Fetal fibronectin as a biomarker of preterm labor: a review of the literature and advances in its clinical use

Spontaneous preterm birth (sPTB) is a challenge in obstetrics today, and is the leading cause of neonatal morbidity and mortality. The ability to predict preterm birth had, until recently, been poor. The biomarker fetal fibronectin (fFN), found at the maternal–fetal interface, when present in high concentrations in cervicovaginal fluid, has been shown to increase the risk of sPTB in symptomatic and asymptomatic women. Recently, further research has been performed into the applicability of such a test to clinical practice, and its effects on management decisions and patient outcomes. Owing to its high negative predictive value, a negative fFN result has been shown to reduce unnecessary interventions, change patient management and reduce healthcare costs, by allowing early reassurance and return to normal care pathways, while care can be concentrated on those at risk. The development of a bedside quantitative fFN test has shown promise to further improve the positive predictive abilities of fFN, as have combined predictive models with cervical length and fFN.

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Significance of preterm birth

Preterm birth (PTB) is currently the leading cause of neonatal morbidity and mortality in developed countries, and despite extensive research efforts, the incidence is increasing [1]. In 2010, 11.1% of all live births were delivered preterm, with 14.9 million premature deliveries worldwide [2]. The rate of PTB varies widely between countries ranging from 5 to 9% of births in Europe, 12% in the USA and up to 18% in Malawi [2]. Defined as delivery before 37 weeks gestation [3], PTB can be divided into three categories: spontaneous preterm labor with intact membranes (50%); preterm premature rupture of membranes (30%); and iatrogenic preterm delivery for maternal or fetal indications, in which labor is either induced or delivery is by prelabor cesarean (20%) [4], The first two categories are often collectively referred to as spontaneous PTB (sPTB) and their etiology may be very similar. Part of the challenge of identifying trends in PTB rates is due to

varying classifications and birth registration processes across countries, complicating the comparison of data. A recent worldwide systematic analysis of PTB rates in 2010 concluded that there was no decrease in rates of PTB in countries studied from 1990 to 2010, with rates either being increased or stable [2].

Furthermore, a Danish study reported a 51% increase in the proportion of sPTB in nulliparous women with singleton pregnancies, and a 20% increase in sPTB in multiparous women at low risk from 1995 to 2004 [5]. An increase in assisted reproductive techniques was also found, resulting in a higher likelihood of multiple pregnancies, and thus an increased risk of preterm parturition for both spontaneous and iatrogenic reasons. Increased rates of PTB are likely to be partly associated with changes in obstetric practice and increased interventions; however, the data from Denmark still highlights that there has been no decrease in sPTB, despite advances in antenatal care and current interventions.

Future
Medicine part of fsg

There are a wide range of factors that influence a woman's likelihood of developing preterm labor and subsequent delivery. These include: socioeconomic status; ethnicity; maternal age; smoking habits; diet; and a short interpregnancy interval [1]. This interplay between biological and environmental factors further complicates the challenge in prediction of sPTB [6]. Complications of PTB are estimated to account for 35% of the world's 3.1 million neonatal deaths annually, and are now the second most common cause of neonatal deaths worldwide in children under 5 years of age, with the first being pneumonia, while PTB is the leading cause of child deaths in almost all high income and middle income countries [2]. The corresponding emotional and economic costs to the public sector are also significant. The Institute of Medicine (DC, USA) estimated costs related to PTB were US$26 billion a year [7], with a reduction in costs as gestational age increases owing to the reduced requirements of neonatal intensive care. The average length of stay in the neonatal intensive care unit for an infant born <32 weeks gestation is 46 days at a cost of US$280,000 in the USA [8]. Moreover the adverse effects of PTB are not only confined to the neonatal period, and despite advances in neonatal care, survival is commonly associated with adverse long-term outcomes (e.g., bronchopulmonary dysplasia, blindness and neurodevelopmental delay) [6,9–12].

PTB is a complex issue with a multifactorial etiology [6]. To date, pathogeneses thought to be involved include cervical weakness, vaginal infection, pre-existing infection in the endometrium, systemic infections accessing the fetal compartment, decidual hemorrhage or in response to other factors (e.g., stretch with multiple pregnancies) [13]. Despite the current interventions, the impact of PTB on society continues to rise [5], largely due to the inability to intervene in the process of preterm labor. Interventions are developing, but directing them to the correct populations is the key, and our ability to predict PTB has until recently been poor.

The original markers of obstetric history, demographic factors and preterm labor symptoms are poor indicators of those women who will go on to deliver preterm [14,15], with almost 90% of women presenting with symptoms and signs of preterm labor not going on to deliver within 7 days, and almost 75% will deliver at term [16]. The identification of at-risk women and accurate prediction of PTB enables targeted interventions, such as administration of steroids and transfer to an appropriate referral centers, to occur in an attempt to minimize neonatal morbidity and mortality. Although possible interventions to prevent PTB occurring are still undergoing research, cerclage, progesterone and cervical pessaries all have shown potential in reducing risks, but identification of those at highest risk will enable researchers and clinicians to better target future research at those most likely to benefit from such interventions.

Fetal fibronectin

Fetal fibronectin (fFN) is a glycoprotein found in placental tissue, amniotic fluid, and between the chorion and decidua, acting like the 'glue' at the maternal–fetal interface. It was first identified in 1985 by Matsuura and Hakomori, due to the presence of the unique monoclonal antibody FDC-6 [17]. fFN is normally present in cervicovaginal fluid in the early weeks of pregnancy before fusion of the decidua and fetal membrane is complete, and again at the end of pregnancy after 35–37 weeks gestation [18], possibly as a normal physiological phenomenon. fFN is thought to be released when the choriodecidual interface is disrupted by mechanical- or inflammatory-mediated injury prior to birth. Therefore, its presence in cervicovaginal fluid in high levels at or after 22 weeks gestation, detected by a swab test in cervicovaginal secretions, has been associated with an increased risk of sPTB [4]. Its detection, even as early as 13 weeks, has some relationship to PTB risk.

