Intrauterine infection is a possible cause of preterm labour and preterm delivery (Hay et al., 1994; Lamont et al., 1986; Romero et al., 2006). It is presumed that the microorganisms from the lower genital tract can ascend towards the amniotic fluid causing intra-amniotic infection, which may lead to preterm labour and delivery. However, the reason why this occurs during pregnancy for a small group of women remains elusive. One of the micro-organisms commonly described as a risk factor for pregnancy complications is *Ureaplasma* spp. Well-defined case reports and studies have shown that *Ureaplasma* spp. may be considered as an agent able to cause adverse pregnancy outcome, such as preterm rupture of the membranes, low birth weight, late miscarriage and preterm birth (Abele-Horn et al., 1997; Breugelmans et al., 2010; Gerber et al., 2003; Oh et al., 2010; Vogel et al., 2006; Yoon et al., 1998). The high colonization rate of normal pregnant women with *Ureaplasma* spp. (40–80 %) impedes the study of the pathogenicity of this micro-organism in pregnancy complications. However, it emphasizes the importance of research on cofactors enabling *Ureaplasma* spp. to cause an ascending infection which ultimately interferes with normal foetal development. One of the cofactors which might facilitate an ascending infection is the association of an abnormal vaginal flora and the presence of *Ureaplasma* spp. colonization; such a combination was shown to be associated with a higher risk for preterm delivery [odds ratio (OR) 2.35] as compared with women with *Ureaplasma* spp. alone (OR 1.64) (Breugelmans et al., 2010). In the current study, we further analysed the association between the two human *Ureaplasma* spp., i.e. *Ureaplasma parvum* and *Ureaplasma urealyticum*, and their presence in normal and abnormal vaginal flora. We sought to answer the following questions. Is there a species which is more often associated with an abnormal flora or not? Is the bacterial load of both *Ureaplasma* spp. different in normal versus abnormal vaginal flora?

A prospective study was conducted in the antenatal clinic of the Universitair Ziekenhuis Brussel, Brussels, Belgium. In total, 596 women agreed to participate. During their first prenatal visit two lower genital tract swabs were obtained: one swab was rolled on a glass slide to evaluate the vaginal flora; the second swab was placed in Universal Transport Medium (Copan) for detection of *Ureaplasma* spp. by real-time (RT)–PCR. Micro-organisms in the clinical specimens transferred onto the glass slides were heat-fixed and Gram-stained. The vaginal flora was evaluated as described previously (Breugelmans et al., 2010): normal vaginal flora was defined as when predominantly lactobacilli were present in the Gram stain. When predominantly Gram-variable rods or mixed flora with reduced or no lactobacilli were present in the Gram stain, this was defined as abnormal vaginal flora.

RT-PCR, allowing detection, quantification and differentiation between the two human *Ureaplasma* spp. (*U. parvum* and *U. urealyticum*) was performed as described previously (Vancutsem et al., 2011). This PCR uses two separate reaction mixtures containing primer pairs and minor groove binder TaqMan probes for detection of the *Ureaplasma* spp. Quantification limits of the assay were between 7.5 × 10³ and 7.5 × 10⁵ copies per specimen. PCRs were performed on the LightCycler 480 II platform (Roche Diagnostics). Interpretation was done by using the second derivative method from LightCycler 480 software version 1.5.

Fisher’s exact test was used for the evaluation of the relationship between the vaginal flora and the presence of *U. parvum* and *U. urealyticum*. Quantitative data were normalized by log₁₀ transformation for comparison of the bacterial loads. The means of log₁₀ copies per sample of *U. parvum* and *U. urealyticum* in positive samples were compared using an unpaired *t*-test. *P*<0.05 was used to indicate statistical significance. All calculations were carried out using InStat version 4 for Mac (GraphPad).

Out of the 596 samples collected, RT-PCR was positive for *Ureaplasma* spp. in 275 (46.1 %) samples. *U. parvum* was the predominant species and was found in 84.7 % of the positive samples, *U. urealyticum* was present in only 22.2 %, whereas both species were found in 19 samples (6.9 %) (Table 1). These results are in agreement with other studies where *U. parvum* was also found to be predominant (Abele-Horn et al., 1997; Kong et al., 2000; Naessens et al., 1988).

