BRIEF REPORT

Detection of *Ureaplasma urealyticum* in Second-Trimester Amniotic Fluid by Polymerase Chain Reaction Correlates with Subsequent Preterm Labor and Delivery

Stefan Gerber,[1,2] Yvan Vial,[2] Patrick Hohlfeld,[2] and Steven S. Witkin[1]

[1]Division of Immunology and Infectious Diseases, Department of Obstetrics and Gynecology, Weill Medical College of Cornell University, New York, New York; [2]Department of Gynecology and Obstetrics, Centre Hospitalier Universitaire Vandois, Lausanne, Switzerland

***Ureaplasma urealyticum* is the microorganism most frequently isolated from the amniotic fluid of women in preterm labor. The relationship between intra-amniotic *U. urealyticum* in healthy second-trimester pregnant women and subsequent pregnancy outcome was investigated. Transabdominal amniotic fluid obtained from 254 asymptomatic women at 15–17 weeks' gestation were tested by polymerase chain reaction (PCR). *U. urealyticum* was identified in 29 subjects (11.4%). A subsequent preterm labor occurred in 17 *U. urealyticum*–positive women (58.6%), compared with 10 (4.4%) *U. urealyticum*–negative women ($P < .0001$). Preterm birth was documented in 7 (24.1%) *U. urealyticum*–positive women compared with only 1 *U. urealyticum*–negative woman (0.4%) ($P < .0001$). *U. urealyticum*–positive women also had a higher prevalence of preterm labor in a prior pregnancy (20.7%) than did the negative women (2.7%; $P = .0008$). PCR testing of second-trimester amniotic fluid for *U. urealyticum* can identify women at risk for subsequent preterm labor and delivery.**

Ureaplasma urealyticum frequently colonizes the lower genital tract of pregnant women, without apparent adverse consequences [1]. In a small subpopulation of women, this microorganism ascends and colonizes the endometrium, either before or after conception [2]. The difficulty resides in identifying the group at risk for ascending colonization. Passage of *U. urealyticum* into the amniotic cavity appears to be an important step for prognosis of the pregnancy. Colonization of the amniotic fluid has been documented as a major risk factor, but usually the diagnosis is made too late for successful intervention [3]. To minimize a possible adverse influence of *U. urealyticum* in pregnancy outcome, a protocol to successfully identify this microorganism early in the pregnancy would be beneficial.

U. urealyticum is the microorganism most frequently cultured by use of standard culture techniques from amniotic fluids of women in preterm labor (PTL) with intact membranes [4] or with preterm premature rupture of membranes (P-PROM) [5]. Whether *U. urealyticum* intra-amniotic colonization in asymptomatic women during the second trimester is also a risk factor for subsequent adverse pregnancy outcome has been examined in only a few studies. *U. urealyticum* culture–positive amniotic fluids have been observed in a small percentage of healthy asymptomatic women in the second trimester of pregnancy [6–9]. In each of these studies, a positive *U. urealyticum* culture was associated with an increased risk of adverse pregnancy outcome in untreated patients, compared with those who were culture-negative or who received antibiotic treatment for this microorganism.

A recent study that used polymerase chain reaction (PCR) to detect *U. urealyticum* in amniotic fluids from women with P-PROM demonstrated that PCR was more sensitive than culture in detecting this microorganism in amniotic fluid and that PCR-positive, culture-negative women were at high risk for adverse pregnancy outcomes [10]. The objective of the present investigation was to perform PCR testing on a large number of second-trimester amniotic fluids from healthy asymptomatic women to evaluate the prevalence of this mycoplasma and whether its detection is associated with P-PROM or preterm birth.

Subjects, materials, and methods. Amniotic fluid samples were collected for analysis from 317 consecutive women who underwent a transabdominal amniocentesis at weeks 15–17 of pregnancy and were stored at −80°C. Indications for amniocentesis were advanced maternal age, family history of chromosomal abnormality, maternal request, or positive detection on "triple test" for alpha fetoprotein, estriol, and beta human chorionic gonadotropin. The same fetal ultrasound team per-

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Informed consent was obtained from patients, and human experimentation guidelines of the US Department of Health and Human Services and Centre Hospitalier, Lausanne, Switzerland, were followed in the conduct of clinical research.

Reprints or correspondence: Dr. Steven S. Witkin, Dept. of Obstetrics and Gynecology, Weill Medical College of Cornell University, 525 E. 68th St., Box 35, New York, NY 10021 (switkin@med.cornell.edu).