The fFN test measures the amount of fFN in the cervicovaginal fluid and assesses the risk of PTB. Most research to date is based on two methods of detection of cervicovaginal fFN, a quantitative laboratory-based ELISA that gives exact concentrations of fFN using the specific FDC-6 monoclonal antibody to bind fFN, and a qualitative rapid bedside test that yields a positive or negative result; both are based on a concentration of 50 ng/ml as the threshold for a positive test [19]. This bedside test has more recently incorporated a quantitative result. The clinical use of fFN has been greatly improved by the introduction of the TLi$_{IQ}$® beside system (Hologic, MA, USA). Prior to its introduction, the specimens needed to be transported and stored in a laboratory for ELISA testing. This meant that results were not usually available for 24–36 h, which could have a significant impact on clinical decision-making. The TLi$_{IQ}$ is performed in hospitals and takes approximately 20 min to yield a result. Testing of fFN is equally accurate for both the rapid test and the ELISA assay, which allows comparison between earlier studies and more recent data using the rapid bedside test [20,21]. The threshold of 50 ng/ml, chosen by the manufacturers, has been further validated by Goepfert et al., who evaluated the relationship between quantitative fFN values and sPTB [22]. fFN specimens were collected at 24, 26, 28 and 30 weeks gestation from 2926 women. ELISA was used to calculate the quantitative fFN

values. Receiver operator characteristic curves were used to identify the optimal threshold for fFN values to predict sPTB within 4 weeks of testing and at <35 weeks gestation. The optimal cutoff point was 45–60 ng/ml for a positive fFN at 24–30 weeks gestation to predict sPTB <35 weeks. As expected, the risk of sPTB increased as levels of fFN increased. This suggests that, the currently accepted value of 50 ng/ml is a good cutoff point for predicting sPTB <35 weeks gestation. However, in clinical practice, sensitivity and specificity are not always equally weighted, and the availability of the new bedside quantitative test may prove useful for clinicians.

fFN in symptomatic women

Lockwood *et al.* were the first to describe fFN in vaginal secretions as a predictor of preterm delivery in 1991 [19]. Shortly after, a multicenter analysis by Iams *et al.* on 192 women from 24 to 34 weeks gestational age with symptoms of preterm labor, found that the fFN assay was most predictive of the likelihood of delivery within the next 7 days with a sensitivity of 93%, specificity of 82%, positive predictive value (PPV) of 29% and negative predictive value (NPV) of 99% [23]. They also reported that fFN was superior to both cervical dilation greater than 1 cm and frequency of uterine contractions in predicting sPTB. Since then, multiple studies have demonstrated that the presence of quantities of fFN >50 ng/ml in cervicovaginal fluid is an independent risk factor for the prediction of PTB in women with symptoms suggestive of preterm labor [23–32].

Peaceman *et al.* also looked into the use of fFN in threatened preterm labor [28]. In total, 763 women between 24^{+0} and 34^{+6} weeks gestation, with uterine contractions, intact membranes and cervical dilatation <3 cm, were enrolled in the double-blinded study. Using 50 ng/ml as a cutoff, 80% of women had negative fFN tests, with the NPV for delivery within 7 and 14 days and at <37 weeks gestation of 99.5, 99.2 and 84.5%, respectively. The PPV was only 13.4% for delivery within 7 days, but rose to 43% for delivery before 37 weeks. These findings have been consistent with both earlier and subsequent publications, with the greatest value of fFN being its high NPV, offering the potential to reduce unnecessary interventions.

Given the numerous number of studies in the healthcare literature regarding fFN, all with varying populations, statistics and reported prediction capabilities, several systematic reviews and meta-analyses have been performed to summarize the large quantity of data available. A large, systematic, quantitative review of test accuracy studies, by Honest *et al.*, looked at 64 observational studies (40 with fFN testing in symp-

tomatic women and 28 in asymptomatic women), with 26,876 patients, to determine the performance of the presence of cervicovaginal fFN in predicting sPTB [20]. Their end points were delivery within 7–10 days from testing, and <34- and <37 weeks gestation. They found that the accuracy of cervicovaginal fFN in predicting sPTB varied considerably for the various gestations studied, but that it is most accurate at predicting sPTB within 7–10 days of testing in women with symptoms of threatened preterm labor. The pooled likelihood ratio for positive results was 5.42 (95% CI: 4.36–6.74) and 0.25 (95% CI: 0.20–0.31) for negative results, with a PPV of 20.6%. Likelihood ratios are valuable in the comparison of studies as they are far less influenced by prevalence, and allow studies to be meaningfully pooled and compared, whereas a PPV can only be interpreted in light of the risk status of the population studied. The use of 7–10 days as an end point is of particular clinical use, as this time frame allows administration of antenatal corticosteroids and necessary transfers to tertiary centers to be made. Being able to identify 'at-risk' patients also avoids unnecessary interventions and potential harm being caused in those who have a low risk of delivery in the coming weeks. If every women with symptoms of threatened preterm labor at 31 weeks gestation was treated with antenatal steroids, 109 women would need to be treated to prevent a single case of respiratory distress syndrome in the newborn. However, if only those with a positive fFN are treated, the number needed to treat is only 17 [20].

