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# A high Nugent score but not a positive culture for genital mycoplasmas is a risk factor for spontaneous preterm birth

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## Abstract

**Objective—**This study was performed to evaluate the relationship among the Nugent score for the diagnosis of bacterial vaginosis (BV), the results of vaginal fluid culture for genital mycoplasma, and the subsequent occurrence of preterm birth.

**Methods—**The Nugent score and culture for genital mycoplasmas were performed in vaginal fluid obtained from 977 pregnant women (gestational age 13–30 weeks). Vaginal samples were obtained with sterile cotton swabs. The relationship among the Nugent score, vaginal fluid culture results and the occurrence of spontaneous preterm birth was examined.

**Results—**(1) Of the 977 women, 14% (137) had a Nugent score of ≥ 8; (2) The prevalence of a positive vaginal culture for genital mycoplasmas was 30% (288); *Ureaplasma urealyticum* was isolated in 252 (88%), *Mycoplasma hominis* in 9 (3%), and both in 27 (9%) women; (3) Cases with a Nugent score of ≥ 8 had a higher rate of a positive vaginal culture for genital mycoplasmas than those with the lower Nugent score (55% vs. 25%; $p<0.001$); (4) Women with a Nugent score of ≥ 8 had a significantly higher rate of spontaneous preterm birth <37 (10% vs. 4%), <34 (5% vs. 2%), and <32 (4% vs. 1%) weeks of gestation than those with the lower Nugent score (At each gestational age, $p<0.05$); (5) In contrast, a positive vaginal culture for genital mycoplasmas was not associated with an increased risk for spontaneous preterm birth; (6) Among patients with a positive culture and a Nugent score of ≥ 8, the frequency of spontaneous preterm delivery (<37 weeks) was 10% (7/72); (7) There was no difference in the incidence of spontaneous preterm delivery according to the results of vaginal culture in patients with a Nugent score of ≥ 8, as well as in those with a lower Nugent score.

**Conclusion—**A high Nugent score (≥ 8) for the detection of BV but not a positive vaginal culture for genital mycoplasmas is a risk factor for spontaneous preterm birth.

### Keywords

Nugent score; bacterial vaginosis; genital mycoplasmas; Ureaplasma urealyticum; Mycoplasma hominis; preterm birth; prematurity

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## INTRODUCTION

Strong evidence supports a relationship between ascending intrauterine infection and spontaneous preterm delivery [1–7].

Bacterial vaginosis (BV), a change in the vaginal ecosystem, is a risk factor for spontaneous preterm birth [6–9]. The Nugent score describes gradations of vaginal flora detected on a Gram stain and is thought to represent changes in the microbial environment of the vagina.

*Ureaplasma urealyticum* is the most common microorganism found in the amniotic cavity [3,10,11]. A multicentre, randomized, double-blind clinical trial showed the antibiotic treatment for vaginal Ureaplasma was not associated with a reduced prematurity [12].

The aim of this study was to evaluate the relationship among the Nugent score, the results of vaginal culture for genital mycoplasmas and the subsequent occurrence of preterm birth.

## MATERIALS AND METHODS

### Study design

A retrospective cohort study was performed to examine the relationship among the Nugent score, the results of vaginal culture for genital mycoplasmas and the subsequent occurrence of preterm birth. The cohort consisted of 977 women who visited Seoul National University Hospital for routine antenatal care (gestational age between 13 and 30 weeks) from April 2002 to December 2004 and in whom vaginal fluid was examined for the Nugent score and cultured for genital mycoplasmas. The Institutional Review Board approved the collection and the use of specimens and clinical information for research purposes. The Seoul National University has a Federal Wide Assurance with the OHRP of the Department of Health and Human Services of the United States.

### Vaginal fluid studies

Vaginal fluid was obtained from the posterior fornix of the vagina with sterile cotton swabs. A swab was placed in a transport medium and sent for culture for mycoplasmas. Vaginal fluid on the other swab was spread on a glass, allowed to air dry and transported to the microbiology laboratory for a Nugent score determination. A Nugent score of 0–10 was assigned by a clinical microbiologist. Mycoplasma culture was performed using the MycoFast® (international Microbio, France) test according to the manufacturer's instructions. The results of vaginal fluid studies were not used in patient management.

