OBSTETRICS

# A comparison of prevaginal and postvaginal manipulation fetal fibronectin

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**BACKGROUND:** Fetal fibronectin (fFN) is used as a biomarker for preterm delivery. Currently, its use is discouraged if there has been vaginal manipulation in the previous 24 hours.

**OBJECTIVE:** Our objective is to determine if there are differences between fFN results before and after vaginal manipulation in the form of sterile vaginal exam or transvaginal ultrasound.

**STUDY DESIGN:** This was a prospective observational cohort study at a single center of women between 22-33 6/7 weeks at risk for preterm delivery due to: (1) a history of preterm delivery, short cervix, or multifetal gestation; or (2) symptoms of preterm labor. We excluded women with vaginal bleeding or infection, placenta previa, ruptured membranes, cervical dilation >3 cm, or any form of vaginal manipulation in the previous 24 hours. Specimen A was collected prior to planned vaginal exam or transvaginal ultrasound and specimen B was collected within 4 hours. The agreement between specimens A and B was assessed using descriptive statistics. Test characteristics of specimens A and B using the outcome of preterm delivery (<37 weeks) were calculated.

**RESULTS:** In all, 310 specimen pairs from 237 women were collected. Specimen A was positive in 37 (12%) instances and negative in 273 (88%) while specimen B was positive in 39 (13%) and negative in 271 (87%). There were discordant results in 26 specimen pairs. Of these, 14 (5%) negative specimen A results subsequently became positive for specimen B, and 12 (32%) positive specimen A results became negative for specimen B. Overall, there was a 92% agreement between specimens A and B (confidence interval, 88—94%). The specificity of specimens A and B for preterm birth was 90% vs 89%, respectively, with a negative predictive value of 87% for both. The false-negative rate was 12.8% for specimen A and 13.3% for specimen B.

**CONCLUSION:** There is a moderately high degree of agreement between prevaginal and postvaginal manipulation fFN results. Their test characteristics for evaluating preterm birth are similar with strong specificity and negative predictive values, and their false-negative rates are not clinically different. Consideration should be made to the utilization of postvaginal manipulation fFN when a prevaginal manipulation specimen is not available.

**Key words:** fetal fibronectin, preterm birth, sterile vaginal exam, transvaginal ultrasound, vaginal manipulation

## Introduction

Fetal fibronectin (fFN) is used as a biomarker for preterm delivery. When compared to clinical history, cervical dilation, and contraction frequency, previous studies have observed a superior ability of fFN to evaluate preterm delivery.[1-4] The strength of fFN testing lies in its high negative predictive value, such that a negative result is reassuring against preterm delivery and thus may lead to a decrease in antenatal interventions, resource utilization, health care costs, and anxiety for women who are destined to deliver at term.[4]

In many institutions, fFN has become part of the standard evaluation of women who present with concerns for preterm labor. Its use, however, is discouraged in several settings, such as the presence of vaginal bleeding, rupture of membranes, or within 24 hours of vaginal manipulation, including sterile vaginal examination (SVE) and transvaginal ultrasound (TVUS). The rationale for deferring testing in the setting of bleeding or rupture of membranes has a physiologic basis, as both maternal serum and amniotic fluid have been shown to contain fFN and may lead to false-positive testing.[5] In contrast, there is no clear basis for deferring testing after vaginal manipulation, and the effect of SVE and TVUS on the expression of fFN in cervicovaginal secretions remains largely unknown. These restrictions limit the utility of fFN to exclude preterm labor in potentially high-risk populations, as many patients who could benefit from this test have undergone a digital examination or TVUS in the previous 24 hours.

To that end, the objective of our study is to determine if there are differences between fFN results obtained before and after vaginal manipulation in the form of SVE or TVUS, and to compare test characteristics of prevaginal and postvaginal manipulation fFN tests in predicting preterm delivery.

## Materials and Methods

We performed a prospective observational cohort study of women between 22 0/7 and 33 6/7 weeks at risk for preterm birth who presented to our institution from May 2014 through August 2015. Women were eligible for inclusion if they had: (1) risk factors for preterm delivery, including a history of spontaneous preterm delivery, sonographically identified short cervix in the current pregnancy, or multifetal gestation; or (2) symptoms of preterm labor, including abdominal and/or back pain, cramping, pelvic and/or vaginal pressure, and contractions. No minimum frequency of contractions or cervical dilation was specified. Women were excluded if they were <18 years of age or had vaginal bleeding, rupture of membranes, abnormal placentation, a

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known vaginal or intrauterine infection, or cervical dilation >3 cm. They were also excluded if they had sexual intercourse, used vaginal medication, or had a TVUS or SVE within the previous 24 hours. Women with cervical cerclage in situ were not excluded.