Smith *et al.* published a systematic review of systematic reviews looking at the predictive capabilities of both fFN and cervical length in PTB [33]. It included five reviews on fFN, with three of them concluding that the presence of fFN in cervicovaginal fluid in symptomatic women, may be useful to predict PTB within 7–10 days of sampling [20,34–35]; this review also included the previously mention paper by Honest *et al.* [20]. One review paper reported that, in symptomatic women, the NPV of fFN for delivery within 7–10 days was a sensitivity of 98–100% and a specificity of 98 and 83%, respectively, across seven studies [34]. A review by Faron *et al.*, also included in the Smith *et al.* analysis, concluded that fFN in cervicovaginal fluid is predictive for PTB in both high-risk and low-risk women for delivery before 34, 35 and 37 weeks gestation [36]. Only one of the reviews included, by Chien *et al.*, concluded that the "presence of fFN in cervicovaginal mucus has limited accuracy in the prediction of preterm labor" [37], with a pooled likelihood ratio in symptomatic women with a positive test for delivery at <37 weeks gestation of 4.6 (95% CI: 3.5–6.1), and the pooled likelihood in the same group for a negative

test of 0.5 (95% CI: 0.4–0.6). However, Chien et al. reported that fFN in symptomatic women relates, at best, to a moderate increase of PTB within 7 days of sampling [37]. Whether the test is good, or of limited accuracy, depends largely on the opinion of the author, as likelihood ratios are consistent over most studies. Actual value depends on whether the predictive ability – that is, to rule in or rule out PTB – impacts on management and outcomes, something not often considered in the literature.

The issue of whether management is affected has partly been addressed by the most recent meta-analysis performed by Berghella et al., looking at five studies with a total of 474 symptomatic women with randomization to knowledge of fFN and no knowledge of result, concluded that management based on the fFN result significantly decreased the rate of PTB before 37 weeks in the studied populations, with rates of 15.6% PTB versus 28.6% in the control group [4]; an almost 50% reduction in PTB rate when the management was based on knowledge of the fFN result. This difference in PTB rates was only seen for the outcome of delivery at <37 weeks gestation. There was no significant difference between the two groups for other outcome measures of rates of PTB <34, 32 or 28 weeks gestation; birthweight <2500 g; maternal hospitalization; perinatal death; tocolysis; or the use of steroids for fetal lung maturity. Given the findings of this review, with a reduction in PTB incidence before 37 weeks gestation based on the fFN result, but other outcomes not reaching significance, further research should be encouraged. This is in contrast to a single study by Grobman et al., which looked at whether fFN results affected patient management and healthcare costs [38]. Their findings suggested that knowledge of the fFN test result made no difference to clinical management or to associated healthcare costs with preterm contractions, reporting that there was no reduction in healthcare costs with knowledge of the fFN result. In this study, although also including only symptomatic women, were fewer positive tests, suggesting the overall risk in this population may have been less and the authors commented that an extended study period may have resulted in increased clinician confidence in the test results.

The issue of healthcare costs associated with PTB is an important one. Currently, women presenting with threatened PTB are transferred to a center appropriate for their gestation, and treated with corticosteroids and tocolytics; in many cases this turns out to be unnecessary. Although, the study by Grobman et al. did not find a reduction in relation to knowledge of fFN, many others have reported promising results [38]. A review in 2010, by Dutta et al., included 10 studies of 5129 patients, looking at the efficacy of fFN testing in minimizing hospital admissions, length of hospital stay and cost savings in women with symptoms of preterm labor, with four out of the ten studies included reporting a significant reduction in the number of hospital admissions and length of stay between the testing and no testing arms [18]. The further six studies showed a significant difference between negative and positive fFN test results. A study by Giles et al. (included in the review), with 151 patients in an 18-month prospective audit, showed that hospital admission was an average of 7.6 days shorter for patients with a negative fFN result, with an average saving of US$2970 [39]. The use of fFN is associated with a 90% reduction in maternal transfer, and a substantial reduction in both costs and inconvenience. A more recent study in 2013, in The Netherlands by van Baaren et al., looked at the cost–effectiveness of a combination of cervical length measurement and fFN testing in women with threatened preterm labor [40]. They performed a model-based cost–effectiveness analysis of seven test treatment strategies. The authors concluded that the most cost-effective strategy for women between 24 and 34 weeks gestation with threatened PTB, is a combination of fFN and cervical length measurement, without compromise to neonatal outcomes. They estimated cost savings of €.8–14.4 million a year in The Netherlands. There are, however, limitations to a model-based analysis, as it is a simplification of a realistic scenario and further research into the application of these models into clinical practice is needed.

fFN in asymptomatic women

The role of identifying asymptomatic women at risk for sPTB has also been extensively studied. In a large, multicenter study, by Goldenberg et al., 2929 asymptomatic women from 22 to 24 weeks gestation were routinely screened for presence of fFN in cervicovaginal secretions every 2 weeks [41]. They concluded that a positive fFN test early in gestation (24–26 weeks) was a more sensitive predictor of sPTB than at a later gestation (28–30 weeks), and that early sampling was able to predict more then 60% of very early PTBs; when the clinical implications are greatest. At all gestational end points used, a positive fFN significantly correlated with sPTB. In an extension of this study, Goldenberg et al. looked at the relationship between fFN, short cervix, previous PTB, bacterial vaginosis and BMI [42]. They concluded that a positive fFN test was the single strongest, independent predictor of PTB with a relative risk (RR) for delivery of 14.1 (95% CI: 9.3–21.4) prior to 32 weeks gestation and a RR of 6.7 (95% CI: 4.9–9.2) prior to 35 weeks gestation. A positive fFN and a short cervical length were the most associated with sPTB

<37 weeks gestation of all the risk factors looked at in this study, with a RR of 3.3 (95% CI: 2.5–4.2) and 3.5 (95% CI: 2.7–4.6), respectively. This was the largest study of asymptomatic screening, but concluded that routine screening was not recommended owing to a lack of effective preventions and treatments. However, these interventions have certainly developed in recent years. A smaller study by Hellemans et al. of 133 asymptomatic women, deemed to be at low risk for sPTB, had similar findings with the presence of cervicovaginal fFN predicting sPTB, with a sensitivity and specificity of 60 and 85%, respectively, and a PPV and NPV of 25 and 96%, respectively [43]. However, they concluded that there was limited clinical value to routine screening for cervical fFN in the general obstetric population, perhaps because of the limited interventions available at this time.

