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Fetal fibronectin in the prediction of preterm birth

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Which factors contribute to false-positive, false-negative and, invalid results in fetal fibronectin testing in women with symptoms of preterm labor?


American Journal of Perinatology 2016
ABSTRACT

Objective We assessed the influence of external factors on false-positive, false-negative, and invalid fibronectin results in the prediction of spontaneous delivery within seven days.

Methods We studied symptomatic women between 24 and 34 weeks' gestational age. We performed uni- and multivariable logistic regression to estimate the effect of external factors (vaginal soap, digital examination, transvaginal sonography, sexual intercourse, vaginal bleeding) on the risk of false-positive, false-negative, and invalid results, using spontaneous delivery within seven days as the outcome.

Results Out of 708 women, 237 (33%) had a false-positive result; none of the factors showed a significant association. Vaginal bleeding increased the proportion of positive fFN results, but was significantly associated with a lower risk of false-positive test results (odds ratio (OR) 0.22; 95% confidence interval (CI): 0.12 to 0.39). Ten women (1%) had a false-negative result. None of the investigated factors was significantly associated with a higher risk of false-negative results. Twenty-one tests (3%) were invalid; only vaginal bleeding showed a significant association (OR 4.5; 95%CI: 1.7 to 12).

Conclusion The effect of external factors on the performance of qualitative fetal fibronectin testing is limited, with vaginal bleeding as the only factor that reduces its validity.
INTRODUCTION

Fetal fibronectin (fFN) is a glycoprotein found at the choriodecidual interface. It is thought to be released through mechanical or inflammatory mediated damage to the membranes before birth and consequently the presence of fFN in cervical and vaginal secretions is a predictor for spontaneous preterm delivery. Using fFN in combination with cervical length measurement has a high negative predictive value (>98%) for delivery within seven days. However, the positive predictive value is modest, resulting in unnecessary referrals and admissions, overtreatment and higher costs.

Concerns have been raised that external factors, like transvaginal sonography and vaginal bleeding can contribute to false-positive, false-negative or invalid fFN test results, which is clearly undesirable. Manufacturers of the bedside fFN kits advise to collect the specimen prior to digital examination or transvaginal sonography, and to avoid testing women who had sexual intercourse within 24 hours prior to testing, because it could lead to iatrogenic release of fFN and therefore cause false-positive results. Furthermore, it is advised to avoid contamination with lubricants, soaps or disinfectants as these substances may interfere with absorption of the specimen by the swab or with the antibody-antigen reaction of fFN tests. Testing women who had moderate or gross vaginal bleeding should be avoided as it may contribute to difficulty in interpreting the fFN test result and may lead to false-positive results.

In clinical practice the fFN test is preferably performed according to manufacturer’s instructions, avoiding external factors that can make the fFN results more difficult to interpret. Yet in some situations this is inevitable. In the Alleviation of Pregnancy Outcome by Suspending of Tocolysis in Early Labour-1 (APOSTEL-I) study, a nationwide cohort study performed in all ten perinatal centers in the Netherlands, symptomatic women underwent fFN testing and cervical length measurement at inclusion to examine the best strategy to identify women who will deliver within seven days and who will not. The fFN test was preferably performed before digital examination or cervical length measurement, according to manufacturer’s instructions. In this study 63% of women were referred to a perinatal center from secondary care centers or primary midwifery practices, where transvaginal sonography or digital examination could have occurred already, and it is unclear whether the fFN test result can still be relied on.

A previous study demonstrated that transvaginal sonography did not affect fFN status in symptomatic women. Several other studies have reported that sexual intercourse increased the risk of false-positive fFN test results in asymptomatic women and suggested that fFN should not be relied on following intercourse within 24, and even 48 hours prior to testing. One study reported that vaginal bleeding was significantly associated with fFN positivity in symptomatic women.

