described by frequency tables and means and standard deviations. Comparisons of proportions for preterm delivery (<37 weeks gestation) were performed with the exact Fisher test; comparison of age was performed by the Wilcoxon signed

TABLE 1
Clinical characteristics of the study population in the *Ureaplasma parvum* positive study group and the *Ureaplasma spp* negative control group

Variable	Total	Study group *U parvum* positive	Control group *Ureaplasma spp* negative	*P* value
Women in each group, n	3216	1247	1969	
Risk factors				
Maternal age, mean (standard deviation)	30.6 (5.9)	29.6 (6.0)	31.2 (5.8)	<.001
Gravidity, mean (standard deviation)	2.67 (1.7)	2.61 (1.7)	2.71 (1.7)	.035
Parity, mean (standard deviation)	1.05 (1.2)	0.97 (1.2)	1.1 (1.2)	<.001
History of preterm birth, n (%)	1099 (34.2)	415 (33.3)	684 (34.7)	.402
Women with available standardized assessment of vaginal smear, n (%)	2455	971 (77.9)	1484 (75.4)	
Bacterial vaginosis, n (%)	215 (8.8)	133 (13.7)	82 (5.5)	<.001
Vaginal candidiasis, n (%)	427 (17.4)	181 (18.6)	246 (16.6)	.192

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rank test in SAS software (version 9.4; SAS Institute Inc, Cary, NC). Smoothed quantiles of GA and comparisons of quantiles between groups were performed by package qcomhd in R (R Core Team; 2013, Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/). Probability values were not adjusted for multiple testing and therefore should be interpreted as exploratory only.

Results

Study population

Samples from 3643 pregnancies were analyzed in the earlier published multicenter study that showed a significant association between first-trimester vaginal isolation of *U parvum* as opposed to *U urealyticum* and subsequent SPB.[11] Of those, 1347 women (37.0%) tested positive for *U parvum*; 214 women (5.9%) tested positive for *U urealyticum*, and 113 women (3.1%) tested positive for both biovars.[11] Of the 1347 *U parvum* positive samples, 31 samples (2.3%) could not be further analyzed to serovar level because there was not enough material left from previous analyses, which left 1316 cases that constitute the *U parvum*-positive study population of the current study. Samples from the 1969 pregnancies that were negative for vaginal *Ureaplasma spp* colonization in the previous study were used as the control group. Clinical characteristics of the study population are shown in Table 1.

Sequencing for validation of high-resolution melt PCR

All 15 random samples that were sequenced for validation of the high-resolution melt assay showed 100% identity with ATCC standard samples in the BLAST database. An additional 94 samples that produced nonstandard melt curve patterns were subjected to DNA sequencing. Of those, 37 samples showed 100% identity with 1 of the ATCC standard samples in the BLAST database and could be assigned to a particular serovar group. Fifty-seven samples had to be excluded because the serovar could not be characterized unambiguously: 13 samples that produced melt curve patterns slightly different from the ATCC positive standard sample for serovar 3 were sequenced and shared the very same single nucleotide polymorphism; 27 samples did not show any signal, and 17 samples showed >1 single nucleotide polymorphism compared with ATCC standard samples.

U parvum serovar colonization

Of the 1316 samples positive for *U parvum*, 1247 samples (94.8%) showed unambiguous serovar results: 1210 samples produced melt curve patterns identical with the ATCC positive standard samples, and an additional 37 samples could be characterized unambiguously via sequencing. Another 12 samples produced high-resolution melt curve patterns identical to standard curves for mixed colonization with serovar 1 and serovar 3. Because of the small sample size, the latter cases were excluded from further analyses.

Of the 1247 samples that showed unambiguous serovar results, 315 samples (25.2%) were positive for serovar 1; 540 samples (43.3%) were positive for serovar 3, and 392 samples (31.4%) were positive for serovar 6. No positive samples were identified for serovar 14.

Vaginal *Ureaplasma* serovar colonization and pregnancy outcome

SPB occurred in 127 women (6.5%) who tested negative for *Ureaplasma spp* colonization and 133 women (10.7%) with the isolation of *U parvum* ($P<.001$). When *U parvum* serovars were considered, SPB occurred in 33 pregnancies

TABLE 2
Effect of colonization with *Ureaplasma parvum* serovars on rates of spontaneous preterm birth in different gestational age groups