Studies have also looked into the usefulness and effects of fFN testing in asymptomatic, high-risk women, with the theory that interventions prior to the development of symptoms of threatened preterm labor may be more successful, as the labor process has not yet commenced.

Nageotte et al. looked at cervicovaginal fFN as a screening test for subsequent PTB in 87 asymptomatic women at high risk for PTB [44]. In total, 31% of patients had a sPTB. The presence of fFN as a predictor for delivery before 34 weeks gestation had a sensitivity of 92.3% and a NPV of 97.8%. A positive fFN test was significantly related to sPTB (odds ratio: 3.8; p < 0.001), and when additional predictive methods, such as the presence of greater then four uterine contractions per hour, tocolytic therapy or cervical dilation, were added, it did not increase the predictive capabilities of a positive fFN alone.

Bittar et al. also looked at the role of cervical fFN in predicting sPTB in asymptomatic patients at high risk of preterm delivery [21]. They performed a prospective cohort study on 102 women deemed at high risk for sPTB based on previous PTB (70 out of 102; 68.62%), prophylactic cervical cerclage (20 out of 102; 19.6%), and uterine malformation (12 out of 102; 11.76%). fFN samples were taken every 2 weeks from 24 to 34 weeks gestation. There were 38 PTBs in the cohort (37.25%). The sensitivity and specificity for predicting sPTB <37 weeks gestation was 73.68 and 92.18%, respectively, with a PPV of 84.84% and NPV of 85.5%. This relatively modest NPV may be explained by the fact that there is a high prevalence of PTB <37 weeks in asymptomatic women in this study (as there is also a corresponding high PPV influenced by the prevalence). While this NPV is lower then that in the previous study (of 97.8%), lower NPV values occur when using later gestations (i.e., 37 weeks as a cutoff). Interestingly, their findings were not as good as other studies on women at less risk, which may also be attributed to the inclusion of women with prophylactic cervical cerclage and other structural abnormalities that may have affected the predictive capabilities of fFN. Studies have demonstrated that the presence of cerclage in asymptomatic women may increase false-positive results, but a good NPV is maintained, which is likely to be useful clinically – discussed later [45].

Bastek et al., in 2013, performed a single-center prospective cohort study looking at 104 asymptomatic women at high risk for sPTB [46]. They had a PTB rate of 24.5% in the cohort. The authors aimed to investigate whether subsequent positive biomarkers from different pathways of sPTB (i.e., inflammatory and mechanical) would be a better predictor of sPTB than a single biomarker (from an isolated pathway) alone. In comparison to the studies mentioned above, Bastek et al. reported that, while a prior history of PTB and cervical length were significantly associated with sPTB, fFN and other biomarkers (elafin, soluble E-cadherin and IL-6) were not. With there being no significant difference or improved predictive capabilities with the inclusion of biomarkers or fFN testing, then prediction is based on cervical length alone. This study is limited by sample size, is at variance with most other studies and may represent a type 2 statistical error due to it's single-center recruitment, and limited follow-up for testing at a second visit, with only complete data from 47 women available for analysis at the second visit. It does, however, highlight the need for further evaluation and research prior to recommending widespread fFN testing of asymptomatic women at high risk for sPTB.