### Pregnancy outcome

Spontaneous preterm birth was defined as a birth following spontaneous labour (regular uterine contractions accompanied by cervical changes), spontaneous preterm premature rupture of membranes or painless cervical dilatation, regardless of the mode of delivery (<37 weeks). Cases with any of the above mentioned conditions before the sampling of vaginal fluid or those who underwent determination of pregnancy after the sampling were not included in the cohort.

### Statistical analysis

Student's *t*-test, chi-square test and Fisher's exact test were used as appropriate. A probability value of <0.05 was considered significant.

# RESULTS

## Study population

The mean gestational age at sampling was 17 ± 1.4 weeks (mean ± SD; range, 12–30 weeks) and at delivery was 39 ± 2.8 weeks (mean ± SD; range, 17–42 weeks). The prevalence of preterm delivery (<37 weeks of gestation was 9% (83/977), and that of spontaneous preterm birth was 5% (48/977). Among the 48 spontaneous preterm births, 23 cases were due to preterm labour, 21 to preterm premature rupture of membranes and 4 to cervical insufficiency. The clinical indications for 35 indicated preterm deliveries included preeclampsia ($n$ = 20), intrauterine fetal death ($n$ = 6; 3 during the anticoagulation therapy for the mechanical heart valve and 3 of unknown etiology), severe systemic lupus erythematosis ($n$ = 3) and others ($n$ = 6).

## Nugent score and vaginal fluid mycoplasma culture

Of 977 women enrolled, 14% (137) had a Nugent score of 8 or greater. The prevalence of a positive vaginal fluid culture for genital mycoplasmas was 30% (288/977); *Ureaplasma urealyticum* was isolated in 252 (88%), *Mycoplasma hominis* in 9 (3%), and both in 27 (9%) cases. Tables I and II display the clinical characteristics of the study population according to the Nugent score and the result of vaginal fluid culture for genital mycoplasmas. The mean maternal age, mean gestational age at sampling and delivery, and proportion of parous women, twin gestation, and those with a history of previous preterm delivery were not different according to the Nugent score above or below 8 or the result of vaginal fluid culture for mycoplasmas. However, the prevalence of a positive vaginal fluid culture for genital mycoplasmas was significantly higher in women with a Nugent score of ≥ 8 than in those with a Nugent score of <8 (Table I). Furthermore, women with a Nugent score had a higher rate of positive vaginal fluid cultures for genital mycoplasmas ($p$ = 0.000 by Pearson chi-square test, Table II).

## Nugent score and subsequent preterm birth

Table III compares the occurrence of preterm birth at different gestational ages between women with a Nugent score of ≥ 8 and those with a lower Nugent score. Women with a Nugent score of ≥ 8 had a significantly increased preterm birth rate at < 37 (14% vs. 8%), < 36 (10% vs. 6%) and <35 (9% vs. 4%) weeks of gestation compared to those with the lower Nugent score (at each gestational age, $p$<0.05). Moreover, the prevalence of subsequent spontaneous preterm birth at < 37 (10% vs. 4%), < 36 (8% vs. 3%), < 35 (7% vs. 2%), < 34 (4% vs. 2%) and < 32 (4% vs. 1%) weeks of gestation was significantly higher in women with a Nugent score of ≥ 8 ($p$ <0.05 at <32 and <34 weeks).

## Vaginal fluid mycoplasma culture and subsequent preterm birth

A positive vaginal culture for mycoplasmas was not associated with an increased rate of subsequent spontaneous preterm birth. Among women with a positive vaginal culture, spontaneous preterm deliveries occurred in 3.2% (9/283) at <34 weeks and in 4.2% (12/283) at 35 weeks of gestation. The frequency was not significantly different among women with a negative vaginal culture for mycoplasmas, which were 1.5% (10/675) at < 34 and 2.1% (14/673) at < 35 weeks of gestation ($p$ = 0.09 at < 34, and $p$ = 0.06 at < 35 weeks of gestation).

## Nugent score combined with a culture for vaginal fluid mycoplasma and subsequent preterm birth

Patients were divided into four groups according to the Nugent score and vaginal mycoplasma culture result; Group 1, women with a Nugent score of ≥ 8 and a positive

vaginal culture ($n$ = 75); Group 2, women with a Nugent score of ≥ 8 but a negative vaginal culture ($n$ = 62); Group 3, women with a Nugent score of < 8 and a positive vaginal culture ($n$ = 213); and Group 4, women with a Nugent score of < 8 and a negative vaginal culture ($n$ = 627). In our population, the proportion of each group was 8, 6, 23 and 64%, respectively (Table IV).