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Table 1. Relationship between *Ureaplasma urealyticum* in amniotic fluid and historical variables.

Variable	*U. urealyticum* status		*P*
	Positive	Negative	
Pregnancy, mean no. (range)	2.3 (1–5)	2.5 (1–9)	
Birth, mean no. (range)	0.9 (0–4)	1.0 (0–4)	
Age, mean years (range)	34.5 (25–42)	34.0 (19–42)	
Previous preterm birth, no. (%)	6 (20.7)	6 (2.7)	.0008

formed all procedures, using the identical method in every case. Before the transabdominal amniocentesis, the skin was disinfected with 10% povidine iodine.

All women were white and of European background. Subsequent pregnancy follow-up revealed that 63 (19.9%) of the subjects had either an elective pregnancy termination or a complicating pregnancy factor that placed them at risk for an adverse outcome: placenta praevia, intrauterine growth restriction, hypertension, cervical incompetence, or twin gestation. The remaining 254 women had no known risk factor for adverse pregnancy outcome.

U. urealyticum in amniotic fluid was detected by PCR using a procedure detailed elsewhere [11], in which primer pairs to a region of the urease gene are used, except that digoxigenin-labeled dUTP was added to the reaction mixture. To ensure specificity, the PCR amplicons were hybridized to a biotinylated oligonucleotide internal probe (5′-biotin-GCC CAC CAA GAC TAT GAT GTT TAG-3′) and the complex detected in duplicate by ELISA using streptavidin-coated wells of a microtiter plate and peroxidase-labeled anti-digoxigenin antibody (Roche Diagnostics).

Stringent precautions used to prevent PCR product carryover were the frequent changing of gloves and performance of the processing, PCR, and hybridization in different rooms. Samples were analyzed without knowledge of pregnancy outcomes.

Clinical pregnancy outcome data were obtained only after the completion of all testing in a blinded fashion directly from the patient and her private obstetrician. "PTL" was defined as regular uterine contractions that indicated the use of a tocolytic drug and bed rest at home after the exclusion of other etiologies (i.e., bladder infection, cervical infection, or cervical incompetence). P-PROM was diagnosed by objective amniotic fluid leakage (based on history, physical examination, and laboratory testing) and/or diminution of amniotic fluid index before 37 weeks of pregnancy. In such cases, the condition of the patient was managed by observation, in the absence of any infection or fetal distress.

The relation between PCR outcome and other variables was analyzed by Fisher's exact test. $P < .05$ was considered to be significant.

Results. During the 6-month study period, the same group of physicians performed 317 amniocenteses. Of these, there was 1 pregnancy loss 2 weeks after, and 2 other patients developed vaginal bleeding 24 and 48 h after the amniocentesis. Both recovered after prolonged bed rest. Subsequent examination of medical records revealed that 16 patients had an elective termination of the pregnancy, 2 had cervical incompetence, and 45 had a major complicating pregnancy factor. All were negative for *U. urealyticum*, except for 2 sets of twin children.

U. urealyticum was detected by PCR in amniotic fluid from 29 (11.4%) of the remaining 254 asymptomatic women with a singleton pregnancy. The relationship between *U. urealyticum* detection and historical variables in these patients is shown in table 1. There was no relationship between *U. urealyticum* colonization and the age or number of previous pregnancies or births. However, women positive for *U. urealyticum* had a higher occurrence of PTL in a prior pregnancy (20.7%) than did the PCR-negative women (2.7%; $P = .0008$). Eight of the amniotic fluid samples were visibly discolored; none of these were positive for *U. urealyticum*.

The relationship between *U. urealyticum* detection and outcome of the pregnancy is shown in table 2. Intra-amniotic carriage of *U. urealyticum* was highly associated with PTL, hospitalization for PTL, P-PROM, and preterm birth before 37 weeks ($P < .0001$). Delivery before 34 weeks' gestation was observed in 2 of the *U. urealyticum*–positive women and in none of the *U. urealyticum*–negative women ($P = .01$).

Discussion. In our study of 254 asymptomatic pregnant patients in their early second trimester, 3% subsequently developed P-PROM and 10% underwent PTL, rates that were in agreement with other studies [12]. Similarly, our incidence of pregnancy loss (0.3%) and complication after midtrimester amniocentesis (0.9%) corresponded to traditional rates [13].