The effect of fFN results on clinicians' management decisions, and hence patient outcomes, has also been discussed in many papers. Bolt et al. looked at 81 women from a single institution between 23 and 34^{+6} weeks gestation, both symptomatic and asymptomatic, with the asymptomatic population being considered at increased risk of PTB based on previous obstetric history or cervical surgery [47]. In total, 15 of the women had a positive fFN test and a total of 97 fFN swabs were performed. Clinician responses were obtained with 28% of tests directly affecting management (in 20 women) and that nearly 90% of the tests had the potential to change management based on the result; see Figure 1. However, this study did not look at outcomes of the chosen management, and further research is required to evaluate whether there is any affect on clinical outcomes. While cervicovaginal fFN has been shown to be a reliable predictor in high-risk asymptomatic women, its use has been associated with increased anxiety in patients [48]. However, a negative test can
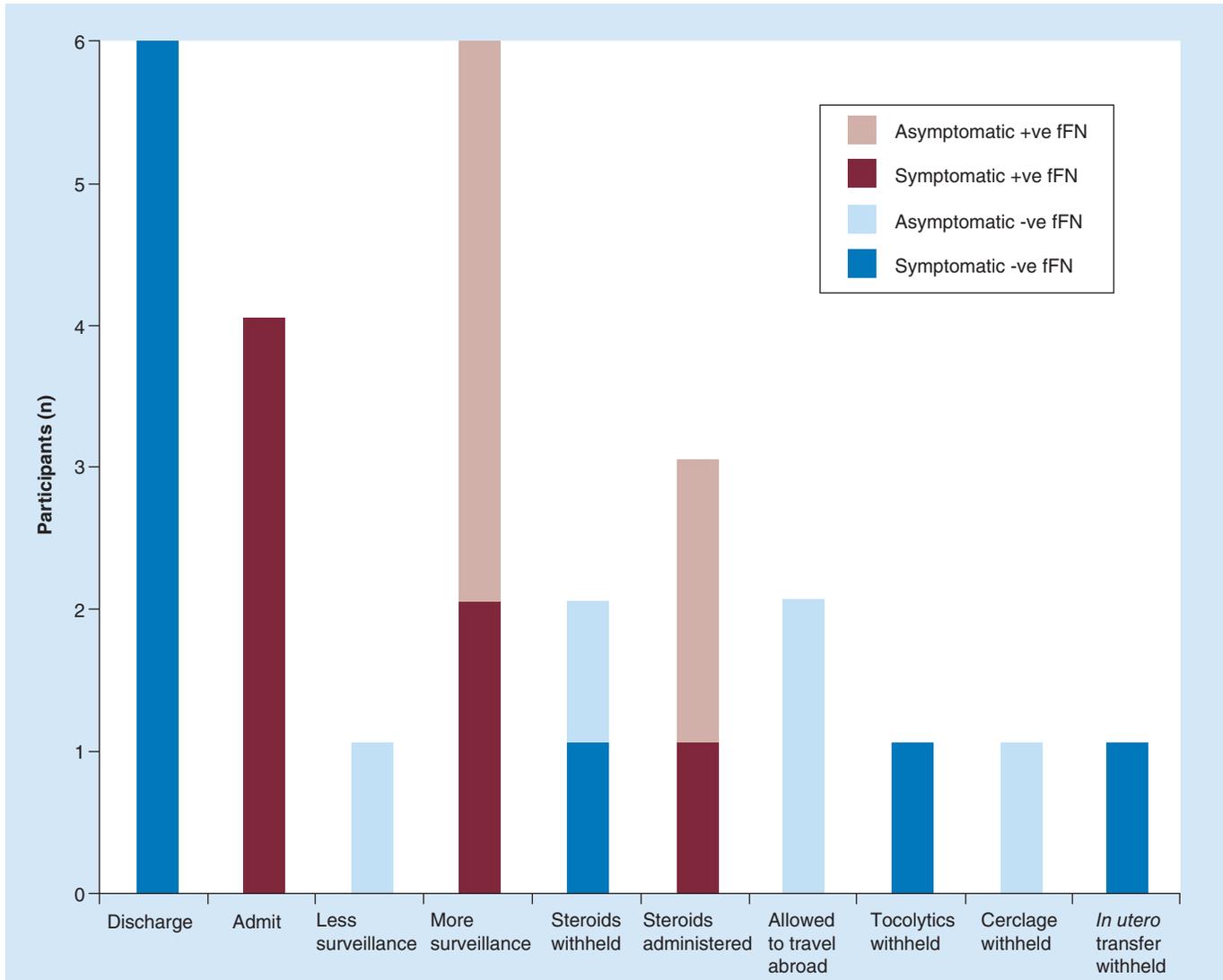
Figure 1. Clinical management changes from fetal fibronectin testing (n = 27).
+ve: Positive; -ve: Negative; fFN: Fetal fibronectin.
Adapted with permission from [47].

also provide reassurance to both the patient and clinician that the risk of delivery in the next 1–2 weeks is low, which allows patients to make informed decisions regarding activities, such as intended travel or work.

The results of these more recent studies highlight how confidence in fFN testing has increased over the past few years, and that clinicians are willing to base significant management decisions solely on the basis of a negative or positive test. With significant decisions being made to discharge home versus admit to hospital for observation, or further interventions, such as cerclage or *in utero* transfer to a tertiary center, being made based on the result [47]. A negative fFN test has been shown to result in fewer hospital admissions and a shorter length of stay in a tertiary center [49]. In 2000, Giles *et al.* found that the use of fFN to determine the need for maternal transfers from the community

hospitals in New South Wales (Australia), resulted in a 90% reduction in transfers, with a negative result negating the need for urgent transfer and allowing for substantial cost savings [39]. This further highlights the benefits of accurately predicting those who need further observation and interventions on an emotional scale for the patients, health outcomes for the fetus and also on a wider economical scale.

Quantitative versus qualitative fFN testing

It has been suggested that further information from a quantitative value, as to the amount of fFN present in the cervicovaginal fluid, could increase predictive capabilities and allow the clinician to make a more informed decision regarding management. Qualitative tests based on one threshold are prone to false-positive and -negative results around that cutoff point. Ear-

lier studies using the quantitative ELISA test suggest that the relationship between fFN and sPTB may be more linear, with increasing concentrations of fFN in cervicovaginal secretions relating to a higher risk of sPTB and, therefore, this knowledge could increase prediction capabilities [22,50–51].

Goldenberg *et al.* determined how patterns of fFN positivity from 24 to 30 weeks gestation predict subsequent fFN results and sPTB [52]. Using the data from the large, multicenter trial with 2929 symptomatic women (mentioned earlier), they concluded that two negative results are required following a positive fFN before the risk of sPTB returns back to the baseline. It would be interesting to see whether with the additional knowledge of the quantitative value of fFN, whether this would change, as some women may go from 49 to 51 ng/ml of fFN, hence changing from a negative to positive result on the qualitative test, however, this is not a great change when the exact value is known. Whereas, knowing a patient's fFN concentration has gone from <10 to 500 ng/ml is more clinically significant, but still yields a negative and then positive result on the standard qualitative test currently most widely used. These studies suggest that quantification of fFN provides additional information that may have more clinical implications than the current qualitative testing.

More recently on a much higher risk cohort, Kurtzman *et al.* looked at quantitative fFN screening in asymptomatic high-risk patients with a single fFN test performed at 24 weeks gestation [50]. Data from 563 women with a previous history of sPTB were analyzed, with a PTB rate of 6.7% at <34 weeks and 19.7% at 37 weeks gestation. In total, 88% of patients had a fFN level of 0 ng/ml at 24 weeks. In contrast to this group (fFN 0 ng/ml), sPTB rates increased progressively as the ng/ml of fFN increased. The RR for sPTB <34 weeks with increasing fFN concentrations compared with 0 ng/ml were 2.42 (fFN 1–49 ng/ml; 95% CI: 0.76–5.66), 4.68 (fFN 50–199 ng/ml; 95% CI: 1.28–10.95), and 9.94 (fFN >200 ng/ml; 95% CI: 2.90–19.67). Goepfert *et al.* provided supporting data that increasing levels of fFN in cervicovaginal fluid up to 300 ng/ml are associated with increasing risks of sPTB [22].

