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Quantitative fetal fibronectin to predict preterm birth in women with previous cervical surgery

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Quantitative fetal fibronectin and cervical length to predict preterm birth in asymptomatic women with previous cervical surgery

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The most appropriate table for print issue is Table 4 (Prediction of spontaneous preterm birth at <34 weeks' gestation)

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Condensation

Prediction of spontaneous preterm birth using cervicovaginal fluid quantitative fetal fibronectin in asymptomatic women with cervical surgery is valid.

Short version of title

Quantitative fetal fibronectin testing after cervical surgery

Abstract

Background: Quantitative fetal fibronectin testing has demonstrated accuracy for prediction of spontaneous preterm birth in asymptomatic women with a prior history of preterm birth. Predictive accuracy in women with previous cervical surgery (a potentially different risk mechanism) is not known.

Objective: To compare the predictive accuracy of cervicovaginal fluid quantitative fetal fibronectin and cervical length testing in asymptomatic women with previous cervical surgery to that in women with one previous preterm birth.

Study Design: A prospective blinded secondary analysis of a larger observational study of cervicovaginal fluid quantitative fetal fibronectin concentration in asymptomatic women measured with a Hologic 10q system (Hologic, Marlborough, MA). Prediction of spontaneous preterm birth (<30, 34 and 37 weeks) with cervicovaginal fluid quantitative fetal fibronectin concentration in primiparous women who had undergone at least one invasive cervical procedure (n=473) was compared with prediction in women who had previous spontaneous preterm birth, preterm prelabour rupture of membranes or late miscarriage (n=821). Relationship with cervical length was explored.

Results: The rate of spontaneous preterm birth <34 weeks' in the cervical surgery group was 3% compared with 9% in previous spontaneous preterm birth group. Receiver operating characteristic curves comparing quantitative fetal fibronectin for

prediction at all 3 gestational end-points were comparable between the cervical surgery and previous spontaneous preterm birth groups; (34 weeks: area under the curve 0.78 [0.64-0.93] vs. 0.71 [0.64-0.78] p=0.39). Prediction of spontaneous preterm birth using cervical length compared with quantitative fetal fibronectin for prediction of preterm birth before 34 weeks of gestation offered similar prediction; area under the curve 0.88 [0.79-0.96] vs. 0.77 [0.62-0.92] p=0.12 in the cervical surgery group; 0.77 [0.70-0.84] vs. 0.74 [0.67-0.81] p=0.32 in the previous spontaneous preterm birth group.

Conclusion: Prediction of spontaneous preterm birth using cervicovaginal fluid quantitative fetal fibronectin in asymptomatic women with cervical surgery is valid, and has comparative accuracy to that in women with a history of spontaneous preterm birth.

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Quantitative fetal fibronectin to predict preterm birth in women with previous cervical surgery

Introduction

Preterm birth (birth before 37 weeks' completed gestation) is responsible for over 1 million neonatal deaths annually. Representing approximately 10% of all deliveries worldwide, rates are continuing to rise[1]. Whilst prior history of spontaneous preterm birth (sPTB) is known to be a limited predictor of subsequent sPTB,[2] currently the two best available predictors for sPTB before 34 weeks of gestation are cervical length (CL) by transvaginal ultrasound scan (TVS) and cervicovaginal fluid (CVF) fetal fibronectin (FFN).[3]

FFN is an adhesive glycoprotein normally found in the fetal membranes and decidua. As the gestational sac implants and attaches to the interior of the uterus in early pregnancy, presence of FFN in the CVF is regarded as 'physiological'.[4] A high concentration of CVF FFN after the 18 weeks' of gestation may indicate mechanical or inflammatory medicated disruption of the attachment of the membranes to the decidua[5]. Detection of CVF FFN has demonstrated accuracy at predicting sPTB in asymptomatic high-risk women (women who have had a previous sPTB or late miscarriage), [6] and there is increasing evidence that the actual concentration of FFN (quantitative FFN, qFFN) measured in CVF allows more accurate discrimination of risk of sPTB. although its role in clinical practice is not yet established. [7–9]

It is well established that women who have undergone invasive cervical surgery (e.g. laser loop excision, or cone biopsy for premalignant changes) are at more than double the risk of sPTB than the background population, although this may differ with procedure and depth of biopsy.[10] Although used increasingly in clinical practice, the ability of qFFN to predict premature birth in women with previous cervical surgery, compared with women at high risk by virtue of their obstetric history, has never been described and routine FFN testing in asymptomatic women with prior cervical surgery is not recommended by NICE.[11] It is possible that the aetiology underlying sPTB may be different in women with previous cervical surgery who have mechanically shortened cervices, compared with women with a previous sPTB, in whom decidual disruption results in activation of inflammatory pathways.[12] The predictive power of qFFN may be different in these two groups.