Variable	*U parvum* positive (n=1247)	*U parvum* serovar 1 positive (n=315)	*U parvum* serovar 3 positive (n=540)	*U parvum* serovar 6 positive (n=392)	*U spp* negative (n=1969)
Term delivery, n (%)	1114 (89.3)	282 (89.5)	481(89.1)	351 (89.5)	1842 (93.6)
Weeks gestation at spontaneous preterm birth, n (%)					
<37	133 (10.7)[a]	33 (10.5)[a]	59 (10.9)[a]	41 (10.5)[a]	127 (6.5)
<32	58 (4.7)[a]	13 (4.1)	29 (5.4)[a]	16 (4.1)	48 (2.4)
<28	45 (3.6)[a]	10 (3.2)	24 (4.4)[a]	11 (2.8)	37 (1.9)
<26	39 (3.1)[a]	8 (2.5)	22 (4.1)[a]	9 (2.3)	30 (1.5)
<24	28 (2.2)[a]	4 (1.3)	17 (3.1)[a]	7 (1.8)	25 (1.3)
Late pregnancy loss 12–20 weeks gestation, n (%)	18 (1.4)	0	13 (2.4)[a]	5 (1.3)	20 (1.0)

[a] $P<.05$ compared with *U parvum* negative group.

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(10.5%) of mothers with vaginal serovar 1 colonization, in 59 pregnancies (10.9%) of mothers with vaginal serovar 3 colonization, and in 41 pregnancies (10.5%) of mothers with vaginal serovar 6 colonization. The statistically significant association between vaginal *U parvum* colonization and preterm delivery at <37 weeks gestation was true for all serovars (serovar 1, $P=.012$; serovar 3, $P<.001$; serovar 6, $P=.007$).

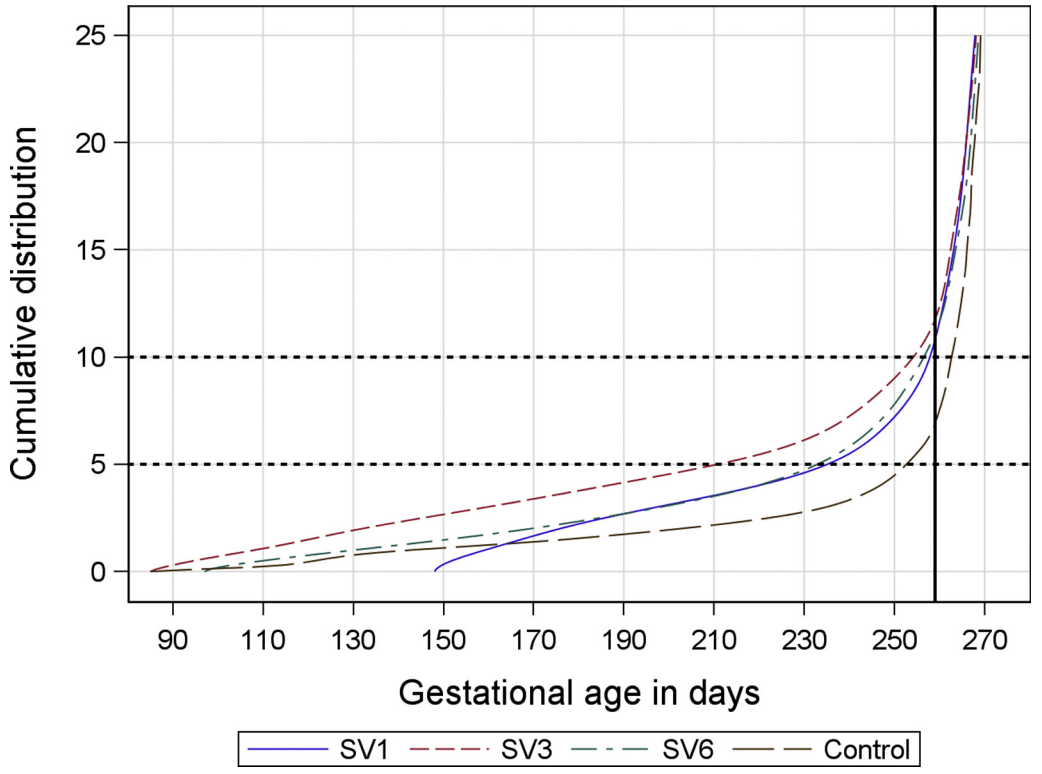
However, when looking at lower GA groups, a statistically significant effect was seen only in serovar 3–positive women when compared with *Ureaplasma* spp negative cases (Table 2): very PTB (<32 weeks gestation; $P<.005$) and extremely PTB (<28 weeks gestation; $P<.005$) were increased significantly when vaginal colonization with *U parvum* serovar 3 was detected as opposed to serovar 1 and serovar 6 positive cases. As many as 4.1% of pregnancies with vaginal isolation of serovar 3 resulted in SPB at <26 weeks gestation ($P<.001$), as opposed to 2.5% in the serovar 1 positive group, 2.3% in the serovar 6 positive group, and 1.5% in the control group. When we analyzed delivery at or before the border of viability at <24 weeks gestation, 3.1% of pregnancies with isolation of serovar 3 resulted in SPB ($P=.007$), as opposed to 1.8% in the serovar 6 positive group and 1.3% both in the serovar 1 positive group and the group with negative culture results. Also, for late pregnancy loss, a significant association was found with *U parvum* serovar 3 ($P=.018$) as opposed to serovar 1 and serovar 6 colonization.