Following this promising data, a bedside test was developed that would produce quantitative results, and Abbott *et al.* performed a prospective blinded study looking at fFN concentrations in cervicovaginal fluid in 300 women with symptoms of preterm labor [53]. Clinicians were blinded to the quantitative result (performed on the Rapid fFN® 10Q analyzer, Hologic, Inc., MA, USA) until after delivery, but knowledge of the qualitative fFN was not blinded. Thresholds of 10, 50, 200 and >500 ng/ml were looked at, with the PPV for sPTB at <34 weeks gestation increasing with each threshold (19, 32, 61 and 75%, respectively). The RR of delivery when each threshold was compared in increasing concentrations with <10 ng/ml was 5.6 (95% CI: 1.05–29.57), 7.9 (95% CI: 1.38–45.0), 22.8 (95% CI: 3.84–135.5), and 51.3 (95% CI: 12.49–211.2; p < 0.01). This study was performed on a bedside quantitative Rapid fFN 10Q analyzer, and its results suggest that further information can be obtained to enable better prediction and the bedside accessibility of the result make it more promising for widespread use in clinical practice.

In effect, as the level increased, the positive prediction and likelihood ratios became much higher, providing a good 'rule in' test (high specificity). Conversely, lower levels provide a good 'rule out' test (high sensitivity and NPV). Clinicians can tailor their decisions to these variable risks, as some decisions may be instigated at different thresholds and circumstances. For example, steroid use may be given at a lower threshold then *in utero* transfer, and both these decisions may vary with gestation.

Potential increased predictive capabilities of a multiple marker test for sPTB

There are currently multiple biomarkers that have been suggested to play a potential role in the prediction of PTB. Cervical length by transvaginal ultrasound and the presence of fFN in cervicovaginal fluid are the two strongest and most established predictors of sPTB. Transvaginal cervical length measurements in asymptomatic women at high risk for sPTB is becoming increasingly popular, with shortening of the cervix below 25 mm proving to be a good predictor of sPTB [54–58]. Given the ability of fFN and cervical length to independently predict sPTB, studies have attempted to combine the two markers of prediction to develop an improved predictive model.

Bolt *et al.* looked at 147 asymptomatic women at high risk for sPTB between 22^{+0} and 30^{+0} weeks gestation who underwent cervical length and fFN testing [59]. A total of 132 women spontaneously labored, with 18% delivering at <37 weeks gestation. Of the 18%, a positive fFN and cervical length of 25 mm or less was associated a 53% risk of sPTB at <37 weeks gestation. Those women with a positive fFN and cervical length >25 mm, only had a 10% risk of sPTB. They also reported significant hazard ratios with the addition of a positive fFN to a known cervical length measurement, regardless of the cervical length, but no significance when cervical length results were added to a known fFN. This suggests the potential use of fFN as an initial screening tool, with cervical length measurement being used only for those with a positive fFN result.

A systematic review by DeFranco *et al.* looked at nine studies with women with symptoms of preterm labor (four studies included both singleton and twin gestations) from 22 to 35 weeks gestation [60]. The overall rate of PTB, <37 weeks gestation, in these studies was 30.2%. The highest levels of sensitivity were reported for studies predicting PTB at <7 or <28 days, with sensitivities of 71.4 (95% CI: 35.9–91.8) and 85.7% (95% CI: 60.1–96.0), respectively. With pooled specificity being high, from 83 to 97%, for all studies included, despite the outcome definition. The PPV and NPV for prediction of sPTB using fFN and cervical length was at <7 days (PPV: 45.4%; 95% CI: 21.3–72, NPV: 98.9%; 95% CI: 96.1–99.7), <28 days (PPV: 63.2%; 95% CI: 41.0–80.8, NPV: 96.8%; 95% CI: 89.1–99.1), and <37 weeks (PPV: 49.4%; 95% CI: 38.6–60.2, NPV: 74.4%; 95% CI: 68.8–79.3). This indicates that, in this cohort, the combined screening tool was more effective at predicting sPTB in short-time intervals and at earlier gestations. The use of these combined techniques in symptomatic women appears to improve the PPV of single-screening methods, while still maintaining a high NPV and sensitivity. These studies that combine multiple predictors were observational, and further research is required in this area to delineate their role in effecting management decisions at the 'point of care'.

Gomez *et al.* also found that a combination of transvaginal cervical length measurements and cervicovaginal fFN testing improved prediction of sPTB in symptomatic women [27]. They performed a prospective cohort study looking at 215 symptomatic women, with a prevalence of sPTB <35 weeks gestation in the cohort of 20%. They further reiterated that both fFN and cervical length are independent predictors of sPTB, however, when combined, the diagnostic performance of each test was improved.

There are many other biological markers that have been suggested as potential predictors for PTB (i.e., estriol, corticotrophin-releasing hormone, β-human chorionic gonadotrophin, AFP, activin, inhibin and relaxin) [61], however, the current data is not as strong for these markers as for fFN and cervical length [62]. Two available bedside tests are, Actim Partus (Medix Biochemica, Kauniainen, Finland), which detects the presence of pIGFBP-1, and Partosure (Qiagen, Hilden, Germany), which detects PAMG, which also show potential as biomarkers of PTB, with rapid bedside tests being available [63,64]. Although their role, and that of other biomarkers in the prediction of PTB, is beyond the scope of this paper. The available data for fFN is considerably larger than these markers.