There was no difference in the incidence of a spontaneous preterm birth according to the vaginal culture result in women with a Nugent score ≥ 8 as well as in those with a lower Nugent score. A significant different was found in the prevalence of spontaneous preterm birth according to the Nugent score in women with a positive vaginal culture (at < 35 weeks), as well as in those with a negative vaginal culture (At < 34, < 35, < 36 and < 37 weeks of gestation).

## DISCUSSION

### Principal findings of this study

(1) A Nugent score ≥ 8 was associated with a positive vaginal culture for genital mycoplasmas, (2) a high Nugent score (≥ 8) without a positive vaginal culture for mycoplasmas was a risk factor for spontaneous preterm birth, (3) a positive vaginal culture for mycoplasmas in women with a high Nugent score did not increase the risk for spontaneous preterm birth.

### Nugent score for the detection of BV and occurrence of preterm birth

In the present study, the Nugent score was used for the detection of BV and was associated with preterm birth in asymptomatic women. Although BV followed by an inflammatory response in the lower genital tract has been considered a risk factor for preterm delivery [6–9], several investigators have been unable to prove a consistent association between BV and preterm birth [8,9,13]. Recently, Vogel et al., reported a lack of association between BV diagnosed using the Amsel's criteria (which is the most popular clinical criteria employed to date) and the rate of preterm birth. They called for the need of a re-examination of the laboratory diagnosis of BV [13].

BV has a syndromic nature and represents a change in the microbial environment of the vagina rather than a disease state. It is asymptomatic in approximately 50% of patients and often not associated with inflammation [6,7]. Therefore, the diagnosis of BV, depending on clinical criteria, has severe limitations. The Nugent score is a standardised system for the interpretation of a Gram stain of vaginal fluid. In addition, it is inexpensive and widely available compared with other laboratory methods. It also has a high intercentre reliability and reproducibility [10].

Although it could be argued that the cut-off for the diagnosis of BV should be a Nugent score of ≥ 8, a score of ≥ 7 was often considered indicative for BV in previous studies [8,10,14]. However, as Nugent mentioned in his original publication, the score provides a method to assess the degree of alteration in vaginal flora as a continuum rather than as a forced dichotomy [10]. After the evaluation of each point of the score, we chose a score of ≥ 8 as a cut-off because it showed the most significant relationship with the occurrence of preterm delivery.

### Detection of mycoplasmas in vaginal fluid and the occurrence of preterm delivery

Several studies have consistently demonstrated an association of *U urealyticum* in amniotic fluid with the occurrence of preterm birth, adverse pregnancy outcome and the development

of chronic lung disease of premature infants [3,11]. Nevertheless, there are few studies reporting the risk of mycoplasmas in vaginal fluid.

Our results demonstrated that a positive vaginal culture for mycoplasmas was not a risk factor for spontaneous preterm birth. A recent study which evaluated the joint effect of vaginal mycoplasmas and BV on adverse pregnancy outcome showed similar results regarding the occurrence of preterm birth [13]. Therefore, a positive vaginal culture for genital mycoplasmas does not need to be performed to assess the risk for spontaneous preterm delivery. This is consistent with our previous observations [15].

We observed a close relationship between the presence of genital mycoplasmas and a high Nugent score. This observation was not unexpected, because BV is known to be associated with genital mycoplasmas. Considering the even higher frequency of the presence of mycoplasma compared to that of a high Nugent score, the presence of mycoplasma possibly precedes the obvious disturbance of vaginal flora. It could be possible that vaginal mycoplasmas might play a role in BV or cause cytokine production. Indeed, Holst et al., demonstrated that the presence of *U urealyticum* in cervical fluid was associated with higher levels of IL-6in the vaginal fluid [5]. Moreover, recent case-control studies using PCR methods indicated that cervico-vaginal mycoplasma colonization might be associated with preterm birth [16,17]. Investigators suggested that the density of colonization [18] or some specific biovar [19] may be associated with preterm delivery. Further investigations based on a consecutive series of patients are required to verify these issues.

