Quantitative fetal fibronectin testing in combination with cervical length measurement in the prediction of spontaneous preterm delivery in symptomatic women

Citation for published version (APA):

DOI:
10.1111/1471-0528.13752

Document status and date:
Published: 01/11/2016

Document Version:
Publisher’s PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:
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Quantitative fetal fibronectin testing in combination with cervical length measurement in the prediction of spontaneous preterm delivery in symptomatic women

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Accepted 17 September 2015. Published Online 15 December 2015.

Objective To evaluate whether in symptomatic women, the combination of quantitative fetal fibronectin (fFN) testing and cervical length (CL) improves the prediction of preterm delivery (PTD) within 7 days compared with qualitative fFN and CL.

Design Post hoc analysis of frozen fFN samples of a nationwide cohort study.

Setting Ten perinatal centres in the Netherlands.

Population Symptomatic women between 24 and 34 weeks of gestation.

Methods The risk of PTD <7 days was estimated in predefined CL and fFN strata. We used logistic regression to develop a model including quantitative fFN and CL, and one including qualitative fFN (threshold 50 ng/ml) and CL. We compared the models’ capacity to identify women at low risk (<5%) for delivery within 7 days using a reclassification table.

Main outcome measures Spontaneous delivery within 7 days after study entry.

Results We studied 350 women, of whom 69 (20%) delivered within 7 days. The risk of PTD in <7 days ranged from 2% in the lowest fFN group (<10 ng/ml) to 71% in the highest group (>500 ng/ml). Multivariable logistic regression showed an increasing risk of PTD in <7 days with rising fFN concentration [10–49 ng/ml: odds ratio (OR) 1.3, 95% confidence interval (95% CI) 0.23–7.0; 50–199 ng/ml: OR 3.2, 95% CI 0.79–13; 200–499 ng/ml: OR 9.0, 95% CI 2.3–35; >500 ng/ml: OR 39, 95% CI 4.4–164] and shortening of the CL (OR 0.86 per mm, 95% CI 0.82–0.90). Use of quantitative fFN instead of qualitative fFN resulted in reclassification of 18 (5%) women from high to low risk, of whom one (6%) woman delivered within 7 days.

Conclusion In symptomatic women, quantitative fFN testing does not improve the prediction of PTD within 7 days compared with qualitative fFN testing in combination with CL measurement in terms of reclassification from high to low (<5%) risk, but it adds value across the risk range.

Keywords Cervical length, prediction, pregnancy, preterm labour, quantitative fetal fibronectin.

Tweetable abstract Quantitative fFN testing adds value to qualitative fFN testing with CL measurement in the prediction of PTD.

Linked article This article is commented on by A Ridout et al., p. 1972 in this issue. To view this mini commentary visit http://dx.doi.org/10.1111/1471-0528.13850.
Introduction

Preterm birth, defined as birth before 37 weeks of gestation, occurs in 10% of all deliveries worldwide and is a major contributor to perinatal morbidity and mortality. In the developed world, women who suffer signs of preterm labour before 32 weeks of gestation are transferred to a perinatal centre. However, most of these women will not deliver within 1 week after onset of symptoms and 50% will continue the pregnancy to term. Accurate identification of women who are at low risk of delivering preterm could avoid unnecessary interventions, and could facilitate targeted interventions such as corticosteroids, magnesium sulphate, tocolysis and intrapartum transfer.

There are several methods to assess the risk of preterm delivery (PTD). Cervical length (CL) measurement is predictive, but not available in an acute setting in each country. Fetal fibronectin (fFN), a glycoprotein found at the chorio–decidual interface, provides an alternative. The most commonly used test is the qualitative fFN bedside test, which provides a positive or negative result based on a threshold of 50 ng/ml. A recently published meta-analysis showed that combining CL measurement and fFN testing improved the prediction of PTD within 7 days. Positive prediction, however, was modest, still resulting in unnecessary referrals and admissions, overtreatment and higher costs.

We recently evaluated the combination of CL measurement and qualitative fFN testing in the assessment of women who will deliver within 7 days and those who will not. Although CL measurement was a good initial predictor, additional fFN testing in women with a CL between 15 and 30 mm improved the identification of women with a low risk (<5%) of delivering within 7 days.