Limitations to fFN testing

As with any test there are limitations and circumstances when it does not perform so well. Most of the limitations of fFN testing have been widely known and, fortunately of the studies performed, many have made attempts to control for these limitations. The swab test (although easily performed) is operator dependent since it is supposed to be held in the posterior vaginal fornix for 10 s to allow maximal absorption of secretions. However, the level of operator skill required is much less then for other potential screening tests (i.e., determining cervical length by ultrasound). Sexual intercourse and digital cervical examination within 24 h of sample collection, have been associated with false-positive results, as has the presence of vaginal bleeding [65]. Licensing recommendations also state that the use of lubricants, soaps or disinfectants may interfere with absorption of the specimen onto the swab, although, this is largely theoretical. These limitations can make it difficult to determine the most effective time to perform the fFN in emergency cases. for example if a result is needed inspite of recent sexual intercourse or cervical manipulation. Clinicians need to be aware of the higher probability of a false-positive result, although, a negative test can be logically relied on even in these 'unlicensed' situations. The availability of the Rapid fFN test now makes it more accessible to smaller regional and remote centers that may not have access to an onsite laboratory to run an ELISA test.

Cervicovaginal fFN use in multiple gestations

The number of multiple gestation pregnancies are increasing [66], particularly higher-order multiples, owing to the increase in assisted reproductive techniques [67]. The higher risk of PTB in this population makes the need for a predictive marker of sPTB clinically relevant, although, prophylactic treatments in twins, such as progesterone, and cervical cerclage have not proved beneficial, but the Arabin pessary (Arabin Dr. GmbH & Co. KG, Witten, Germany) has had variable success [68]. A systematic review and meta-analysis by Conde-Agudelo and Romero, 2010, looked at 15 studies with a total of 1221 women with multiple pregnancies [69]. The analysis included both symptomatic and asymptomatic women. For asymptomatic women, the pooled sensitivities, specificities and positive and negative likelihood ratios for predicting sPTB at <32 weeks gestation were 35 (95% CI: 17–59), 94 (95% CI: 86–97), 5.5 (95% CI: 2.8–11.1) and 0.69% (95% CI: 0.47–1.06), respectively. For sPTB <37 weeks gestation, they were 40 (95% CI: 32–52), 85 (95% CI: 78–93), 2.7 (95% CI: 1.5–4.9)

and 0.71% (95% CI: 0.55–0.85), respectively. The area under summary receiver operator characteristic (ROC) curves for asymptomatic women with multiple gestations were 0.82 for delivery <37 weeks gestation and 0.78 for delivery <32 weeks gestation. For symptomatic women with multiple pregnancies, the pooled sensitivities, specificities and positive and negative likelihood ratios for cervicovaginal fFN were most accurate within 7 days of testing; 85 (95% CI: 51–98), 78 (95% CI: 66–90), 3.9 (95%: CI 2.5–5.6) and 0.20% (95% CI: 0.05–0.72), respectively. The area under the ROC curves for symptomatic women with multiple gestations were also greatest for delivery within 7 days of testing (0.85), with an area under the ROC curve of 0.57 for delivery <37 weeks gestation. The low likelihood ratios found in this review suggest that fFN may only play a minimal role in predicting sPTB in multiple gestations, and that it appears to be most useful in predicting sPTB before 32 weeks in asymptomatic women and within 7 days in those with symptoms of threatened PTB. The later gestational end points may not be so relevant in twins, as the presence of fFN may be due to stretch and a more physiological mechanism.

Fox et al., 2009, examined a retrospective cohort of 155 asymptomatic twin pregnancies, using a combined predictive model of fFN testing and measurement of cervical length between 22 and 32 weeks gestation [70], with findings that a combination of positive fFN and cervical length <20 mm had a significantly higher PPV for delivery at <37, <34, <32, <30 and <28 weeks gestation. They found that with a single positive test (either cervical length or fFN), the rates of sPTB at <28, <30 and <32 weeks gestation were 13.3, 9.5 and 8.3%, respectively. However, if both tests were positive, these rates were significantly increased at 50, 33.3 and 54.5%, respectively. Cox proportional hazard models were used showing an adjusted hazard ratio of 5.86 (95% CI: 3.11–11.05) for fFN positive versus fFN negative, and 4.75 (95% CI: 1.98–11.35) for positive fFN and cervical length <20 mm versus negative fFN and cervical length >20 mm. This study suggests that, perhaps a combined screening model may be useful in predicting sPTB in twin pregnancies, but further research needs to be performed to confirm these findings.

Many of the multiple gestation studies include both twins and higher gestations making it difficult to interpret the results. Roman et al. looked specifically at fFN for prediction of sPTB in women with asymptomatic triplet pregnancies [71]. A total of 56 patients were retrospectively analyzed. The outcomes of sPTB prior to 28, 30 and 32 weeks gestation, and delivery within 2 and 3 weeks of testing, were examined. Their findings suggested a moderate-to-high prediction of sPTB in

women with triplets. Sensitivity, specificity, PPV, NPV and positive likelihood ratio for delivery <30 weeks were 75, 85.4, 46.2, 95.3 and 5.13%, respectively, and for delivery within 3 weeks of testing were 53.3, 95.8, 53.3, 95.8 and 12.7%, respectively.

In 1996, Tolino et al. performed a prospective cohort study on 68 asymptomatic women with multiple pregnancies, with the use of qualitative fFN determined by ELISA assays [72]. Using a single positive sample, the sensitivity, specificity, PPV and NPV of sPTB at <37 weeks gestation was 90.9, 68.5, 71.3 and 88.8%, respectively. If a positive test was considered as two consecutive positive samples, predictive capabilities improved to 86.6, 78.9, 76.4 and 88.2%, respectively. Although these findings are from a single study, it is something to consider that perhaps the test is still valid in patients with multiple gestations, but the definitions and thresholds may be need to altered. Studies are underway considering quantitative fFN in twins, and this is likely to add to the value as per the data in singleton pregnancies.