We hypothesized that qFFN could be used to predict subsequent PTB in women with previous cervical surgery. The primary aim was to compare the predictive value of qFFN in a cohort of primiparous women with previous invasive cervical surgery, compared to women with prior sPTB.

Materials and Methods

This was a pre-defined prospective masked secondary analysis of a larger observational study of CVF qFFN concentration (ng/ml) in asymptomatic women using commercially available rapid bedside FFN testing (Hologic™).[9,13] Samples

were taken between 22^{+0} -27^{+6} weeks' gestation. The primary end-point of the study was sPTB (including premature prelabour rupture of membranes [PPROM]) with delivery before 34 weeks', with secondary end points of delivery before 30 and 37 weeks' gestation. The study was conducted from October 2010 through July 2014 at 5 teaching hospitals in the United Kingdom.

The study was approved by the South East London Research Ethics Committee, and all local research ethics committees that were associated with participating centers. Informed written consent was obtained from all participants. Gestational age was confirmed by early obstetric ultrasound examination. According to hospital protocol and the licensing recommendations on qFFN testing, women with cervical dilation >3 cm, frank bleeding, or rupture of membranes (on speculum examination) were excluded from the study because of interference with qFFN measurement (Figure 1). Samples that were blood stained, or from women who had undergone sexual intercourse or vaginal examination within the past 24 hours were excluded (due to known interference with qFFN measurement[14]), and the next eligible qFFN test was used if one was available within the gestational age limit of $22\text{-}27^{+6}$ weeks.

Categorical TLIQ data (positive/negative) were provided to clinicians, but quantitative 10Q results remained masked (a random result code was generated by the analyzer) until after delivery. Thresholds of 10, 50, 200, and 500 ng/mL were predefined before analysis, based on the literature [5,8]. A sterile speculum examination was performed, and a polyester swab was inserted for 10 seconds into the posterior fornix of the vagina to collect a sample of CVF. The swab was placed

into the test buffer solution and two tests were run concurrently. The first aliquot (200 µl) of this solution was analysed immediately with the conventional qualitative TLi Rapid FFN analyser (Hologic). Another aliquot was analysed with the quantitative Rapid 10Q FFN analyser (Hologic) by placing on a test cassette and inserting into the analyser; after 10 minutes a numerical result is displayed electronically. All clinicians were trained in the use of the FFN analyser. After FFN swabs were taken, at the same appointment, CL was measured (mm) using TVS performed by trained staff; the mean of three values was used in this analysis. All sonographers were formally trained by the institution's governance requirements, which included formal assessment prior to accreditation. The mean value was taken to be consistent with our previous evidence.[9] Cervical length measurements were taken and recorded prior to any FFN assay.

From the cohort of asymptomatic high risk women who met the criteria for analysis (n= 1294), 2 groups of women were selected; primiparous women (no previous pregnancy lasting >14 weeks' duration) with singleton pregnancies at 22-27^{+6} weeks of gestation who had undergone invasive cervical surgery (e.g. LLETZ, cone/loop biopsy) (n= 473) prior to their index pregnancy, and women with singleton pregnancies at 22-27^{+6} weeks of gestation, with prior sPTB <37 weeks', premature rupture of membranes (PPROM) or miscarriage >14 weeks' gestation, who had never undergone any cervical surgery (n= 821). Where multiple visits had occurred, the first eligible visit in the gestational age bracket was used. A formal power calculation was not performed as this was a planned subgroup analysis of a previously powered study. We chose not to include multiparous women without

prior sPTB into the cervical surgery group to limit the number of variables affecting the risk of PTB.

Descriptive characteristics were calculated for baseline demographics in both groups. Logistic regression and interaction tests were used to assess whether the meaning of the FFN test results varied according to the women's risk group. Calculations were carried out in Stata version 11.2 (StataCorp, College Station, Texas). The performance of qFFN as a predictor of sPTB was tested for different gestational age limits (prior to 34 weeks (primary outcome), 30 and 37 weeks) and predictive statistics for sPTB were calculated and compared between the previous cervical surgery and previous PTB groups; ROC areas were calculated and compared,[15] and sensitivity, specificity, predictive values, likelihood ratios and relative risk were assessed at pre-specified endpoints: 10, 50, 200, 500 ng/mL. The utility of CL measurement following stratification by qFFN category was explored, as well as the utility of qFFN following stratification by the presence and absence of a short cervix (<25 mm). A composite score was developed by using logistic regression for the continuous variables of CL and the log of qFFN concentration to predict prematurity before 34 weeks. The composite score was compared with 2 continuous measurements of qFFN and CL using ROC curve analysis[15]. Iatrogenic PTB prior to the gestational age outcome under consideration were excluded from statistical analysis (Figure 1).