The aim of this study was to estimate the effect of external factors on false-positive, false-negative, and invalid fFN test results for the outcome preterm delivery within seven days in women with threatened preterm birth.
MATERIALS AND METHODS

We analyzed data collected in the APOSTEL-I study, a nationwide cohort study conducted in all ten perinatal centers in the Netherlands between December 2009 and August 2012 studying women with threatened preterm birth. The design and results of the study have been reported in detail elsewhere. Women with signs of preterm labor between 24 and 34 weeks of gestational age with intact membranes were included. Signs of preterm labor were contractions ($\geq 3/30$ min), vaginal bleeding and abdominal or back pain. Exclusion criteria were cervical dilatation more than three centimetres, previous treatment with tocolysis within seven days before inclusion and contra-indications for tocolysis, such as suspected intra-uterine infections, fetal distress or lethal congenital abnormalities. Women who had iatrogenic delivery within seven days after inclusion were excluded. At inclusion, all women underwent a qualitative fFN test, using the qualitative Rapid fFN TLI10 analyzer (Hologic, Marlborough, MA) with a 0.050 $\mu$g/mL positivity cut-off.

There was no strict protocol for treatment decisions, but recommendations were provided. It was recommended to start tocolysis in women with a cervical length below 10 mm and in women with a cervical length between 10 and 30 mm with a positive fFN result, and to withhold tocolysis in women with a cervical length above 30 mm. For women with a cervical length between 10 and 30 mm in combination with a negative fFN test, the clinician on call decided whether to start tocolysis in these women.

Before the study started, physicians and midwives were trained on how to collect a fFN specimen from the posterior fornix of the vagina. In six participating centers, the fFN test was directly performed within the obstetric departments. In the other four centers, the fFN test was done at the hospital’s central laboratory. The involved personnel were all trained to perform the fFN test. Secondly, cervical length measurement was done by transvaginal sonography. The use of ultrasound gel was not described in the protocol.

A specimen had to be collected preferably before digital examination or cervical length measurement. Yet women referred to the participating perinatal centers by their primary gynecologist in a general hospital (secondary care) or by a general practitioner or midwife (primary care) could already have had a transvaginal sonography or digital examination within 24 hours before performance of the fFN test. In addition, data collection included information on other factors that might influence the fFN result, such as the use of vaginal soap or sexual intercourse within 24 hours before performance of the fFN test and vaginal bleeding during testing. All women provided written informed consent before entering the study.

The outcomes of interest were (1) false-positive fFN results, (2) false-negative fFN results, and (3) invalid fFN results. The false-positive and false-negative fFN results were based on the primary outcome spontaneous delivery within seven days after inclusion. A positive fFN result, but no spontaneous delivery within seven days was considered as a false-positive result, while a negative fFN result with a spontaneous delivery within seven days was considered as a false-negative result.
Approximately 457 data points (11%) for the factors were missing, ranging from 0% missing values for ‘bedside test’ to 31% for sexual intercourse within 24 hours before test performance. Missing data were interpreted as missing at random (MAR). Since it is well documented that a complete case analysis can yield biased results, we corrected the missing values using multiple imputation (10 times).\textsuperscript{10-12}

We performed uni- and multivariable logistic regression analysis to estimate the effect of the external factors on the fFN results (false-positive, false-negative, and invalid). The factors under study were the use of vaginal soap (yes or no), digital examination (yes or no), transvaginal sonography (yes or no) and sexual intercourse (yes or no) (all four within 24 hours prior to performance of the IFN test), vaginal bleeding during testing (yes or no) and whether the IFN test was done within the obstetric department or at the laboratory (‘bedside test’: yes or no). Data analyses were performed in SPSS version 22.0 (IBM corporation, Armonk, NY).

RESULTS

In the APOSTEL-I study 714 women were included. Six women were excluded because they had an iatrogenic delivery within seven days after inclusion for fetal or maternal reasons, leaving 708 women for the final analysis. Baseline characteristics of the study population are presented in Table 1.