Evaluation of a new bedside quantitative fFN test showed that quantitative information has added value over the qualitative fFN test. Increasing fFN concentrations were associated with an increasing risk of PTD. Changing the threshold from 50 ng/ml to 200 ng/ml led to an increase in the positive predictive value for the prediction of delivery within 14 days from 20% to 37%, with minimal effect on the negative predictive value. However, until now the predictive value of the quantitative fFN test has not been evaluated in combination with CL measurement.

The aim of this study was to evaluate whether quantitative fFN testing improves the prediction of PTD within 7 days compared with qualitative fFN testing, in combination with CL measurement.

Methods

We performed a post hoc analysis on frozen fFN samples obtained during the APOSTEL-1 study. The APOSTEL-1 study is a nationwide cohort study conducted in all ten perinatal centres in the Netherlands between December 2009 and August 2012. Its objective was to determine the predictive capacity of qualitative fFN in addition to CL measurement. Informed written consent was obtained from all participants. Further description of the study design and main results have been presented elsewhere.

In short, symptomatic women between 24 and 34 weeks of gestation and with intact membranes underwent fFN screening and CL measurement. Exclusion criteria were cervical dilatation >3 cm, previous treatment with tocolysis within 7 days before inclusion and contraindications for tocolysis, such as suspected intrauterine infections, fetal distress or lethal congenital abnormalities. Because the primary outcome was spontaneous PTD within 7 days after presentation, iatrogenic deliveries within 7 days were excluded. No strict protocol was available for treatment decisions, but recommendations were provided. It was recommended to start tocolysis in women with a CL <10 mm and in women with a CL between 10 and 30 mm with a positive fFN result, and to withhold tocolysis in women with a CL >30 mm. For women with a CL between 10 and 30 mm in combination with a negative fFN test, the clinician on call decided whether to start tocolysis. Clinicians could prescribe nifedipine, indomethacin, atosiban, ritodrine, or all of these. Corticosteroids were given to women at the discretion of the clinician on call.

Testing of fFN was performed using the qualitative Rapid fFN TLI IQ analyser (Hologic, Marlborough, MA, USA) according to the manufacturer’s instructions. A 50 ng/ml cut-off was used for the qualitative result of the fFN test (positive, negative). Cervical length was measured by transvaginal ultrasound. After obtaining the qualitative result, the fFN samples were frozen for future analyses, initially at a temperature of −20°C. Within 6 months, all samples were transferred to the Academic Medical Centre in Amsterdam, where they were all stored at a temperature of −80°C.

For this post hoc analysis, only samples from women with a CL <30 mm were selected, as women with a CL >30 mm are known to be at low risk for PTD (1% delivered within 7 days). Women without a frozen fFN sample available or women with more than one frozen fFN sample were excluded from the study population. After thawing of selected samples, quantitative fFN testing was performed using the Rapid fFN 10Q analyser (Hologic).
Analysis

Descriptive characteristics were obtained for baseline demographics. To check for mismatches, the original qualitative (positive/negative) fFN result from the APOSTEL-1 cohort was compared with the post hoc qualitative fFN result.

For risk stratification, thresholds of 10, 50, 200 and 500 ng/ml for the quantitative fFN test were predefined before analysis, and CL was divided into groups of 5 mm. Subsequently, the predefined fFN and CL strata were related to the risk of PTD within 7 days, which was the primary endpoint. A risk <5%, corresponding to the risk for women with a CL >25 mm, was considered as low risk. This threshold has been derived from the interpretation of CL because it is currently applied in women with signs of preterm labour. To assess if more low-risk women could be identified using the quantitative fFN test with various thresholds, we compared the accuracy to that of the qualitative fFN test (threshold 50 ng/ml), which was according to the APOSTEL-1 study most useful in women with a CL between 15 and 30 mm.

Furthermore, we used univariable and multivariable logistic regression analyses to develop and compare three multivariable models to predict PTD within 7 days; one model including the variables CL and qualitative fFN, one model including the variables CL and quantitative fFN, and one model with quantitative fFN alone. The variables CL and quantitative fFN were analysed as continuous variables, whereas qualitative fFN was dichotomous. First, linearity between these continuous variables and the outcome was checked using cubic spline analyses on the logit scale. Variables were categorised in case of significant nonlinearity. We used bootstrapping techniques for internal validation to avoid a too optimistic performance of the models. Two hundred bootstrap samples of equal size to the original data set were drawn from the original data set with replacement. This resulted in a shrinkage factor, which we used to adjust the regression coefficients. The models were compared in terms of overall fit and discrimination. Model fit was expressed with Nagelkerke $R^2$. The area under the receiver operating characteristics curve (AUC) was used to express the models’ ability to discriminate between women who delivered within and after 7 days.