Women were recruited from 2 general areas: obstetric ultrasound and the labor and delivery triage unit. At our institution, women with risk factors for preterm delivery, including a history of spontaneous preterm delivery, sonographically identified short cervix in the current pregnancy, or multifetal gestation are offered cervical length screening every 2 weeks between 16-28 weeks' gestation. Women with symptoms of preterm labor are evaluated in the labor and delivery triage unit. In these settings, women who met the inclusion criteria were approached by trained research staff and offered enrollment in a consecutive fashion when research staff were available. A woman could be enrolled >1 time in the study if they presented on a different occasion during the same pregnancy and continued to meet inclusion criteria.

Once consent was obtained, fFN sample was collected by an obstetric care provider or research staff member prior to vaginal manipulation, in the form of SVE, TVUS, or both in some instances. This was labeled "specimen A" and considered to be the reference standard. At the obstetrical provider's discretion, this specimen could be sent to the inpatient laboratory and the result used to guide clinical decision making. If the obstetric provider did not think the result would aid in clinical management, specimen A was instead analyzed in the research laboratory with the provider blinded to results. A second fFN—" specimen B"—was collected within 4 hours following vaginal manipulation. This specimen was considered to be the index test and used solely for the purpose of this study.

The Rapid fFN Specimen Collection Kit (Hologic, Sunnyvale, CA) was used for all specimen collection. Specimens were generally collected without the use of a sterile speculum, as blind collections have been shown to have excellent agreement with those collected with the use of a sterile speculum in previous studies.[6,7] A sterile polyester-tipped applicator was introduced into the posterior vagina for 10 seconds. The applicator was then returned to its tube, which was then labeled with the patient's study number and specimen number. Specimens sent to the inpatient hospital laboratory were run upon receipt using a solid-phase enzyme-linked immunosorbent assay in which cervicovaginal samples are incubated in microtiter wells coated with FDC-6, a monoclonal antibody specific for fFN. The resulting antibody-antigen complex is washed to remove nonspecifically bound material and then reacted with an enzyme-labeled antibody directed against human fibronectin. The microtiter well is then washed again to remove unbound labeled antibody and incubated with an enzyme substrate. The presence or absence of fFN is determined spectrophotometrically at a wavelength of 550 nm. The results are reported as negative if the concentration of fFN is <0.050 $\mu$g/mL and positive if the concentration is $\geq$0.050 $\mu$g/mL. Only qualitative results were reported for these specimens. Study specimens were stored in a freezer at −80.0°C within 10 minutes of collection until run in the research laboratory. Prior to analysis, these specimens were placed in a 37°C water bath for 20 minutes, mixed, and then equilibrated to room temperature for 30 minutes before testing was performed. These specimens were then analyzed in the research laboratory on a RapidfFN10Q system (Hologic, Sunnyvale, CA), which uses the same solid-phase enzyme-linked immunosorbent assay described above but provides both qualitative and quantitative results.

Data abstraction included gestational age at specimen collection; time and date of specimen collection, storage, and analysis; qualitative results of all specimens; quantitative results if available; type of vaginal manipulation (SVE or TVUS); indication for vaginal manipulation; and cervical dilation and/or cervical length as available. Maternal and obstetric characteristics and delivery information were also collected and entered into a deidentified secure data entry system. The study was performed in accordance with the Standards for Reporting of Diagnostic Accuracy (STARD) guidelines for the reporting of studies of diagnostic accuracy.[8]

A priori sample size calculations were performed. Using the Clopper-Pearson method and an estimated agreement of 97.5%, a sample size of 290 would be required to produce a 2-sided 95% confidence interval (CI). Descriptive statistics, proportion agreement with CI calculation, and kappa statistics were used to assess the agreement between specimens A and B. Test characteristics—including sensitivity, specificity, and positive and negative predictive values—were calculated to evaluate specimen B using specimen A as the gold standard. Test characteristics were also calculated to evaluate specimens A and B compared to the outcomes of preterm (<37 weeks' gestation) and spontaneous preterm delivery.[9,10] Analyses were performed using the specimen pairs as the unit of analysis based on the assumption that specimens obtained from the same patient at different time points are independent. Other analyses were performed using the patient as the unit of analysis, taking only the first specimen pair into account for patients who had multiple enrollment, for variables that are specific to the patient. Statistical analyses were performed using Stata 12.0 (StatCorp, College Station, TX). This study received institutional review board approval from Columbia University Medical Center.

## Results

During the study period of May 2014 through August 2015, 310 specimen pairs were collected from 237 women who were seen in the obstetric ultrasound and labor and delivery triage units at our institution. Of these specimen pairs, 222 were obtained in the setting of TVUS only, 72 in the setting of SVE only, and 16 in the setting of both SVE and TVUS.

Maternal and obstetric characteristics, as well as delivery information, for the 237 women comprising the cohort, are presented in Table 1. In all, 185 women had 1 specimen pair collected during the