Results

A total of 473 women with previous cervical surgery were eligible for analysis and comparison was made with 821 women with one previous sPTB. The mean gestational age of testing was 23^{+3} weeks (SD ±1.90). Demographics for study participants, and the nature of cervical surgery are illustrated in Figure 2. The proportion of women of African and Afro-Caribbean origin was lower in the cervical surgery group than previous sPTB.

Concentrations of qFFN were obtained for all women with the use of the 10Q test. There were no adverse events related to the test. Overall, the rate of sPTB <34 weeks' after iatrogenic deliveries were excluded in the cervical surgery group was 14/468 (3%) compared with 71/809 (9%) in the previous sPTB group. The number of women and proportion of preterm deliveries assigned to each of the pre-specified qFFN categories is shown in Table 2. 82% of women in the cervical surgery group and 61% in the sPTB group had CVF qFFN concentrations of <10ng/ml category. However, more than four times as many women with a previous sPTB had a qFFN concentration >200 ng/ml compared to those with prior cervical surgery (Table 2).

The diagnostic accuracy of qFFN for predicting sPTB <30 weeks, <34 and <37 weeks' gestation in each group using pre-specified thresholds is shown in Tables 3, 4 and 5. Using a qFFN concentration threshold of 10ng/ml gave a high negative predictive value for prediction of sPTB at all 3 gestational end-points in both groups. A qFFN concentration threshold of ≥200 ng/ml had high specificity for prediction of sPTB at all gestational end-points in both groups, although the optimal balance between sensitivity and specificity remains at 50ng/ml.

The relative risk (RR) of sPTB at each gestational endpoint for each qFFN concentration is shown in Table 6. In the cervical surgery group, the relative risk (RR) of sPTB <34 weeks for qFFN concentration threshold of 200g/ml was 21.8 (95% CI 5.5-86.8), almost triple the relative risk at the same threshold in the previous sPTB group; RR 6.7 (3.6-12.6).

Receiver operator characteristic (ROC) curves for prediction of sPTB < 30, < 34 and <37 weeks of gestation are shown in Figure 2. ROC curves for prediction at each gestational end-point were comparable between the cervical surgery and previous sPTB groups, and the differences between the groups were not statistically significant; (34 weeks: 0.78 [0.64-0.93] vs. 0.71 [0.64-0.78], p=0.39; 30 weeks: 0.82 [0.64-0.99] vs. 0.73 [0.62-0.84], p=0.42; 37 weeks: 0.62 [0.50-0.74] vs. 0.65 (0.60-0.70], p=0.69).

Of the included cohort, 1,256 women had paired TVS CL measurements. Table 7 illustrates the proportion of women who delivered prematurely, stratified according to CL measurement, qFFN concentration and risk factor (cervical surgery or previous sPTB). Women with iatrogenic PTB were excluded. Of these women, 32/463 (7%) in the cervical surgery group, and 104/793 (13%) in the previous sPTB group had a CL <25mm.

Both FFN and CL were consistently strong and highly significant predictors of prematurity, as a test based on the combination, with little evidence that any test or

combination performed better than any other. As expected, women with a short cervix (CL <25 mm) had a higher rate of sPTB before 34 weeks compared to women with CL >25mm; 7/32 (21.9%) vs. 7/431 (1.6%) in the cervical surgery group and 33/104 (31.7%) vs. 38/689 (5.5%) in the previous sPTB group. Among those women with a short cervix, stratification according to qFFN concentration further discriminated risk of sPTB (Table 7). For example, 2/4 (50%) of women in the previous cervical surgery group with short cervix and a qFFN value >200 delivered <34 weeks compared with 3/23 (13%) in the same group with a qFFN <10 ng/ml (Risk difference: 37%, CI: 14% to 88%, P=0.079). Likewise 13/26 (50%) of women with previous sPTB and short cervix delivered spontaneously prior to 34 weeks, compared with 5/33 (15.2%) of women in the same group with the lowest qFFN concentrations (<10ng/ml) (Risk difference: 35%, CI: 12% to 58%, P=0.0039).