This effect was also demonstrated by calculation of cumulative distribution curves of GA at birth depending on serovar colonization. We encountered a significant difference between serovar groups in a way that the curve for serovar 3 was shifted significantly towards extremely low GA compared with the control group (Figure 1). Concerning the 5th percentile of the cumulative distribution curve (5% of all deliveries that took place before this GA), there was a difference in GA at birth of 42 days between women who tested positive for serovar 3 (211 days; approximately 30 weeks gestation) and women who tested negative for *U parvum* (253 days; 36 weeks gestation; $P<.001$). This difference was not significant for serovar 1 (235 days; approximately 34 weeks gestation) and serovar 6 (233 days; approximately 33 weeks gestation).

Combined effect of *U parvum* serovar 3 colonization and history of PTB on pregnancy outcome

Figure 2 shows the cumulative distribution curve of the combined effect of *U parvum* serovar 3 colonization and a history of PTB, which is an independent risk factor for SPB in this cohort, as published earlier.[11] Although the cumulative distribution curve of *U parvum* serovar 3–negative women with a history of PTB was comparable with the curve of women who tested positive for *U parvum* serovar 3 but without a history of PTB, the combination of these 2 risk factors revealed a statistically highly significant increased risk of SPB. Concerning the 5th percentile, there was a difference in GA at birth of >13 weeks (95 days) between the group with and without those 2 risk factors (163 vs 258 days gestation; $P<.001$). For the 10th percentile, a difference in GA of >4 weeks (32 days) between those 2 groups was still highly significant (232 vs 264 days gestation; $P<.001$). Only 5.5% of women who tested negative for *U parvum* serovar 3 and without a history of PTB gave birth at <37 weeks gestation (259 days) compared with 18.0% of women who tested positive for *U parvum* serovar 3 and a history of PTB ($P<.001$).

FIGURE 1
Gestational age at birth depending on *Ureaplasma parvum* serovar colonization



Cumulative distribution curves represent cumulative frequencies of gestational age at birth, depending on vaginal *U parvum* serovar colonization. The 5th percentile indicates that 5% of all deliveries in the respective group are taking place before this gestational age.

SV, serovar.

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Combined effect of *U parvum* serovar 3 colonization and BV on pregnancy outcome

The effect of the combined risk factors diagnosis of BV, which was an independent risk factor for SPB in our previous study, and the isolation of *U parvum* serovar 3 was analyzed. Again, the cumulative distribution curve for *U parvum* serovar 3−negative women with diagnosis of BV was comparable with the curve of women who tested positive for *U parvum* serovar 3 and negative for BV. There was, however, a highly significantly increased risk for PTB if both risk factors were present in early pregnancy (Figure 3). Concerning the 5th percentile, there was a difference in GA at birth of >10 weeks (73 days) between these 2 groups (181 vs 254 days gestation; P=.005). For the 10th percentile, a difference in GA of >6 weeks (44 days) was still highly significant (219 vs 263 days gestation; P=.001). Only 7.0% of women who tested negative for *U parvum* serovar 3 and without a diagnosis of BV gave birth at <37 weeks gestation (259 days) compared with 22.0% of women who tested positive for *U parvum* serovar 3 and a diagnosis of BV (P=.008).