In the present study, detection of *U. urealyticum* by PCR in second-trimester amniotic fluid of asymptomatic women was highly correlated with subsequent PTL and preterm delivery. The findings are similar to earlier investigations that examined the impact on pregnancy of having a positive second-trimester

Table 2. Relationship between *Ureaplasma urealyticum* in amniotic fluid and pregnancy outcome.

Outcome variable	*U. urealyticum* status		*P*
	Positive ($n = 29$)	Negative ($n = 225$)	
PTL	17 (58.6)	10 (4.4)	<.0001
Hospital stay for PTL	9 (31.0)	6 (2.7)	<.0001
Birth			
Before 37 weeks	7 (24.1)	1 (0.4)	<.0001
Before 34 weeks	2 (6.9)	0	.01
P-PROM	6 (20.7)	1 (0.4)	<.0001
Cesarean section	9 (31.0)	56 (24.9)	NS

NOTE. Data are no. (%) of subjects. NS, not significant; P-PROM, preterm premature rupture of membranes; PTL, preterm labor.

amniotic fluid culture for *U. urealyticum* [6–9]. However, the percentage of *U. urealyticum*–positive amniotic fluid samples in our study exceeded that found in previous investigations that used culture techniques. Possible explanations include the enhanced sensitivity of PCR over culture for detection of this microorganism in amniotic fluid [10] and/or population differences in lower genital tract colonization or migration of *U. urealyticum* to the upper genital tract. The extent of *U. urealyticum* colonization is known to vary widely among populations in different geographic areas [8]. *U. urealyticum* cultures were not performed on amniotic fluids in the present study, so we are unable to compare the sensitivity of our PCR with culture for these samples.

Women in the present study who were positive for intra-amniotic *U. urealyticum* also had a higher rate of PTL in previous pregnancies than did PCR-negative women. This observation is consistent with the possibility that *U. urealyticum* might have been present in the endometrium as a persistent colonizer before the current conception. This raises the interesting possibility that it might be of value to sample the endometrium for *U. urealyticum* prior to attempted conception in women with a prior preterm birth. Detection and treatment might improve subsequent pregnancy outcome. It has been proposed elsewhere that preexisting infection of the uterine cavity was a predisposing factor for subsequent adverse pregnancy outcomes [14].

It remains uncertain whether *U. urealyticum* directly induced the observed pregnancy complications or whether this organism was merely a marker for another infection. It has been suggested that the significance of *U. urealyticum* in amniotic fluid is mainly its association with other microorganisms [15]. However, when standard culture techniques are used, *U. urealyticum* is often the sole microorganism identified in the amniotic fluid of women in PTL and, furthermore, is associated with elevated intra-amniotic levels of proinflammatory cytokines [4, 6, 9, 10]. Studies that use more refined gene amplification analyses are necessary to definitively rule out the presence of additional microorganisms in *U. urealyticum*–positive amniotic fluid samples and their relationship to adverse pregnancy outcome.

It should be noted that 76% of our patients with intra-amniotic *U. urealyticum* gave birth at $\geqslant$37 weeks, whereas only 21% of the *U. urealyticum*–positive patients developed P-PROM and 24% gave birth preterm. It would be of great interest to identify markers predictive of adverse pregnancy outcomes in the *U. urealyticum*–positive group. Perhaps a quantitative PCR or a combination of PCR and culture to identify the extent of infection, would be of value. Evaluation of the inflammatory response in amniotic fluid, determined by levels of proinflammatory cytokines and matrix metalloproteinases, or the extent of a stress response, measurable by heat shock protein determination, could provide details of the fetal response to this microorganism. In recent unpublished studies from our laboratory, possession of allele 2 of the polymorphic interleukin-1 receptor antagonist gene also appeared to increase the rate of adverse pregnancy outcome after *U. urealyticum* infection. A detailed evaluation of a combination of such markers may eventually lead to a method to define the high-risk group in whom antibiotic treatment may prove beneficial.

Nevertheless, the highly significant association between intra-amniotic *U. urealyticum* and adverse pregnancy outcome strongly suggests the potential value of PCR testing of pregnant women in their second trimester for this organism. Routine screening for *U. urealyticum* in amniotic fluid at the time of midtrimester amniocentesis can identify the group of patients who are infected with this microorganism. Further studies are needed to assess whether all women with risk factors for prematurity, or all women who undergo a second-trimester amniocentesis for other indications, might benefit from *U. urealyticum* amniotic fluid testing. In addition, the possible use of new antibiotic treatments such as azithromycin, which exhibits an increased ease of transplacental passage, to improve pregnancy outcome in *U. urealyticum* intra-amniotic infection awaits further investigation.

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