Prediction of sPTB < 34 weeks using CL combined with qFFN offered superior prediction than using qFFN or CL alone, although this difference was only statistically significant in the previous cervical surgery group, and only for qFFN compared to the combination of CL and qFFN (P=0.021). For women with previous cervical surgery, prediction of sPTB using CL had ROC AUC of 0.87 (0.78-0.96) whereas qFFN in this cohort had a ROC AUC of 0.75 (0.759-0.91), p=0.11 for the difference; both tests combined had a ROC AUC of 0.89 (0.79-0.98) (p=0.02 compared to FFN alone, p= 0.49 compared to CL alone). In the previous sPTB group, CL had a ROC AUC of 0.76 (0.69-0.84), qFFN a ROC AUC of 0.72 (0.65-0.80), p=0.29 for the difference, and the combined tests had a ROC AUC of 0.78 (0.70-0.85) (p=0.08 compared to FFN alone, p=0.40 compared to CL alone). 0.6% of women in the previous cervical surgery group

and 2.4% of women in the previous PTB group underwent cervical cerclage during their pregnancy. It is therefore possible that the predictive ability of the tests have been underestimated.

Comment

These data demonstrate that CVF qFFN measurement using the rapid bedside 10Q analyser is a valuable and valid screening tool for assessing sPTB risk in asymptomatic primiparous women who have undergone prior cervical surgery, comparable to that in women with previous premature birth (in whom qFFN testing is well established).[8] Based on these results, a primiparous woman who has undergone previous cervical surgery and found to have a raised qFFN concentration of any value ≥10ng/ml has a greater than double the risk of delivering spontaneously before 34 weeks' gestation compared to a woman with a history of sPTB with the same qFFN test result, although absolute risks in this group remained lower. It is difficult to compare the absolute risks of cervical surgery and prior history given the different demographic of the populations. In spite of this the predictive ability remained good and therefore is likely to be generalizable.

Furthermore, the results confirm the increasingly recognised value of using quantitative, as opposed to qualitative fetal fibronectin; risk of sPTB is clearly related to the concentration of FFN in the CVF of women who have undergone previous cervical surgery. There may be value in using alternative risk thresholds at <10 ng/ml to define 'low risk' and >200 ng/ml to identify those women at higher risk,

rather than the traditional 'positive/negative' threshold of 50 ng/ml. Although 50ng/ml is an appropriate single threshold, sensitivity and specificity are rarely equally weighted in clinical practice and quantification allows the clinician to tailor their decision-making.

A clinical tool that can be used to differentiate so-called 'high-risk' women is highly desirable, due to the likely outcome being a term birth even amongst women with previous poor obstetric histories. A test with a high sensitivity is desirable in these circumstances, when considering the balance of benefit and harm. For women with a previous preterm birth, a threshold of 10ng/ml has high sensitivity and specificity for prediction of spontaneous preterm birth. As such, a test with high sensitivity allows clinicians to reassure high-risk women with low qFFN concentration that they are likely to have a term birth. At all thresholds, sensitivity, specificity, positive and negative predictive values in both the cervical surgery group and previous sPTB are comparable; thus this test can be used with comparable accuracy in both asymptomatic high risk groups.

This is the largest study of qFFN to date in high-risk women with data obtained from a prospectively collected masked dataset. In terms of generalizability of results, the study population and sPTB rate was comparable to previously published high-risk studies. The study population was of varied ethnic origin and thus the test can be considered transferable to a broad high-risk population. The difference in ethnicities between the comparison groups may reflect the aetiology of the diseases being investigated, but there is no evidence to suggest the predictive

ability of the test is related to ethnicity. Although this generalizability is limited to nulliparous women, the mechanism in multiparous women is likely to be similar.

A limitation of the study design was that women involved with a history of pregnancy loss or early preterm birth were routinely offered cerclage if a short cervix was detected, a management compliant with UK Royal College of Obstetrician and Gynaecologist guidelines (progesterone is not routinely prescribed). The use of cerclage may have influenced outcome (a likely 50% reduction in risk of sPTB <34 weeks' in these circumstances). A proportion of women in both groups underwent cervical cerclage during their pregnancy. As so few women received intervention (0.6% of women in the previous cervical surgery group and 2.4% of women in the previous PTB group) in both groups, this is unlikely to have had a major effect on the comparison between them. It is therefore possible that the predictive ability of the tests have been underestimated, as the cerclage may make a positive test a false positive result, and therefore the sensitivity could be even higher.[16]