Comment

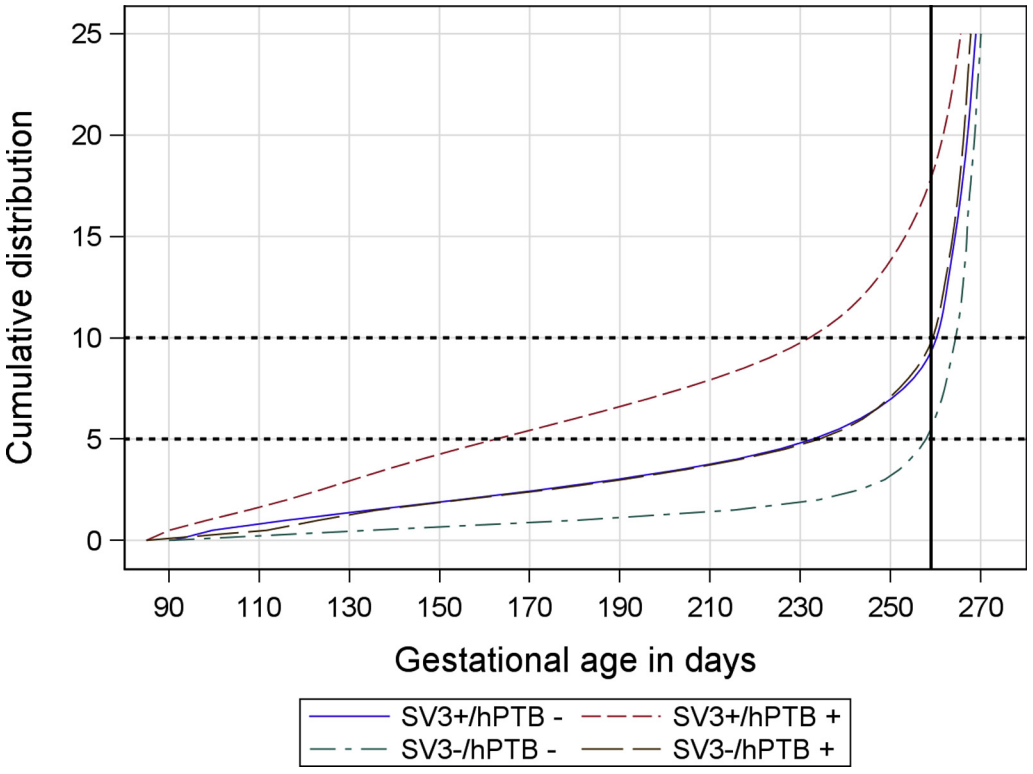
Principal findings of the study

The results of the present study indicate a statistically significant and clinically relevant effect of vaginal *U parvum* serovar 3 colonization in early pregnancy on SPB at very and extremely low GA. Although a significant association for SPB at <37 weeks gestation was also found for serovar 1 and serovar 6, this was not true for the clinically relevant subgroup of very and extremely PTBs. This is important because these extremely premature infants are at the highest risk for death and short- and long-term morbidities, which creates a high burden also in terms of economic costs.[25] Moreover, we could show that the combination of vaginal *U parvum* serovar 3 colonization and a history of PTB or BV significantly increased the risk for adverse pregnancy outcome.

Results of the study in context

Because ascending infection might occur very early in pregnancy and procedures for intrauterine detection are invasive and thus not applicable as screening tools, there is ongoing discussion about the potential impact of screening and treating vaginal *Ureaplasma spp* colonization.[26,27] We previously were able to demonstrate that vaginal *U parvum*, but not *U urealyticum*, colonization in the first trimester of pregnancy increases the

FIGURE 2
Combined effect of *Ureaplasma parvum* serovar 3 colonization and history of preterm birth



Cumulative distribution curves represent cumulative frequencies of gestational age at birth, depending on the combination of the risk factors vaginal *U parvum* serovar 3 colonization and history of preterm birth. The 5th and 10th percentiles, respectively, indicate that 5% or 10% of all deliveries in the respective group are taking place before this gestational age.

hPTB, history of preterm birth; *SV*, serovar.