### The importance of the detection of cervico-vaginal inflammation

There has been a growing body of evidence that cervico-vaginal inflammation represented by elevated cytokine levels in cervico-vaginal secretion is related to microbial invasion of amniotic fluid, intra-amniotic inflammation and preterm birth [1,2,4,5,20,21]. Clinically, not all BV is associated with obvious inflammation. Moreover, not all cervico-vaginal inflammation is caused by BV. Therefore, the detection of cervico-vaginal inflammation, rather than BV, should be considered for the prediction of preterm birth. Romero et al. indicated the need for the understanding of the vaginal ecosystem in respect to the host response as well as the vaginal flora [7]. Indeed, investigators have reported a wide range of changes in vaginal flora as well as inflammatory cells by simply studying a smear of vaginal fluid. Moreover, heterogeneity in the cytokine profile concentration in vaginal fluid of women with BV has been demonstrated [6,7,20]. Donders et al. reported that the number of lactobacilli on a smear correlated with the concentrations of several cytokines in vaginal fluid [6]. Hedges et al. suggested that “an intermediate flora” on the vaginal smear may precede the changes diagnosed as BV on a Gram stain. The progression or regression between “an intermediate flora” and BV seems to be correlated with changes in the concentrations of cytokines in vaginal fluid, and therefore, in the mucosal immune response [20].

The results reported in this study suggest that a high Nugent score (developed by Robert Nugent and Sharon Hillier) is associated with the occurrence of spontaneous preterm birth. The original description of the score was intended to be a grading system of the microbial flora. It is possible and consistent with previous observations that a subset of women with a high Nugent score (representing an abnormal vaginal ecosystem) have a proinflammatory cytokine profile in vaginal fluid, and that these patients are at risk for spontaneous preterm labor related to intrauterine infection and inflammation, as previously proposed by Romero et al. [7]. The inflammatory and cytokine response is under genetic control, and therefore, the maternal genotype for specific genes implicated in the immune response may play a key role in determining what patients with an abnormal flora will develop an inflammatory response leading to spontaneous preterm delivery (for a detailed discussion, see reference 7).

### Clinical implications of this study

Our study reports a relationship between the Nugent score alone (without combining other criteria, such as vaginal pH) and preterm delivery in asymptomatic pregnant women. This indicates a Nugent score, which is an inexpensive, rapid and widely available test, can be applied in practice for identifying women at risk for preterm birth.

### Strengths and limitation compared with other studies

Our data complement previous studies. Ours was a homogenous study population for socioeconomic and demographic risk factors (Race, ethnicity, economic status and prenatal care). This may contribute to lower frequencies of BV (14%) and of positive cultures for genital mycoplasmas (30%) in the present study than those of previous studies [5,7,8,22]. Actually, a recent prospective study based on a rather homogenous population in Denmark showed similar frequencies in BV and the rate of positive mycoplasma cultures, which were 14 and 41% respectively [13].

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## Table I

Clinical characteristics of the study population according to the Nugent score.

| | Nugent score | | |
|---|---|---|---|
| Characteristics | ≥ 8 (*N* = 137) | < 8 (*N* = 840) | *p*-value |
| Maternal age (years, mean ± SD) | 31 ± 3.5 | 31 ± 3.4 | NS |
| Primiparity | 85 (62%) | 446 (53%) | NS |
| Previouspreterm birth | 10 (7%) | 65 (8%) | NS |
| Twin gestation | 5 (4%) | 33 (4%) | NS |
| Gestational age at sampling (weeks, mean ± SD) | 16.6 ± 1.1 | 16.7 ± 1.4 | NS |
| Gestational age at delivery (weeks, mean ± SD) | 38.5 ± 3.3 | 38.9 ± 2.7 | NS |
| Positive vaginal discharge culture for mycoplasmas | 75 (55%) | 213 (25%) | < 0.001 |

NS, not significant.