It is well known that women with previous cervical surgery are at increased risk of preterm birth (RR 1.25-2.61 depending on the height of conization)[17–19]. There are several proposed mechanisms to explain their increased risk; structurally, the removal of cervical tissue may produce a mechanically weakened, shortened cervix which reduces its functioning during pregnancy, an effect that increases with increasing proportion of tissue removed.[20] It has also been suggested that previous cervical conisation leads to an impaired inflammatory response during pregnancy

due to the loss of cervical glands, increasing the susceptibility to infection as well as altering the cervical flora to increase the risk of preterm premature rupture of the membranes (PPROM).[12,19] In contrast, infection and inflammation are thought to play a major etiological role in sPTB without a history of cervical surgery, particularly at early gestations.[21]

A recent paper by the RCOG Science Advisory Committee has stressed the need to determine antenatal interventions that could reduce the risk of sPTB for women who have had previous cervical surgery. Prediction of PTB using qFFN allows a clinician to reassure those women at low risk and direct them back to routine antenatal care, whilst identifying those at the highest risk, allowing for targeted interventions. These include providing antenatal corticosteroids and in utero transfer, as well as potentially guiding prophylactic intervention such as cervical cerclage or vaginal progesterone, but further trials are needed to confirm their efficacy in these groups.

CVF qFFN testing is a useful adjunct to CL measurement to accurately discriminate those women with a short cervix who were destined to deliver early; in the cervical surgery group, 3/23 (13%) of women who had a CL of <25mm and qFFN <10 ng/mL delivered spontaneously prior to 34 weeks' gestation, compared with 2/4 (50%) of those with a qFFN >200ng/mL. However, in contrast to TVS measurement of CL, qFFN can be performed with little training and depending on service provision, it may prove to be as useful and cheaper used as a stand-alone test. In the absence of CL measurement availability, a qFFN value of <10 ng/mL in a woman who is otherwise high risk having had previous cervical surgery will be immensely

reassuring (<1.5% risk of sPTB <34 weeks). Currently NICE recommends consideration of prophylactic cervical cerclage for women with CL< 25 mm at 16-24 weeks gestation who have either previous sPTB or a history of cervical trauma.[11] Given that many women with a short cervix do not deliver early, qFFN may be able to discriminate those women who may benefit from cerclage, and avoid over-treatment. This can be relied on as the risk of sPTB is lower than background risk if qFFN measurement is low and cervical length is long.

In the overall cohort study[9], (which included both women with cervical surgery and women with previous pre-term birth, as well as uterine abnormalities) from which these women were selected, we demonstrated that the relative risk of sPTB increased as concentrations of qFFN increased and that thresholds of <10ng/ml and >200 ng/ml were particularly clinically relevant. The current study confirms that these observations are valid in women with previous cervical surgery alone.

Currently, women with previous cervical surgery are not routinely offered antenatal CVF screening for risk of PTB.[11] As this group has higher PTB rates than background rates, we believe screening is justified in this population. The implementation of qFFN, a clinically useful, low-risk, test has the potential to discriminate between women with a history of cervical surgery who no longer need intensive surveillance (the majority) from those whose risk is elevated, particularly if combined with cervical length surveillance and who may ultimately require intervention. The challenge remains to identify the most appropriate prophylactic intervention in those considered at high risk.

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Table 1: Demographic and obstetric characteristics of high-risk asymptomatic women tested for cervicovaginal fluid fetal fibronectin concentration from 22^{+0} – 27^{+6} weeks' gestation

Characteristic	**Cervical surgery (n= 473)**	**Previous sPTB (n=821)**
Age (years, mean ± SD)	33 ±4.2	32 ±5.5
Body mass index (kg/m^2, mean ± SD)	23 ±3.5	26.8 ±5.6
Ethnicity, n (%):		
European	411 (87)	321 (39)
African & Afro-Caribbean	9 (2)	332 (41)
Other	53 (11)	168 (20)
Smoking history, n (%):		
Current	10 (2)	50 (6)
Ex-smoker	127 (27)	101 (12)
Never	336 (71)	670 (82)
Obstetric history:		
Any previous pregnancy, n (%)	155 (32.8)	821 (100)
Gestation of previous pregnancy (weeks, mean ± SD)	8.1 (2.3)	32.3 (7.6)
Outcome of previous birth		
sPTB, n (%)	-	566 (69)
PPROM, n (%)	-	290 (35)
Late miscarriage (16-23 weeks gest), n (%)	-	315 (38)
Cervical surgery, n (%):		
Cone	90 (19)	-
LLETZ	370 (78.2)	-
Laser	51 (10.7)	-
Trachelotomy	2 (0.4)	-
Other	34 (7.1)	-
Combination	19 (4)	