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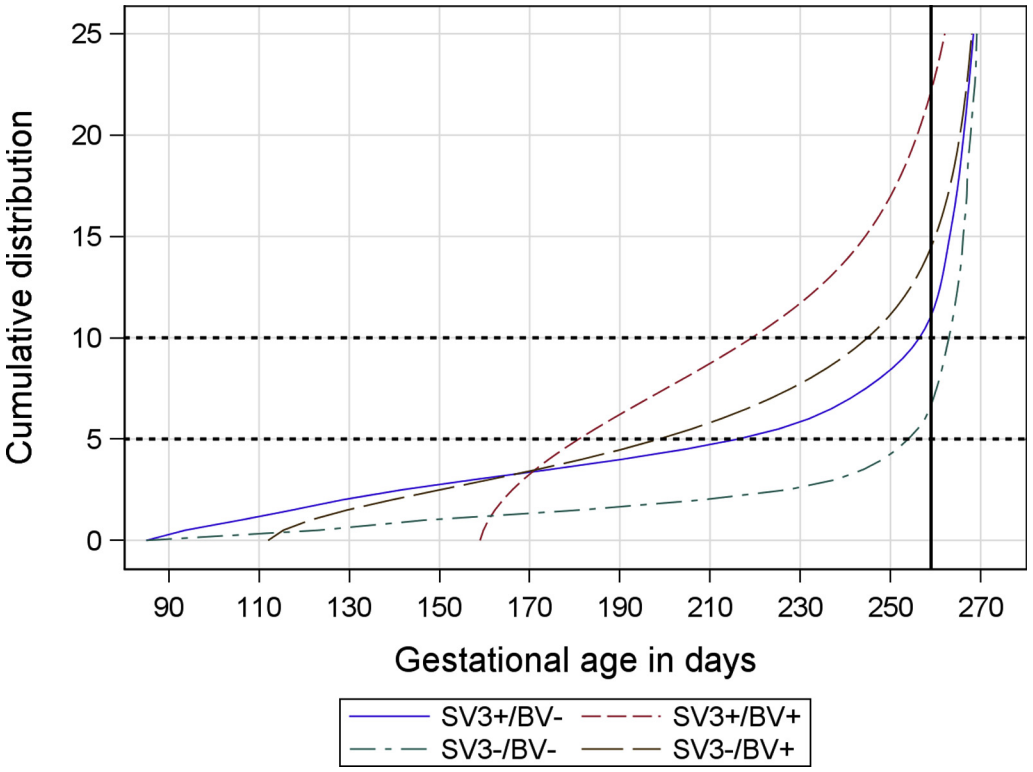
risk for SPB.[11] The current study adds that serovar 3 colonization might pose the highest risk for SPB, especially at very low and extremely low GA. With regard to *U parvum* serovar pathogenicity, DeFrancesco et al[20] found an association between vaginal colonization with serovar 3 and genital infections and loss of Lactobacilli, whereas serovar 6 was associated with a normal vaginal flora and asymptomatic women. In contrast, Payne et al[17] reported a strong association between vaginal serovar 6 as opposed to serovar 1 and serovar 3 and subsequent SPB. Reasons for these divergent results are unclear but might include different ethnic collectives. Although Payne et al found serovar 6 to be the most common isolate in their study collective, our data with serovar 3, which is the most common *U parvum* serovar, are consistent with most reports in the literature;[20,21,28] however, previous studies were based on small sample sizes. In general, there is ongoing debate whether different pathogenicity of *ureaplasmas* relates to the serovar level. Xiao et al[21] proposed that *Ureaplasma spp* virulence does not depend on the serovar specificity but rather on genes horizontally transferred between *ureaplasmas* that might be present in pathogenic and absent in commensal *ureaplasmas*. Furthermore, they hypothesize that pathogenicity depends on the specific immune response. Size variations in the multiple banded antigen, a surface exposed lipoprotein, and potential major virulence factors of *ureaplasmas* have been suspected to affect the host immune response against *Ureaplasma spp* infection.[29–31] Based on those findings and the fact that 40% of their clinical isolates represented genetic mosaics, Xiao et al concluded that serotyping might be of limited value. However, in our collective, 94.8% of samples showed results that were identical to the ATCC standard samples. One possible explanation for this discrepancy is the fact that we exclusively serotyped samples that were positive for *U parvum*, which is more stable than *U urealyticum*.[16]

Clinical and research implications

Several studies have investigated the association between the vaginal microbiome during pregnancy and the risk of SPB.[32–35] Although the data are not consistent, these studies reported some evidence for an association between an alteration of the vaginal microbiome and SPB.[32,33] Recently, a

FIGURE 3
Combined effect of *Ureaplasma parvum* serovar 3 colonization and bacterial vaginosis



Cumulative distribution curves represent cumulative frequencies of gestational age at birth, depending on the combination of the risk factors vaginal *U parvum* serovar 3 colonization and bacterial vaginosis. The 5th and 10th percentiles, respectively, indicate that 5% or 10% of all deliveries in the respective group are taking place before this gestational age.

BV, bacterial vaginosis; *SV*, serovar.

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potential association between a *Lactobacillus*-poor vaginal microbiome with a high abundance of *ureaplasmas* and an elevated risk of SPB was reported, although this study was based on a very small number of patients.[32] In line with these findings, in our cohort, the risk for SPB was especially high in women who tested positive for vaginal *U parvum* serovar 3 colonization and a diagnosis of BV. Because vaginal *ureaplasmas* raise the pH by hydrolyzing urea into carbon dioxide and ammonia,[36] it is tempting to hypothesize that *Ureaplasma spp* colonization supports the emergence of mixed infections with different organisms as seen in BV.[36,37] Moreover, it was proposed that alterations in the vaginal microbiome, normally acting as a barrier against colonization by other bacteria, might enable organisms that include *Ureaplasma* spp or particular *Ureaplasma* serovars to overgrow and ascend from the vagina into the uterine cavity.[33,35,38]