To determine whether quantitative fFN compared with qualitative fFN improves the capability of the model to identify low-risk women (<5% risk), we compared the two models in terms of reclassification. A reclassification table summarises the number of women who were correctly reclassified from high risk in the model with CL and qualitative fFN to low risk in the model with CL and quantitative fFN, and vice versa. Statistical analyses were performed in SPSS version 20.0 (Chicago, IL, USA) and R version 2.10.0 (The R foundation for Statistical Computing, 2009, Vienna, Austria, www.R-project.org).

Results

In 714 women participating in the APOSTEL-1 study, CL measurement and fFN testing were performed. Six women who underwent induction of labour or had an elective caesarean section within 7 days after study entrance, were excluded. Samples of 121 women were not available and 20 women with more than one frozen sample were excluded, because we did not know the reason for repetition of the test. In 11 women the CL was not recorded. Another 201 women were excluded because they had a CL >30 mm, of whom two (1%) delivered within 7 days. In total, 355 samples were available for testing. Five samples appeared to contain an insufficient amount of fluid for testing. Therefore, a total of 350 fFN samples from symptomatic women were eligible for this post hoc analysis (see Figure S1). Baseline characteristics of the study population are shown in Table 1. The complete demographics of the overall study population are presented elsewhere.

In total, 69 of these 350 women (20%) delivered within 7 days after presentation. Of the 350 samples tested with the quantitative Rapid fFN 10Q analyser, 162 (46%) had a positive (>50 ng/ml) fFN result. Of the samples that were initially positive at inclusion in the APOSTEL-1 study using the qualitative Rapid fFN TLIQ analyser, 46 samples (24%) had a negative fFN result using the quantitative Rapid fFN 10Q analyser. None of these 46 women delivered within 7 days after inclusion. On the other hand, ten samples

| Table 1. Baseline characteristics of the study population (n = 350) |
|------------------|---------------------|---------------------|---------------------|
| Characteristic   | Mean/C6             | SD/C6              | IQR/C6              |
| Preterm delivery ≤7 days, n (%) | 69 (20%)          | 207 (59%)        |                     |
| Nulliparous, n (%)             | 29.9 ± 5.4        | 29.0 ± 2.7        |                     |
| Age (years)                  | 71 (20%)          | 79 (23%)         |                     |
| Gestational age (weeks)       | 202 (58%)         | 202 (58%)        |                     |
| Multifetal gestation, n (%)   | 216 (61%)         | 216 (61%)        |                     |
| Previous preterm delivery, n (%) | 19 (5-216)    | 19 (5–216)       |                     |
| Caucasian race, n (%)         | 193 (54%)         | 193 (54%)        |                     |
| Body mass index (kg/m²), mean ± SD | 23.1 ± 4.3   | 23.1 ± 4.3       |                     |
| Cervical length (mm), median (IQR) | 19 (13–24)    | 19 (13–24)       |                     |
| Fibronectin status positive, n (%) | 189 (54%)    | 189 (54%)        |                     |
| Fibronectin quantitative result (ng/ml), median (IQR) | 35 (5–216)   | 35 (5–216)       |                     |

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.
(7%) had initially been negative using the qualitative Rapid fFN TL4Q analyser and were now positive. Two of these ten women (20%) delivered within 7 days after presentation.

Table 2 shows the risk stratification of PTD within 7 days. The risk of PTD within 7 days after presentation increased with rising fFN concentration from 2% in the lowest fFN group (<10 ng/ml) to 71% in the highest fFN group (>500 ng/ml), and shortening of the CL from 5% in the group with the longest cervix (25–30 mm) to 78% in the group with the shortest cervix (<5 mm). In total, 97 women had a CL <15 mm, of whom 50 (52%) delivered within 7 days. The risk of PTD within 7 days in women with a CL <15 mm was in all fFN strata above the acceptable 5%, ranging from 10% in the group with an fFN concentration <10 ng/ml, to 80% in the group with an fFN concentration >500 ng/ml. One hundred and sixty-five women had a CL >15 mm and an fFN concentration <50 ng/ml, of whom three (2%) delivered within 7 days. Forty-one women had a CL >15 mm and an fFN concentration between 50 and 200 ng/ml, of whom three (7%) delivered within 7 days, which is above the acceptable 5%.