Table 2: Spontaneous pre-term birth in asymptomatic high risk women according to quantitative fetal fibronectin (qFFN) categories, for both the cervical surgery group (non-shaded) and previous spontaneous preterm birth (sPTB) group (shaded)

qFFN category (ng/ml)	Test	Total N (%)	Spontaneous Preterm Birth		
			< 30 weeks N (%)	<34 weeks N (%)	<37 weeks N (%)
Less than 10	Cervical Surgery	389 (82.2)	3 (0.8)	6 (1.5)	21 (5.4)
	Previous sPTB	504 (61.4)	9 (1.8)	24 (4.8)	68 (13.5)
10-49	Cervical Surgery	43 (9.1)	0	1 (2.3)	4 (9.3)
	Previous sPTB	165 (20.1)	5 (3.0)	18 (10.9)	36 (21.8)
50-199	Cervical Surgery	21 (4.4)	1 (4.8)	4 (19.0)	6 (28.6)
	Previous sPTB	82 (10.0)	4 (4.9)	14 (17.0)	23 (28.0)
200-499	Cervical Surgery	6 (1.3)	1 (16.7)	2 (33.3)	3 (50)
	Previous sPTB	34 (4.1)	8 (23.5)	11 (32.4)	15 (44.1)
500 or greater	Cervical Surgery	4 (0.8)	1 (25)	1 (25)	1 (25)
	Previous sPTB	8 (1.0)	3 (37.5)	4 (50.0)	4 (50.0)
Total *	Cervical Surgery	473 (100)	6 (1.3)	14 (3.0)	35 (7.4)
	Previous sPTB	821 (100)	29 (3.5)	71 (8.6)	146 (17.8)

***** Women with iatrogenic deliveries before the gestation of analysis were excluded (cervical surgery group; n=1 less than 30 weeks of gestation, n=4 less than 34 weeks of gestation, n=5 less than 37 weeks of gestation. Previous sPTB group; n=8 less than 30 weeks of gestation, n=4 less than 34 weeks of gestation, n=16 less than 37 weeks of gestation).

† All comparisons for each gestational endpoint are statistically significant (P<.01) except 10–49 ng/mL compared with 50–199 ng/mL and 200–499 compared with 500 or greater (P>.1 for all gestational endpoints).

Table 3: Prediction of spontaneous preterm birth at <30 weeks' gestation according to fetal fibronectin concentration for both the cervical surgery group (non-shaded) and previous sPTB (shaded)

		Fetal fibronectin threshold (ng/ml)			
Predictive variable (95% CI)	**Test**	**≥10**	**≥50**	**≥200**	**≥500**
Sensitivity (%)	Cervical Surgery	50 (11.8-88.2)	50 (11.8-88.2)	33.3 (4.3-77.7)	16.7 (0.4-64.1)
	Previous sPTB	69.0 (49.2-84.7)	51.7 (32.5-70.6)	37.9 (20.7-57.7)	10.3 (2.2-27.4)
Specificity (%)	Cervical Surgery	84.1 (80.5-87.3)	94.0 (91.4-96.0)	98.3 (96.6-99.3)	99.4 (98.1-99.9)
	Previous sPTB	64.7 (61.2-68.0)	85.7 (83.1-88.1)	95.8 (94.1-97.1)	99.4 (98.5-99.8)
PPV (%)	Cervical Surgery	3.9 (0.8-11.0)	9.7 (2.0-25.8)	20.0 (2.5-55.6)	25.0 (0.6-80.6)
	Previous sPTB	6.7 (4.2-10.2)	11.8 (6.8-18.7)	25.0 (13.2-40.3)	37.5 (8.5-75.5)
NPV (%)	Cervical Surgery	99.2 (97.8-99.8)	99.3 (98.0-99.9)	99.1 (97.8-99.8)	98.9 (97.5-99.7)
	Previous sPTB	98.3 (96.7-99.2)	98.0 (96.6-98.9)	97.7 (96.3-98.6)	96.8 (95.3-97.9)
LR +	Cervical Surgery	3.15 (1.4-7.2)	8.32 (3.5-20.0)	19.42 (5.2-73.0)	25.89 (3.1-214.6)
	Previous sPTB	1.95 (1.5-2.5)	3.62 (2.5-5.4)	9.01 (5.1-16.0)	16.22 (4.1-64.6)
LR –	Cervical Surgery	0.59 (0.3-1.3)	0.53 (0.2-1.2)	0.68 (0.4-1.2)	0.84 (0.6-1.2)
	Previous sPTB	0.48 (0.3-0.8)	0.56 (0.4-0.8)	0.65 (0.5-0.9)	0.90 (0.8-1.0)
ROC area	Cervical Surgery	0.82 (0.64-0.99)			
	Previous sPTB	0.73 (0.62-0.84)			