Table 1 Baseline demographic and clinical characteristics of the total study population

<table>
<thead>
<tr>
<th></th>
<th>Missings (n (%))</th>
<th>Total N=708*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age – years</td>
<td>0</td>
<td>29.7 ± 5.3</td>
</tr>
<tr>
<td>Caucasian race</td>
<td>0</td>
<td>413 (58)</td>
</tr>
<tr>
<td>Body-mass index - kg/m(^2)</td>
<td>83 (12)</td>
<td>22.4 (20.3 – 25.2)</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>54 (8)</td>
<td>81 (14)</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>0</td>
<td>369 (52)</td>
</tr>
<tr>
<td>Previous preterm delivery &lt;37 wks</td>
<td>0</td>
<td>154 (22)</td>
</tr>
<tr>
<td>Multifetal gestation</td>
<td>0</td>
<td>108 (15)</td>
</tr>
<tr>
<td>Cervical length – mm</td>
<td>13 (2)</td>
<td>25.2 ± 12.5</td>
</tr>
<tr>
<td>Fibronectin status positive</td>
<td>9 (1)</td>
<td>314 (44)</td>
</tr>
<tr>
<td>False-positive fibronectin results</td>
<td></td>
<td>237 (33)</td>
</tr>
<tr>
<td>False-negative fibronectin results</td>
<td></td>
<td>10 (1)</td>
</tr>
<tr>
<td>Invalid fibronectin results</td>
<td></td>
<td>21 (3)</td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>16 (2)</td>
<td>99 (14)</td>
</tr>
<tr>
<td>Vaginal soap &lt;24 hs</td>
<td>144 (20)</td>
<td>169 (24)</td>
</tr>
<tr>
<td>Digital examination &lt;24 hs</td>
<td>41 (6)</td>
<td>365 (52)</td>
</tr>
<tr>
<td>Transvaginal sonography &lt;24 hs</td>
<td>40 (6)</td>
<td>378 (53)</td>
</tr>
<tr>
<td>Sexual intercourse &lt;24 hs</td>
<td>216 (31)</td>
<td>86 (12)</td>
</tr>
<tr>
<td>Bedside fibronectin test</td>
<td>0</td>
<td>444 (63)</td>
</tr>
<tr>
<td>Preterm delivery &lt; 7 days</td>
<td>0</td>
<td>96 (14)</td>
</tr>
</tbody>
</table>

Data are presented as number of patients (%) of the total study population for categorical and dichotomous variables and mean ± standard deviation (SD) or median (IQR) for continuous variables. *After multiple imputation
Of 708 women, 96 (14%) delivered within seven days after presentation. After imputation, there were 169 (24%) women who used soap, 365 (52%) women who had a digital examination, 378 (53%) women who had a transvaginal sonography and 86 (12%) women who had sexual intercourse within 24 hours prior to testing. Ninety-nine (14%) women had vaginal bleeding during testing and 444 (63%) fFN tests were done within the obstetric department.

Of 314 women with a positive fFN test, 237 (75%) did not deliver within seven days; these had a false-positive fFN result. Of 373 women with a negative fFN test, only 10 (2.7%) delivered within seven days; these had a false-negative result. Twenty-one fFN test results out of 708 tests (3%) were invalid. The sensitivity, specificity, positive and negative predictive value, and positive and negative likelihood ratio for the fFN test were 80%, 59%, 25%, 97%, 2.07 and 0.18, respectively.

Table 2 shows the results of the univariable and multivariable analyses; pooled estimates based on multiple imputation are given. Because of the low incidence of false-negative and invalid fFN results, we only performed multivariable logistic regression analysis with the false-positive fFN result as outcome. None of the factors was associated with a higher risk of false-positive test results. Vaginal bleeding during testing was significantly associated with a lower risk of a false-positive test result in univariable analysis (OR 0.22; 95% CI: 0.12 to 0.39) and multivariable analysis (OR 0.22; 95% CI: 0.12 to 0.40). Despite a lower risk of false-positive results in case of vaginal bleeding, there were more positive fFN results in women with vaginal bleeding; of 99 women with vaginal bleeding 76 (77%) had a positive fFN result, compared to 237 out of 609 women (39%) without vaginal bleeding. The sensitivity, specificity, positive and negative predictive value, and positive and negative likelihood ratio for vaginal bleeding were 45%, 91%, 43%, 91%, 4.90 and 0.61, respectively.