## Table II

Clinical characteristics of the study population according to the result of a vaginal fluid culture for mycoplasmas.

| | Vaginal fluid for mycoplasmas | | |
|---|---|---|---|
| Characteristics | Negative ($N$ = 689) | Positive ($N$ = 288) | $p$-value |
| Maternal age (years, mean ± SD) | 31 ± 3.3 | 31 ± 3.7 | NS |
| Primiparity | 364 (53%) | 167 (58%) | NS |
| Previous preterm birth | 54 (8%) | 21 (7%) | NS |
| Twin gestation | 28 (4%) | 10 (4%) | NS |
| Gestational age at sampling (weeks, mean ± SD) | 16.7 ± 1.3 | 16.7 ± 1.4 | NS |
| Gestational age at delivery (weeks, mean ± SD) | 38.9 ± 2.8 | 38.9 ± 2.8 | NS |
| Nugent score | | | < 0.001 |
| 0–3 | 577 (76%) | 178 (24%) | |
| 4–7 | 50 (59%) | 35 (41%) | |
| 8–10 | 62 (45%) | 75 (55%) | |

NS, not significant.

## Table III

The frequency of preterm birth according to the Nugent score.

| | Nugent score | | Odds ratio and 95% confidence interval |
|---|---|---|---|
| | **≥ 8 (*N* = 137)** | **< 8 (*N* = 840)** | |
| Preterm birth (*n*) | | | |
| < 32 weeks | 7 (5%) | 20 (2%) | 2.2 (0.9–5.3) |
| < 34 weeks | 9 (7%) | 29 (3%) | 2.0 (0.9–4.3) |
| < 35 weeks | 12 (9%) | 35 (4%) | 2.2 (1.1–4.4) * |
| < 36 weeks | 14 (10%) | 47 (6%) | 1.9 (1.0–3.6) * |
| < 37 weeks | 19 (14%) | 64 (8%) | 2.0 (1.1–3.4) * |
| Spontaneous preterm birth (*n*/*N*) | | | |
| < 32 weeks | 5/135 (4%) | 9/829 (1%) | 3.5 (1.2–10.6) * |
| < 34 weeks | 6/134 (4%) | 13/824 (2%) | 2.9 (1.1–7.8) * |
| < 35 weeks | 9/134 (7%) | 17/822 (2%) | 3.4 (1.5–7.8) * |
| < 36 weeks | 10/133 (8%) | 24/817 (3%) | 2.7 (1.3–5.8) * |
| < 37 weeks | 13/131 (10%) | 35/811 (4%) | 2.4 (1.3–4.8) * |

* $p < 0.05$

**Table IV**

The frequency of preterm birth according to the Nugent score and the result of vaginal fluid culture for mycoplasmas.

| | Group 1 | Group 2 | Group 3 | Group 4 | |
|---|---|---|---|---|---|
| | **Nugent score ≥ 8 (+)Culture (*N* = 75)** | **Nugent score ≥ 8 (−) Culture (*N* = 62)** | **Nugent score < 8 (+)Culture (*N* = 213)** | **Nugent score < 8 (−) Culture (*N* = 627)** | **Total** |
| Preterm birth (*n*) | | | | | |
| < 32 weeks | 4 (5%) | 3 (5%) | 5 (2%) | 15 (2%) | 27 (3%) |
| < 34 weeks | 6 (8%) | 3 (5%) | 8 (4%) | 21 (3%) * | 38 (4%) |
| < 35 weeks | 8 (11%) | 4 (6%) | 9 (4%) * | 26 (4%) * | 47 (5%) |
| < 36 weeks | 8 (11%) | 6 (10%) | 11 (5%) | 36 (6%) | 61 (6%) |
| < 37 weeks | 10 (13%) | 9 (15%) | 18 (8%) | 46 (7%) | 83 (9%) |
| Spontaneous preterm birth (*n/N*) | | | | | |
| < 32 weeks | 3/74 (4%) | 2/61 (3%) | 2/210 (1%) | 7/619 (1%) | 14/964 (1%) |
| < 34 weeks | 4/73 (5%) | 2/61 (3%) | 5/210 (2%) | 8/614 (1%) * | 19/958 (2%) |
| < 35 weeks | 6/73 (8%) | 3/61 (5%) | 6/210 (3%) * | 11/612(2%) * | 26/956 (3%) |
| < 36 weeks | 6/73 (8%) | 4/60 (7%) | 6/208 (3%) | 18/609 (3%) * | 34/950 (4%) |
| < 37 weeks | 7/72 (10%) | 6/59 (10%) | 10/205 (5%) | 25/606 (4%) *,** | 48.942 (5%) |

* $p < 0.05$ compare to Group 1.

** $p < 0.05$ compare to Group 2.