Table 4: Prediction of spontaneous preterm birth at <34 weeks' gestation according to fetal fibronectin concentration for both the cervical surgery group (non-shaded) and previous sPTB (shaded)

		Fetal fibronectin threshold (ng/ml)			
Predictive variable (95% CI)	**Test**	**≥10**	**≥50**	**≥200**	**≥500**
Sensitivity (%)	Cervical Surgery	57.1 (28.9-82.3)	50.0 (23.0-77.0)	21.4 (4.7-50.8)	7.1 (0.2-33.9)
	Previous sPTB	66.2 (54.0-77.0)	40.8 (29.3-53.2)	21.1 (12.3-32.4)	5.6 (1.6-13.8)
Specificity (%)	Cervical Surgery	85.0 (81.4-88.2)	94.7 (92.2-96.6)	98.5 (96.8-99.4)	99.3 (98.1-99.9)
	Previous sPTB	66.4 (62.9-69.8)	86.9 (84.2-89.2)	96.2 (94.6-97.5)	99.5 (98.6-99.9)
PPV (%)	Cervical Surgery	10.5 (4.7-19.7)	22.6 (9.6-41.1)	30.0 (6.7-65.2)	25.0 (0.6-80.6)
	Previous sPTB	15.9 (11.9-20.6)	23.0 (16.0-31.4)	34.9 (21.0-50.9)	50.0 (15.7-84.3)
NPV (%)	Cervical Surgery	98.5 (96.7-99.4)	98.4 (96.7-99.4)	97.6 (95.7-98.8)	97.2 (95.3-98.5)
	Previous sPTB	95.3 (93.1-97.0)	93.9 (91.8-95.5)	92.7 (90.6-94.4)	91.6 (89.5-93.5)
LR +	Cervical Surgery	3.8 (2.3-6.3)	9.5 (4.9-18.2)	13.9 (4.0-48.2)	10.8 (1.2-97.5)
	Previous sPTB	2.0 (1.6-2.4)	3.1 (2.2-4.6)	5.6 (3.1-9.9)	10.4 (2.7-40.7)
LR –	Cervical Surgery	0.5 (0.3-0.9)	0.5 (0.3-0.9)	0.8 (0.6-1.1)	0.9 (0.8-1.1)
	Previous sPTB	0.51 (0.4-0.7)	0.7 (0.6-0.8)	0.8 (0.7-0.9)	1.0 (0.9-1.0)
ROC area	Cervical Surgery	0.78 (0.64-0.93)			
	Previous sPTB	0.71 (0.64-0.78)			

Table 5: Prediction of spontaneous preterm birth at <37 weeks' gestation according to fetal fibronectin concentration for both the cervical surgery group (non-shaded) and previous sPTB (shaded)

		Fetal fibronectin threshold (ng/ml)			
Predictive variable (95% CI)	**Test**	**≥10**	**≥50**	**≥200**	**≥500**
Sensitivity (%)	Cervical Surgery	40.0 (23.9-57.9)	28.6 (14.6-46.3)	11.4 (3.2-26.7)	2.9 (0.1-14.9)
	Previous sPTB	53.4 (45.0-61.7)	28.8 (21.6-36.8)	13.0 (8.0-19.6)	2.7 (0.8-6.9)
Specificity (%)	Cervical Surgery	86.0 (82.3-89.1)	95.1 (92.6-96.9)	98.6 (97.0-99.5)	99.3 (98.0-99.9)
	Previous sPTB	67.4 (63.6-71.0)	87.3 (84.5-89.8)	96.4 (94.7-97.7)	99.4 (98.4-99.8)
PPV (%)	Cervical Surgery	18.9 (10.7-29.7)	32.3 (16.7-51.4)	40.0 (12.2-73.8)	25.0 (0.6-80.6)
	Previous sPTB	27.0 (22.0-32.5)	33.9 (25.6-42.9)	45.2 (29.8-61.3)	50.0 (15.7-84.3)
NPV (%)	Cervical Surgery	94.6 (91.9-96.6)	94.2 (91.6-96.2)	93.2 (90.4-95.3)	92.6 (89.8-94.8)
	Previous sPTB	86.5 (83.2-89.4)	84.5 (81.5-87.1)	83.1 (80.2-85.7)	81.9 (79.0-84.5)
LR +	Cervical Surgery	2.9 (1.8-4.6)	5.8 (3.0-11.4)	8.2 (2.4-27.5)	4.1 (0.4-38.2)
	Previous sPTB	1.6 (1.4-2.0)	2.3 (1.6-3.1)	3.7 (2.1-6.5)	4.4 (1.1-17.5)
LR –	Cervical Surgery	0.7 (0.5-0.9)	0.8 (0.6-0.9)	0.9 (0.8-1.0)	1.0 (0.9-1.0)
	Previous sPTB	0.7 (0.6-0.8)	0.82 (0.73-0.91)	0.9 (0.9-1.0)	1.0 (1.0-1.0)
ROC area	Cervical Surgery	0.62 (0.50-0.74)			
	Previous sPTB	0.65 (0.60-0.70)			