Cubic spline analyses showed a nonlinear relationship of quantitative fFN with the risk of PTD. Therefore, we analysed this variable as a categorical variable (categorised as <10, 10–49, 50–199, 200–499 and >500 ng/ml). Cervical length was analysed as a continuous variable, because cubic spline analyses showed linearity with the risk of PTD. In univariable analysis a higher fFN concentration was associated with a higher risk of PTD [10, 10; 23, 95% CI 6.5–83]; >500 ng/ml: OR 10.5, 95% CI 2.9–499]. CL was associated with a lower risk of PTD (OR 0.84 per cm, 95% CI 0.80–0.87), a positive fibronectin test was associated with a higher risk of PTD (OR 19, 95% CI 8.1–46).

Table 3 shows the two multivariable models. The model with CL and qualitative fFN, and the model with CL and quantitative fFN are presented after shrinkage with an average shrinkage factor of 0.98 and 0.93, respectively. The model with CL and qualitative fFN had an AUC of 0.89 (95% CI 0.85–0.93) and a Nagelkerke $R^2$ of 0.50. The model with CL and quantitative fFN had an AUC of 0.91 (95% CI 0.88–0.95) and a Nagelkerke $R^2$ of 0.58. Quantitative fFN alone had an AUC of 0.85 (95% CI 0.81–0.90) and a Nagelkerke $R^2$ of 0.41.

Using our multivariable model with CL and quantitative fFN compared with CL and qualitative fFN, a total of 21 women (6%) were reclassified; three women (1%) were reclassified from low risk (risk <5% of delivery within 7 days) to high risk, of whom 0 (0%) women delivered within 7 days. Conversely, 18 women (5%) were reclassified from high risk to low risk, of whom one (6%) woman delivered within 7 days. The reclassification is shown in Table 4.

**Discussion**

**Main findings**

We assessed the novel quantitative fFN bedside test in combination with CL measurement in the prediction of spontaneous PTD within 7 days. We demonstrated an association between the fFN concentration and the risk of spontaneous PTD, with an increase from 2% in the lowest fFN group (<10 ng/ml) to 71% in the highest fFN group (>500 ng/ml). However, both the stratified analyses and the logistic regression analyses showed that in combination with CL measurement, the quantitative fFN test did not
improve the prediction of spontaneous PTD within 7 days, compared with the qualitative fFN test, in terms of discrimination between low (<5%) and high risk.

Strengths and limitations
A strength of our study is that the data were derived from a well-described, large, nationwide cohort of women, in which qualitative fFN was collected according to protocol, while quantitative fFN samples were collected in a blinded and hence unbiased way. It is the first study that combines the quantitative fFN bedside test and CL measurement. All fFN samples were stored according to the manufacturer’s instructions (Hologic).

A potential limitation is the number of mismatches between the original fFN results from the APOSTEL-1 study and the post hoc fFN results. In total, the post hoc fFN result of 56 samples (16%) did not match the original fFN result. The change from a positive result to a negative result could possibly be explained by degradation of the protein as a result of storage of the samples. On the other hand, the change from a negative to a positive result is not explained by an increase in the quantity of the protein, whereas inappropriate initial test performance is more likely. As two out of ten women (20%) with a positive fFN result with the quantitative 10Q analyser, which was initially negative, delivered within 7 days after presentation, the predictive value of the post hoc fFN test was more accurate.

Furthermore, we did not take twin pregnancies into account, which we did include in our analyses (71/350, 20%). The quantitative fFN test may behave differently in multiple pregnancies. It would be preferable to repeat all analyses in multiple pregnancies, but there were too few women with PTD to develop a separate prediction model for this group (16/71, 23%). In addition, larger sample sizes are needed to evaluate the effect of other factors, such as ethnicity, body mass index or age on the accuracy of fFN levels.

A final limitation is the potential influence of tocolysis on the number of women who had PTD within 7 days. There is no good evidence that tocolytics, given during the first 48 hours after admission, can delay delivery by this amount of time. Hence, we think that it is unlikely that tocolysis could have influenced the results of this study.