Table 2

<table>
<thead>
<tr>
<th>Factors</th>
<th>False-positive fFN result* (n=237)</th>
<th>False-negative fFN result* (n=10)</th>
<th>Invalid fFN results (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable</td>
<td>Multivariable</td>
<td>Univariable</td>
</tr>
<tr>
<td></td>
<td>OR  95%CI</td>
<td>OR  95%CI</td>
<td>OR  95%CI</td>
</tr>
<tr>
<td>Vaginal soap**</td>
<td>1.30 0.65 to 2.60</td>
<td>0.96 0.42 to 2.22</td>
<td>1.36 0.25 to 7.49</td>
</tr>
<tr>
<td>Sexual intercourse**</td>
<td>1.92 0.59 to 6.28</td>
<td>1.84 0.51 to 6.68</td>
<td>0.98 0.12 to 7.95</td>
</tr>
<tr>
<td>Vaginal bleeding**</td>
<td>0.22 0.12 to 0.39</td>
<td>0.22 0.12 to 0.40</td>
<td>3.13 0.67 to 26.8</td>
</tr>
<tr>
<td>Vaginal examination**</td>
<td>0.85 0.48 to 1.50</td>
<td>0.79 0.41 to 1.51</td>
<td>1.22 0.35 to 4.30</td>
</tr>
<tr>
<td>Transvaginal sonography**</td>
<td>1.24 0.72 to 2.12</td>
<td>1.48 0.77 to 2.85</td>
<td>2.47 0.60 to 9.36</td>
</tr>
<tr>
<td>Bedside test#</td>
<td>0.61 0.35 to 1.08</td>
<td>0.70 0.37 to 1.32</td>
<td>0.99 0.27 to 3.57</td>
</tr>
</tbody>
</table>

* Outcome = preterm delivery < 7 days , ** Within 24 hours prior to testing, # Instead of in laboratory
Since the incidence of false-negative test results was low (2.7%) confidence intervals around estimates in subgroups were wide. None of the factors was significantly associated with a higher risk on false-negative test results, but the risk was higher in case of vaginal bleeding during testing (OR 3.1, 95%CI 0.67 to 27) and transvaginal sonography prior to testing (OR 2.5, 95% CI 0.60 to 9.4).

The low incidence of invalid test results (3%) also led to wide confidence intervals around the estimates as well. Point estimates suggested that all factors contributed to invalid test results, but statistical significance was only observed for vaginal bleeding during testing (OR 4.5 - 95% CI 1.7 to 12).

DISCUSSION

In this study we estimated the potential effect of external factors that could contribute to false-positive, false-negative and invalid fFN test results for the outcome preterm delivery within seven days in symptomatic women. The use of vaginal soap, digital examination, transvaginal sonography, sexual intercourse and the test performed within the obstetric department were not significantly associated with the risk of specific IFN results. Only vaginal bleeding during testing was found to lead to more positive fFN results, and was significantly associated with a lower risk of false-positive test results and a higher risk of invalid test results.

To our knowledge, this is the first cohort study to estimate the effect of several external factors on potentially misleading fFN results combined in one dataset on symptomatic women. A strength of the study is that the data are derived from a well-described, large, nationwide cohort. A possible limitation of the study is that the external factors we studied were dichotomous variables (within 24 hours prior to fFN testing; yes or no). We did not take the interval between digital examination, transvaginal sonography or sexual intercourse and performance of the fFN test into account. We also did not quantify the amount of vaginal blood loss and did not record whether gel was used during transvaginal sonography and digital examination. It is reasonable to think that a greater amount of both vaginal blood loss and ultrasound gel has a higher effect on the possible change of fFN concentration than a minimal volume.

Unfortunately, because of the low number of false-negative and invalid test results, multivariable regression analysis was not possible for these outcomes. However, the low rate of these outcomes despite the fairly frequent occurrence of the different external factors before fFN testing suggests that the impact of these factors is limited and that the test result can still be relied on.