Table 6: Relative risk of sPTB at < 30, <34 and <37 weeks' gestation according to quantitative fetal fibronectin concentration for both the cervical surgery group (non-shaded) and previous spontaneous preterm birth group (shaded)

		Spontaneous Preterm Birth		
qFFN category (ng/ml)	**Test**	**<30 weeks**	**<34 weeks**	**<37 weeks**
		Relative Risk (95% CI)		
Less than 10	Cervical surgery	1	1	1
	Previous sPTB	1	1	1
10-49	Cervical Surgery	1	1.5 (0.2-11.8)	1.7 (0.62-4.8)
	Previous sPTB	1.7 (0.6-5.0)	2.3 (1.3-4.1)	1.7 (1.7-3.5)
50-199	Cervical Surgery	6.3 (0.7-57.7)	12.4 (3.8-40.8)	5.3 (2.4-11.7)
	Previous sPTB	2.8 (0.9-8.8)	3.6 (2.0-6.7)	2.4 (1.7-3.5)
200-500	Cervical Surgery	21.9 (2.6-181.9)	21.8 (5.5-86.8)	3.3 (2.1-5.1)
	Previous sPTB	12.7 (5.2-31.0)	6.7 (3.6-12.6)	3.7 (2.5-5.6)
>500	Cervical Surgery	32.9 (4.3-252.5)	16.3 (2.5-106.4)	3.7 (1.8-7.7)
	Previous sPTB	21.5 (7.1-64.9)	10.7 (4.8-23.7)	3.9 (2.0-7.7)

Table 7: Proportion of women who delivered prematurely when analysed according to cervical length (CL) measurement (above and below 15 mm) and quantitative fetal fibronectin (qFFN) category.

				Spontaneous Preterm Birth		
Cervical length	qFFN category (ng/ml)	**Test**	**N***	**<30 weeks**	**<34 weeks**	**<37 weeks**
				N(%)	**N(%)**	**N(%)**
≥25 mm	<10	Cervical Surgery	366	2 (0.4)	3 (0.6)	13 (2.7)
		Previous sPTB	471	6 (0.7)	19 (2.3)	60 (7.3)
	10-199	Cervical Surgery	59	0 (0.0)	3 (0.6)	8 (1.7)
		Previous sPTB	202	3 (0.4)	17 (2.1)	35 (4.2)
	≥200	Cervical Surgery	6	1 (0.2)	1 (0.2)	2 (0.4)
		Previous sPTB	16	1 (0.1)	2 (0.2)	4 (0.5)
	Total*	Cervical Surgery	431	3 (0.6)	7 (1.4)	23 (4.9)
		Previous sPTB	689	10 (1.2)	38 (4.6)	99 (12.1)
<25 mm	<10	Cervical Surgery	23	1 (0.2)	3 (0.6)	8 (1.7)
		Previous sPTB	33	3 (0.4)	5 (0.6)	8 (1.0)
	10-199	Cervical Surgery	5	1 (0.2)	2 (0.4)	2 (0.4)
		Previous sPTB	45	6 (0.7)	15 (1.8)	24 (2.9)
	≥200	Cervical Surgery	4	1 (0.2)	2 (0.4)	2 (4.2)
		Previous sPTB	26	10 (1.2)	13 (1.6)	15 (1.8)
	Total*	Cervical Surgery	32	3 (0.6)	7 (1.4)	12 (2.5)
		Previous sPTB	104	19 (2.3)	33 (4.0)	47 (5.7)

***** Women with iatrogenic deliveries before the gestation of analysis were excluded (cervical surgery group; n=1 less than 30 weeks of gestation, n=4 less than 34 weeks of gestation, n=5 less than 37 weeks of gestation. Previous sPTB group; n=8 less than

30 weeks of gestation, n=4 less than 34 weeks of gestation, n=16 less than 37 weeks of gestation).

Figure Legend:

Figure 1. Standards for the reporting of diagnostic accuracy studies (STARD) flow diagram illustrates the number of participants who were involved in study and those who were excluded according to defined exclusion criteria.

Figure 2. ROC curves for prediction of sPTB before 30, 34 and 37 weeks gestation