Regarding therapy of *U parvum* infections, it remains controversial whether antibiotic treatment might help to reduce rates of SPB and adverse neonatal outcomes because studies showed contradictory results and because a randomized trial failed to show any benefit of antibiotic treatment.[27,39-41] This partly could be due to the fact that most treatment studies did not discriminate between *Ureaplasma* biovars and serovars and have been conducted with erythromycin or clindamycin, which have a low rate of transplacental passage.[42,43] Animal studies suggest that more potent antibiotics, such as azithromycin or solithromycin, are needed to effectively eradicate *U parvum* from the amniotic fluid and fetal key organs, but these antibiotics have not yet been tested in clinical trials in pregnant women.[44–46] Our results identify a group of women at particularly high risk for SPB at low GA. These findings might integrate into protocols for intervention studies, testing whether screening and treatment with antibiotics effective against *Ureaplasma spp* and BV reduce the rate of SPB in this high-risk population.

Strengths and weaknesses

The use of cumulative distribution curves, which indicate delivery at a particular GA in relation to risk factors, provided a very meaningful representation of clinical significance because a cutoff point of GA was not used. Therefore, we were able to demonstrate that colonization with *U parvum* serovar 3 has an adverse impact mainly on SPB at very small GA. Moreover, the application

of the high-resolution melt PCR assay with excellent validity and reliability as published by Payne et al[23] shows that *U parvum* serovar differentiation is possible in a large clinical collective.

As a limitation of the assay, we must acknowledged that our experiments that produced standard curves for samples that contained multiple serovars showed that, as soon as serovar 6 was included in a mixed ATCC serovar sample, the melt curve pattern could not be differentiated from serovar 6 alone. Hence, the actual percentage of mixed serovar samples could be higher than found in our study, and a certain amount of serovar 6 cases could represent mixed infections, also reported by Payne et al.[47] Still, the frequency of mixed colonization with *U parvum* serovars seems to be low, and the clinical significance is completely unknown.[23,47,48] Moreover, the high-resolution melt assay does not allow for quantitative measurement; therefore, we are not able to make any statement on bacterial load and its potential association with SPB.[49,50]

Another limitation of the study is the fact that we did not assess the colonization status with bacteria other than *ureaplasmas*.

Conclusion

Because rates of vaginal *Ureaplasma spp* colonization in pregnant women are high, screening for *Ureaplasma* biovars alone does not appear suitable to identify women at highest risk of SPB for potential treatment studies. Whether a screen-and-treat approach for BV in pregnancy is able to reduce the rate of SPB remains controversial.[51–54] The results of the present study indicate that the combination of vaginal isolation of *U parvum* serovar 3 and a history of PTB or a diagnosis of BV in early pregnancy identifies a group of women who are at a particularly high risk for SPB at low GA, which is a finding that might be incorporated into the design of future intervention studies.