One other limitation is the potential influence of tocolysis on the number of women who had spontaneous preterm delivery within seven days, and therefore on the number of women with a false-positive or false-negative fFN results. In general, tocolysis is only given for the first 48 hours after admission to postpone delivery until the steroids for fetal lung maturation are given and have a short half-life.13, 14 There is no good evidence that tocolytics, given during the first 48 hours after admission,
can delay delivery after seven additional days have passed.\textsuperscript{15} Thus, we think that it is unlikely that tocolysis could have influenced the results of this study.

Transvaginal sonography prior to fFN testing did not significantly increase the risk of false-positive fFN results. This is in line with a previous study, in which a fFN test was performed before and immediately after transvaginal sonography in 25 symptomatic women.\textsuperscript{6} Although not significant, we found that transvaginal sonography led to more false-negative fFN test results. This could possibly be caused by the water-based ultrasound gel diluting the vaginal fluid containing fFN, but this is not more than a hypothesis.

The APOSTEL-I study showed that the best strategy to identify women at risk for delivery within seven days and those who are not is to perform additional fFN testing in women with a cervical length between 15 and 30 millimeters.\textsuperscript{5} Not performing the fFN test in women with a cervical length beyond this range leads to less unnecessary interventions and lower costs. We support the recommendation in the manufacturer’s instructions that the fFN test should preferably be done before transvaginal sonography. For this reason, a swab should be taken before cervical length measurement, and the fFN test should only be performed in case of a cervical length between 15 and 30 millimeters. However, in situations where this is inevitable, for example when the transvaginal sonography has already been done in another hospital, the fFN test result can still be relied on.

Previous studies have demonstrated an increase in fFN concentration and an increase in false-positive fFN test results in pregnant women with a history of sexual intercourse within 24 hours prior to testing and even up to 36 hours prior to testing.\textsuperscript{7, 16} This could be explained by the presence of seminal fibronectin in the cervicovaginal secretions after sexual intercourse, which is rich in α\textsubscript{1,2}-linked fucosylated glycoprotein. α\textsubscript{1,2}-linked fucosylated glycoprotein is a fucose containing molecule that acts like a natural ligand and is important in cell recognition and adhesion. fFN derived from placental, decidua and amnion cells is covered in α\textsubscript{1,2}-linked fucose.\textsuperscript{17} During fFN testing, a cross-reaction of the α\textsubscript{1,2}-linked fucose containing seminal fibronectin with the fFN immunoassay is possible. However, our data does not confirm this assumption, showing no association between sexual intercourse within 24 hours prior to testing and false-positive fFN test results. An explanation for our results could be that in our data we did not distinguish between protected or unprotected intercourse. Nevertheless, our results reject the hypothesis of sexual intercourse causing more false-positive results by iatrogenic release of fFN through cervical manipulation.

We believe that results of the fFN test are not influenced by location of testing; the fFN test can be done within the obstetric department as a bedside test or in the central laboratory of the hospital.

In our study, vaginal bleeding was the only factor significantly associated with a lower risk of false-positive and a higher risk of invalid fFN test results. A previous study reported that vaginal bleeding was a significant predictor of positivity for fFN in symptomatic women.\textsuperscript{9} Feinberg et al. explains the possible action of vaginal bleeding causing false-positive results by the presence of FDC-6-reactive fibronectin in
human blood independent of gender or pregnancy state. In this way it is possible that overt or occult blood in the cervix or vagina could predispose a patient to testing positive for cervicovaginal FDC-6-reactive fibronectin. Even though vaginal bleeding caused fewer false-positive fFN results in our study, the total percentage of positive fFN results increased in the group with vaginal bleeding compared to the group without vaginal bleeding, which is in line with Feinberg’s explanation. Vaginal bleeding itself can be seen as a strong predictor of preterm delivery. Compared to women without vaginal bleeding, fewer women with vaginal bleeding had a negative fFN result with slightly higher false-negative fFN results. Moreover, there was an increased risk of an invalid fFN result. Based on these data, we agree with the manufacturer’s recommendation not to use fFN testing in women with vaginal bleeding.
REFERENCES