Interpretation
Our data confirm previous studies that report an increasing risk of PTD with higher fFN concentrations. A previous study included 300 symptomatic women and showed a PTD rate of 5.7% within 14 days of testing, much lower than the 20% we found. This could partially be explained by the fact that we only selected women with a CL <30 mm and the fact that women with a CL >30 mm are known to be at low risk for PTD. Moreover, the total study population of the APOSTEL-1 was already a high-risk population with a PTD rate of 12%.

Table 3. Multivariable models for predicting spontaneous preterm delivery within 7 days in symptomatic women, including CL and quantitative fFN or qualitative fFN as predictors

<table>
<thead>
<tr>
<th></th>
<th>Beta*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>–0.72</td>
<td></td>
</tr>
<tr>
<td>Cervical length (mm)</td>
<td>–0.15</td>
<td>0.86 (0.83–0.90)</td>
</tr>
<tr>
<td>Qualitative fFN</td>
<td>2.2</td>
<td>9.4 (3.8–24)</td>
</tr>
</tbody>
</table>

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<tbody>
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<td></td>
</tr>
<tr>
<td>Cervical length (mm)</td>
<td>–0.15</td>
<td>0.86 (0.82–0.90)</td>
</tr>
<tr>
<td>Qualitative fFN &lt;10 ng/ml</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>10–49 ng/ml</td>
<td>0.24</td>
<td>1.3 (0.23–7.0)</td>
</tr>
<tr>
<td>50–199 ng/ml</td>
<td>1.2</td>
<td>3.2 (0.79–13)</td>
</tr>
<tr>
<td>200–499 ng/ml</td>
<td>2.2</td>
<td>9.0 (2.3–35)</td>
</tr>
<tr>
<td>&gt;500 ng/ml</td>
<td>3.7</td>
<td>39 (9.4–164)</td>
</tr>
</tbody>
</table>

*Coefficients were shrunken with an average shrinkage factor 0.93; AUC = 0.89 (95% CI 0.85–0.93); Nagelkerke $R^2 = 0.50$.

**Coefficients were shrunken with an average shrinkage factor 0.98; AUC = 0.91 (95% CI 0.88–0.95); Nagelkerke $R^2 = 0.58$.

Table 4. Reclassification table, showing the number of women reclassified from high risk to low risk and vice versa using the model with CL and qualitative fFN compared with the model with CL and quantitative fFN

<table>
<thead>
<tr>
<th>Model CL and qualitative fFN</th>
<th>Model CL and quantitative fFN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk*</td>
<td>High risk</td>
<td></td>
</tr>
<tr>
<td>Low risk*</td>
<td>151 (3 PTD – 2%)</td>
<td>154 (3 PTD – 2%)</td>
</tr>
<tr>
<td>High risk</td>
<td>18 (1 PTD – 6%)</td>
<td>196 (66 PTD – 3%)</td>
</tr>
<tr>
<td>Total</td>
<td>169 (4 PTD – 2%)</td>
<td>350 (69 PTD – 20%)</td>
</tr>
</tbody>
</table>

*Low risk is defined as a risk of PTD <5%.
Abbott et al. demonstrated that the quantitative fFN test added value over the currently used qualitative fFN bedside test. However, their study did not evaluate CL measurement. Discrimination of both the model with CL and qualitative fFN, and the model with CL and quantitative fFN was significantly better than the quantitative fFN test alone (AUC 0.89, 95% CI 0.85–0.93; AUC 0.91, 95% CI 0.88–0.95; and AUC 0.85, 95% CI 0.81–0.90, respectively).

The results of the APOSTEL-1 study showed that fFN testing in women with a CL between 15 and 30 mm was helpful to stratify women as low risk or high risk of delivery within 7 days, using a 5% risk threshold. This threshold is based on a 25-mm cut-off for CL, which corresponds to a risk of 5% of delivery within 7 days. Using the fFN test in women with a CL between 15 and 30 mm, an additional 10% of all women could be prevented from unnecessary treatment, referrals and admissions, compared with CL with a 25-mm cut-off. We examined whether the quantitative fFN test could decrease the number of false-positive results, so improving decision making. However, the results of this study demonstrated that changing the threshold from the conventional 50 ng/ml to 200 ng/ml in the presence of a CL between 15 and 30 mm, gave a 7% risk of PTD in this group. On the other hand, more stringent prediction of low-risk women in the case of a CL <15 mm using the 10 ng/ml threshold, showed a 10% risk of PTD in this group. Both risks are above the threshold of an acceptable 5% risk to deliver within 7 days. Furthermore, logistic regression analyses showed that a model with CL and quantitative fFN compared with CL and qualitative fFN, reclassified 18 women from high risk to low risk. However, one of these women (6%) delivered within 7 days, which implies an incorrect reclassification as the risk is above the threshold of 5%. Hence, using the quantitative fFN test compared with the qualitative fFN test, no additional women could be saved from unnecessary referral and overtreatment if we rely on the 5% risk threshold.