References

1. Digiulio DB, Romero R, Kusanovic JP, et al. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. Am J Reprod Immunol 2010;64:38–57.
2. Sweeney EL, Kallapur SG, Gisslen T, et al. Placental infection with Ureaplasma species is associated with histologic chorioamnionitis and adverse outcomes in moderately preterm and late-preterm infants. J Infect Dis 2016;213:1340–7.
3. Romero R, Miranda J, Kusanovic JP, et al. Clinical chorioamnionitis at term: I, microbiology of the amniotic cavity using cultivation and molecular techniques. J Perinat Med 2015;43:19–36.
4. Oh KJ, Lee SE, Jung H, Kim G, Romero R, Yoon BH. Detection of ureaplasmas by the polymerase chain reaction in the amniotic fluid of patients with cervical insufficiency. J Perinat Med 2010;38:261–8.
5. Yoon BH, Romero R, Kim M, et al. Clinical implications of detection of Ureaplasma urealyticum in the amniotic cavity with the polymerase chain reaction. Am J Obstet Gynecol 2000;183:1130–7.
6. Yoon BH, Romero R, Lim JH, et al. The clinical significance of detecting Ureaplasma urealyticum by the polymerase chain reaction in the amniotic fluid of patients with preterm labor. Am J Obstet Gynecol 2003;189:919–24.
7. Yoon BH, Romero R, Park JS, et al. 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 1998;179:1254–60.
8. Goldenberg RL, Andrews WW, Goepfert AR, et al. The Alabama Preterm Birth Study: umbilical cord blood Ureaplasma urealyticum and Mycoplasma hominis cultures in very preterm newborn infants. Am J Obstet Gynecol 2008;198:43.e1–5.
9. Breugelmans M, Vancutsem E, Naessens A, Laubach M, Foulon W. Association of abnormal vaginal flora and Ureaplasma species as risk factors for preterm birth: a cohort study. Acta Obstet Gynecol Scand 2010;89:256–60.
10. Vogel I, Thorsen P, Hogan VK, Schieve LA, Jacobsson B, Ferre CD. The joint effect of vaginal Ureaplasma urealyticum and bacterial vaginosis on adverse pregnancy outcomes. Acta Obstet Gynecol Scand 2006;85:778–85.
11. Rittenschober-Bohm J, Waldhoer T, Schulz SM, et al. First trimester vaginal ureaplasma biovar colonization and preterm birth: results of a prospective multicenter study. Neonatology 2017;113:1–6.
12. Robertson JA, Stemke GW, Davis JW Jr, et al. 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 2002;52:587–97.
13. Kim M, Kim G, Romero R, Shim SS, Kim EC, Yoon BH. Biovar diversity of Ureaplasma urealyticum in amniotic fluid: distribution, intrauterine inflammatory response and pregnancy outcomes. J Perinat Med 2003;31:146–52.
14. Abele-Horn M, Wolff C, Dressel P, Pfaff F, Zimmermann A. Association of Ureaplasma urealyticum biovars with clinical outcome for neonates, obstetric patients, and gynecological patients with pelvic inflammatory disease. J Clin Microbiol 1997;35:1199–202.
15. Kataoka S, Yamada T, Chou K, et al. Association between preterm birth and vaginal colonization by mycoplasmas in early pregnancy. J Clin Microbiol 2006;44:51–5.
16. Xiao L, Glass JI, Paralanov V, et al. Detection and characterization of human Ureaplasma species and serovars by real-time PCR. J Clin Microbiol 2010;48:2715–23.
17. Payne MS, Ireland DJ, Watts R, et al. Ureaplasma parvum genotype, combined vaginal colonisation with Candida albicans, and spontaneous preterm birth in an Australian cohort of pregnant women. BMC Pregnancy Childbirth 2016;16:312.
18. Martinez MA, Ovalle A, Santa-Cruz A, Barrera B, Vidal R, Aguirre R. Occurrence and antimicrobial susceptibility of Ureaplasma parvum (Ureaplasma urealyticum biovar 1) and Ureaplasma urealyticum (Ureaplasma urealyticum biovar 2) from patients with adverse pregnancy outcomes and normal pregnant women. Scand J Infect Dis 2001;33:604–10.
19. Paralanov V, Lu J, Duffy LB, et al. Comparative genome analysis of 19 Ureaplasma urealyticum and Ureaplasma parvum strains. BMC Microbiol 2012;12:88.
20. De Francesco MA, Negrini R, Pinsi G, Peroni L, Manca N. 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 2009;28:641–6.
21. Xiao L, Paralanov V, Glass JI, et al. Extensive horizontal gene transfer in ureaplasmas from humans questions the utility of serotyping for diagnostic purposes. J Clin Microbiol 2011;49:2818–26.
22. Ireland DJ, Keelan JA. The maternal serological response to intrauterine ureaplasma sp. infection and prediction of risk of pre-term birth. Front Immunol 2014;5:624.
23. Payne MS, Tabone T, Kemp MW, Keelan JA, Spiller OB, Newnham JP. High-resolution melt PCR analysis for genotyping of Ureaplasma parvum isolates directly from clinical samples. J Clin Microbiol 2014;52:599–606.
24. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991;29:297–301.
25. Stephens AS, Lain SJ, Roberts CL, Bowen JR, Nassar N. Survival, hospitalization, and acute-care costs of very and moderate preterm infants in the first 6 years of life: a population-based study. J Pediatr 2016;169:61–8.e3.
26. Morency AM, Bujold E. The effect of second-trimester antibiotic therapy on the rate of preterm birth. J Obstet Gynaecol Can 2007;29:35–44.