Cervicovaginal fFN use in women with cervical cerclage

The presence of a cervical cerclage already highlights a women at high risk for sPTB, and the ability to predict impending sPTB in this particularly high-risk cohort could have significant impacts on ongoing obstetric observation and management. fFN is currently not recommended by the manufacturers for use in the presence of cervical cerclage, based on insufficient data to prove its efficacy at the time of licensing for this indication. An early retrospective study, which was small and uncontrolled, suggested that the presence of a cervical cerclage reduced the PPV and NPV of fFN for PTB [73]. Since then, further, larger studies have been performed. Duhig et al. carried out a retrospective observational study of 910 asymptomatic high-risk women having fFN tests performed between 23^{+0} and 27^{+6} weeks gestation [45]. They found that for delivery <30 weeks gestation, the presence of a cervical cerclage reduced the specificity of fFN testing compared with the control group, 77 versus 90% (p < 0.00001), but that the sensitivity remained similar between the two groups, 78.6% with no cerclage and 60% with cerclage (p > 0.4). The NPV for both groups also remained high, >98% for prediction of delivery at <30 weeks gestation. This suggests that, although the presence of cervical cerclage increases the chances of a false-positive fFN test, the predictive value of a negative test does not change significantly to alter clinical utility. Another smaller study by Benson et al. looked at 48 women with symptoms of threatened preterm labor with a cervical cerclage in situ, and also suggested that

negative prediction remained good enough to use clinically [74]. The sensitivity, specificity, PPV and NPV for delivery <34 weeks gestation were 91, 78, 56 and 97%, respectively, with values being even higher for prediction of delivery within 2 weeks of testing (100, 77, 28 and 100%, respectively).

This suggests that, although the rate of false-positive tests may be higher, clinicians may still be able to make reliable management decisions based on a negative test, despite the presence of a cervical cerclage. This potentially has significant implications for clinical practice, as women with a cervical cerclage *in situ* are those considered at very high risk for sPTB and these studies suggest that a negative fFN test may still be relied on in this group, allowing reassurance where most needed, as these women often have poor histories and considerable anxiety.

Conclusion & future perspective

Several strategies for the identification of women at risk for sPTB have been proposed – that is, risk scoring systems and numerous biochemical markers. The aim of these prediction markers is to decrease unnecessary interventions for patients at low risk for sPTB and to enable identification of those who would most benefit from current interventions, such as administration of tocolysis, antenatal corticosteroids and transfer to a tertiary care facility.

The role of fFN in the prediction of sPTB has been extensively studied, and in populations of both

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Executive summary

Significance of preterm birth
- Preterm birth (PTB) is the leading cause of neonatal morbidity and mortality in the developed world.
- The incidence of PTB is increasing.
- There are substantial associated healthcare costs, often for long-term neonatal complications.

Fetal fibronectin as a predictor
- Fetal fibronectin (fFN) is a glycoprotein that acts as the 'glue' at the maternal–fetal interface.
- It is not normally found in cervicovaginal secretions after 22 weeks gestation.
- The presence of fFN in vaginal secretions is a good predictor of spontaneous PTB (sPTB) in both asymptomatic and symptomatic women.
- Its negative predictive value (NPV) is >98%, making it a useful tool to provide reassurance, and avoid unnecessary and potentially harmful interventions.

Quantitative versus qualitative fFN
- Studies highlight that relationships between fFN concentrations in cervicovaginal fluid are linear, with increased concentrations inferring a greater risk of delivery.
- New bedside quantitative tests are available, superseding the previous time-consuming laboratory-based ELISA testing.
- Quantitative testing improves the positive predictive value and likelihood ratios of the test, while maintaining the high NPV.
- Clinicians can tailor their decisions to these variable risks, as some decisions may be instigated at different thresholds and circumstances.

Multiple marker test for PTB
- Many biomarkers have been suggested.
- Transvaginal cervical length and fFN are the best independent predictors of sPTB.
- A combination of these two markers (fFN and cervical length) appears to improve predictive capabilities further.

Other indications for fFN
- The use of fFN in multiple gestations has suggested a predictive role, but different thresholds may need to be used. The addition of quantitative values in this population is currently undergoing research and may add value.
- fFN has been shown to maintain a high NPV in women with a cervical cerclage *in situ*.

Future perspective
- Further studies into the added value of quantitative fFN in improving the ability to predict preterm birth are needed.
- Combining fFN and cervical length to predict the risk of sPTB and aid management decisions is a focus of future research.
- It is still unclear which interventions are likely to be the most beneficial in preventing or stopping the process of preterm labor, however, being able to identify those at highest risk will enable future studies of interventions to be better targeted at those most likely to benefit.
- To date, few clinical trials of intervention have used fFN, but this may be a way of better targeting interventions in the future.

symptomatic and asymptomatic women, it is one of the best predictors of PTB. All studies to date have shown a relationship between the detection of fFN in cervicovaginal fluid and a shorter period to delivery. A negative fFN result has been shown to reduce unnecessary interventions, change patient management and reduce associated healthcare costs. The addition of quantitative measures and the inclusion of further parameters, such as cervical length, have shown promise in improving the reliability of a positive fFN test and, hence, improving the PPV. Although, it is still unclear which interventions are likely to be the most beneficial in preventing or stopping the process of preterm labor. Being able to identify those at highest risk will enable future studies of interventions to be better targeted at those most likely to benefit. To date, few clinical trials of interventions to prevent sPTB, or improve outcomes, have used fFN, but this may be a way of better targeting interventions in the future, and more research is needed to evaluate this further. The

high NPV in asymptomatic, high-risk women enables early reassurance and return to normal care pathways, while care can be concentrated on those at risk. Prophylactic treatment may be more beneficial earlier, and current work is establishing the role of quantitative fFN at earlier gestations in combination with other biomarkers.

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