We think that the 5% risk threshold is a useful cut-off in clinical decision making and our results showed that from this point of view the quantitative fFN test had no added value. However, the quantitative test could be valuable in further risk discrimination across the risk range. The result showed that the risk of PTD escalated from 2% in the group with the lowest fFN concentration (<10 ng/ml) to 71% in the group with the highest fFN concentration (>500 ng/ml). Moreover, in the group with the shortest cervix (<5 mm) the risk even increased from 0% to 100%. Women with a 10% risk of delivery within 7 days could be treated differently from women with a 90% risk.

Obviously, quantitative fFN testing alone performed better than an isolated qualitative fFN test. Hence, in settings where sonographic CL measurement is not available, quantitative fFN testing could be an alternative. In our setting, the initial sonographic CL measurement could prevent fFN additional testing in 54% of the women, as their cervix was >30 mm or <15 mm. Decisions on the most cost-effective approach are dependent on local settings.

In this study, we focused on the 5% risk threshold of PTD within 7 days regardless of the gestational age, as this threshold corresponds to current management. However, it would be very interesting as well to assess the risk of PTD along with the gestational age; i.e. clinicians might choose a different treatment of women with a 20% risk of PTD at 26 weeks than at 33 weeks. This evokes a discussion about defining different risk thresholds at different gestational ages, which goes beyond the scope of this study.

**Conclusion**

In conclusion, the quantitative fFN bedside test does have added value in the risk assessment of PTD within 7 days across the risk range, allowing personalized decision making. However, it does not improve discrimination between low risk (<5%) and high risk of spontaneous PTD within 7 days compared with the currently used qualitative fFN bedside test in combination with CL measurement. Future analyses should focus on expanded individualized risk assessment using additional information such as vaginal examination, laboratory findings and patient characteristics to facilitate clinical decision making regarding admission, *in utero* transfer and administration of antenatal corticosteroids, magnesium sulphate for neuroprotection and/or tocolysis.

**Disclosure of interests**

Ben Mol has presented on prevention of preterm birth at a Hologic sponsored meeting in Glasgow, 2011. Travel costs were reimbursed. Full disclosure of interests available to view online as supporting information.

**Contribution to authorship**

BM JV MO BO and JvdP conceived and designed the experiments, MB and GvB analysed the data. MB wrote the first draft of the manuscript. MB, JV, FW, MO, AK, MP, GO, HS, MS, KB, MH, AB, FV, MDW, CB, JC, HD, BNB, JvE, MF, KS, JvdP, PB, BO, MK, BM and GvB contributed to the writing of the manuscript and agree with manuscript results and conclusions. JV, FW, MO, AK, MP, GO, HS, MS, KB, MH, AB, FV, MW, CB, JC, HD, BNB, JvE, MF, KS, JvdP, PB, BO, MK, BM and GvB enrolled patients. JV, BO, JvdP and BM obtained funding for the trial.

**Details of ethics approval**

The study protocol was approved by the ethics committee of the Amsterdam Medical Centre (MEC 08/363 # 09.17.0658) on 21 April 2009 and it was published thereafter.
Funding
This investigator-initiated study was funded by The Netherlands Organisation for Health Research and Development (ZonMw, grant number 80-82310-98-09056). All decisions concerning the study design, execution, analyses and reports were made solely by the investigators. Hologic (Marlborough, MA) provided the quantitative fetal fibronectin tests for this study free of charge.

Acknowledgements
First and most important, we would like to thank all women that participated in the study. We also greatly acknowledge the efforts of all Dutch obstetric residents, gynaecologists and midwives in the perinatal centres who helped us to include women during their shifts. We are grateful for the help of all the laboratory specialists and analysts who performed the fibronectin tests. Furthermore, this study would not have been possible without the great effort of the research staff and research nurses and midwives of the Dutch Obstetrical Consortium.

Supporting Information
Additional Supporting Information may be found in the online version of this article:
Figure S1. Participant flow diagram.

References