27. Eschenbach DA, Nugent RP, Rao AV, et al. A randomized placebo-controlled trial of erythromycin for the treatment of Ureaplasma urealyticum to prevent premature delivery: The Vaginal Infections and Prematurity Study Group. Am J Obstet Gynecol 1991;164:734–42.
28. Naessens A, Foulon W, Breynaert J, Lauwers S. Serotypes of Ureaplasma urealyticum isolated from normal pregnant women and patients with pregnancy complications. J Clin Microbiol 1988;26:319–22.
29. Dando SJ, Nitsos I, Kallapur SG, et al. The role of the multiple banded antigen of Ureaplasma parvum in intra-amniotic infection: major virulence factor or decoy? PLoS One 2012;7:e29856.
30. Knox CL, Dando SJ, Nitsos I, et al. 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 2010;83:415–26.
31. Sweeney EL, Kallapur SG, Meawad S, et al. Ureaplasma species multiple banded antigen (MBA) variation is associated with the severity of inflammation in vivo and in vitro in human placentae. Front Cell Infect Microbiol 2017;7:123.
32. Digiulio DB, Callahan BJ, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci U S A 2015;112:11060–5.
33. Hyman RW, Fukushima M, Jiang H, et al. Diversity of the vaginal microbiome correlates with preterm birth. Reprod Sci 2014;21:32–40.
34. Romero R, Hassan SS, Gajer P, et al. The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term. Microbiome 2014;2:1–15.
35. Romero R, Hassan SS, Gajer P, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome 2014;2:4.
36. Harada K, Tanaka H, Komori S, et al. Vaginal infection with Ureaplasma urealyticum accounts for preterm delivery via induction of inflammatory responses. Microbiol Immunol 2008;52:297–304.
37. Donders GG, Ruban K, Bellen G, Petricevic L. Mycoplasma/Ureaplasma infection in pregnancy: to screen or not to screen. J Perinat Med 2017;45:505–15.
38. Foschi C, Salvo M, D'Antuono A, et al. Distribution of genital Mollicutes in the vaginal ecosystem of women with different clinical conditions. New Microbiol 2018;41:225–9.
39. Gomez R, Romero R, Nien JK, et al. Antibiotic administration to patients with preterm premature rupture of membranes does not eradicate intra-amniotic infection. J Matern Fetal Neonatal Med 2007;20:167–73.
40. Antsaklis A, Daskalakis G, Michalas S, Aravantinos D. Erythromycin treatment for subclinical Ureaplasma urealyticum infection in preterm labor. Fetal Diagn Ther 1997;12:89–92.
41. Vouga M, Greub G, Prod'hom G, et al. Treatment of genital mycoplasma in colonized pregnant women in late pregnancy is associated with a lower rate of premature labour and neonatal complications. Clin Microbiol Infect 2014;20:1074–9.
42. Dando SJ, Nitsos I, Newnham JP, Jobe AH, Moss TJ, Knox CL. Maternal administration of erythromycin fails to eradicate intrauterine ureaplasma infection in an ovine model. Biol Reprod 2010;83:616–22.
43. Witt A, Sommer EM, Cichna M, et al. Placental passage of clarithromycin surpasses other macrolide antibiotics. Am J Obstet Gynecol 2003;188:816–9.
44. Acosta EP, Grigsby PL, Larson KB, et al. Transplacental transfer of Azithromycin and its use for eradicating intra-amniotic ureaplasma infection in a primate model. J Infect Dis 2014;209:898–904.
45. Keelan JA, Kemp MW, Payne MS, et al. 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 2014;58:447–54.
46. Miura Y, Payne MS, Keelan JA, et al. 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 2014;58:5413–20.
47. Payne MS, Furfaro LL, Tucker R, Tan LY, Mokany E. One-step simultaneous detection of Ureaplasma parvum and genotypes SV1, SV3 and SV6 from clinical samples using PlexPCR technology. Lett Appl Microbiol 2017;65:153–8.
48. Sung TJ, Xiao L, Duffy L, Waites KB, Chesko KL, Viscardi RM. Frequency of ureaplasma serovars in respiratory secretions of preterm infants at risk for bronchopulmonary dysplasia. Pediatr Infect Dis J 2011;30:379–83.
49. Kasper DC, Mechtler TP, Reischer GH, et al. The bacterial load of Ureaplasma parvum in amniotic fluid is correlated with an increased intrauterine inflammatory response. Diagn Microbiol Infect Dis 2010;67:117–21.
50. Aaltonen R, Heikkinen J, Vahlberg T, Jensen JS, Alanen A. Local inflammatory response in choriodecidua induced by Ureaplasma urealyticum. BJOG 2007;114:1432–5.
51. Kiss H, Petricevic L, Husslein P. Prospective randomised controlled trial of an infection screening programme to reduce the rate of preterm delivery. BMJ 2004;329:371.
52. Kekki M, Kurki T, Pelkonen J, Kurkinen-Raty M, Cacciatore B, Paavonen J. Vaginal clindamycin in preventing preterm birth and peripartal infections in asymptomatic women with bacterial vaginosis: a randomized, controlled trial. Obstet Gynecol 2001;97:643–8.
53. Subtil D, Brabant G, Tilloy E, et al. Early clindamycin for bacterial vaginosis in pregnancy (PREMEVA): a multicentre, double-blind, randomised controlled trial. Lancet 2018;392:2171–9.
54. Ugwumadu A, Manyonda I, Reid F, Hay P. Effect of early oral clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: a randomised controlled trial. Lancet 2003;361:983–8.

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