Gram stain classified 486 (81.5 %) samples as normal vaginal flora and 110 (18.5 %) samples as abnormal vaginal flora. *Ureaplasma* spp. were significantly more often isolated from patients with an abnormal vaginal flora than from women with a normal vaginal flora (55.5 versus 44.0 % respectively, *P*=0.034) (Table 1). The significant difference is mainly attributed to *U. urealyticum*: this species was found in only 8.4 % of the women with a normal vaginal flora and in 18.2 % of the samples showing an abnormal vaginal flora (*P*=0.005). For *U. parvum*-positive samples, no difference in detection between normal and abnormal vaginal flora was found (*P*=0.39) (Table 1). This might indicate a higher pathogenic potential for *U. urealyticum* as compared with *U. parvum*. Previous reports also indicate a more pathogenic potential for *U. urealyticum*: the presence of *U. urealyticum* has more frequently been associated with recurrent miscarriage, persistent and recurrent urethritis, pelvic inflammatory disease and preterm delivery (Abele-Horn et al., 1997; Naessens et al., 1988).

Genital tract colonization with *Ureaplasma* spp. and its association with abnormal vaginal flora
Nineteen samples contained both group and 6 in the group with abnormal vaginal flora).

Ureaplasma spp. Overall quantitative mal vaginal flora and the bacterial load of high bacterial load and chorioamnionitis significant independent correlation with values for , , (2000) investigated the bacterial coloniza-
tion rate of (2007). Not only the micro-organism by itself, but as a factor of virulence: Abele-Horn et al. (2004) and found a significant independent correlation with high bacterial load and chorioamnionitis and preterm delivery. In our study, we did not detect any correlation between abnormal vaginal flora and the bacterial load of Ureaplasma spp. Overall quantitative values for Ureaplasma spp. ranged from 3.88 to 7.87 log10 copies per sample with a mean of 5.51 log10 copies per sample, and were statistically not different in samples with normal and abnormal vaginal flora (P>0.05). The mean concentration of Ureaplasma spp. in the group of positive women was 4.88 (range <3.88–7.64) log10 copies per sample for U. urealyticum and 5.67 (range <3.88–7.87) log10 copies per sample for U. parvum. No significant difference in bacterial load of any Ureaplasma spp. was seen between women with normal or abnormal vaginal flora.

To date, a good marker for prediction of prematurity and adverse pregnancy outcome does not exist. The presence of abnormal vaginal flora in combination with the presence of Ureaplasma spp. appears to be a risk factor (OR 2.35) (Breugelmans et al., 2010). However, the specificity of this co-existence of abnormal vaginal flora and Ureaplasma spp. is still too low and a more specific marker for adverse pregnancy outcome is necessary. As we have been able to establish a relationship between U. urealyticum and an abnormal vaginal flora in this study, this could be an interesting parameter to investigate as a marker for prematurity. A possible explanation for the pathogenic effect of this association during pregnancy may be due to the loss of the protective role of the commensal flora. This enables other micro-organisms such as Ureaplasma spp. to cause an ascending infection which leads to adverse pregnancy outcome. Further studies should be performed to evaluate if species determination of Ureaplasma spp. with PCR in combination with the evaluation of the vaginal flora could be valuable as a marker for prematurity.

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Abbreviations: OR, odds ratio; RT, real-time.


<table>
<thead>
<tr>
<th>Samples*</th>
<th>All samples (N=596) [n (%)]</th>
<th>Normal vaginal flora (N=486) [n (%)]</th>
<th>Abnormal vaginal flora (N=110) [n (%)]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ureaplasma spp.</td>
<td>275 (46.1)</td>
<td>214 (44.0)</td>
<td>61 (55.5)</td>
<td>0.034</td>
</tr>
<tr>
<td>U. parvum</td>
<td>233 (39.1)</td>
<td>186 (38.3)</td>
<td>47 (42.7)</td>
<td>0.389</td>
</tr>
<tr>
<td>U. urealyticum</td>
<td>61 (10.2)</td>
<td>41 (8.4)</td>
<td>20 (18.2)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Nineteen samples contained both U. parvum and U. urealyticum (13 in the normal vaginal flora group and 6 in the group with abnormal vaginal flora).