Fetal Fibronectin

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OBJECTIVES: The objectives of this review paper are to: describe the fetal fibronectin assay, its purpose, and clinical significance; evaluate the sensitivity and specificity of the fetal fibronectin test; describe the specimen collection and measurement of the fetal fibronectin test; and present the advantages and disadvantages of incorporating fetal fibronectin testing in routine prenatal care.

DATA SOURCES: Current literature.

DATA SYNTHESIS: Fibronectin proteins function in plasma and extracellular matrix in cell adhesion and migration. Recently, a fibronectin protein has been evaluated and proposed as a predictor of preterm delivery. A simple, qualitative assay detects this protein, fetal fibronectin, in cervicovaginal secretions of women who are at risk for or have symptoms of preterm delivery. The test is positive when there has been a rupture in the membranes attaching the fetus to the uterus, thus indicating pending preterm delivery. Sensitivity and specificity studies have been performed to evaluate its reliable prediction of preterm delivery.

CONCLUSION: Evaluation of sensitivity and specificity studies document that the fetal fibronectin test predicts preterm delivery. For symptomatic women, a sensitivity of 89% and specificity of 86% was found.

ABBREVIATIONS: fFN = fetal fibronectin; PTD = preterm delivery.

INDEX TERMS: cervicovaginal secretions; fetal fibronectin; fibronectin; preterm delivery.

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A 25-year-old pregnant female, 26 weeks gestation, presented to her obstetrician with onset of uterine contractions, backache, and abdominal discomfort. She had delivered a healthy baby at preterm, 32 weeks gestation, three years previously. Both she and her physician were concerned because of prior preterm delivery and the gestational week of this episode. A digital cervical examination was performed and vaginal cultures for microbiology were collected. Before the examination and culture collection, a fetal fibronectin collection kit was obtained. The swab was used to collect a sample of cervicovaginal secretions; it was placed in the tube of buffer provided and was sent to the laboratory for a fetal fibronectin test.

Fetal fibronectin (fFN) is detected in the cervicovaginal fluid of pregnant women as a predictor of risk for preterm delivery (PTD). A recently developed qualitative solid-phase immunosorbent assay for fFN may significantly reduce hospital stay and costs for these women. Presently in the United States, delivery before 37 weeks gestation, PTD, occurs in approximately 10% of births and is a leading cause of neonatal morbidity and mortality.[1,2,3] This rate has not changed significantly in the past forty years despite advances in perinatal care.[4] Early detection of PTD risk will hopefully allow clinicians delivering prenatal care to reduce its occurrence and resulting morbidity and mortality. At the same time, healthcare costs should be reduced by designating those with symptoms of PTD from those that are truly at risk and require intervention.

Fibronectin proteins are found in plasma and extracellular matrix and function as components of cell adhesion and migration. They also play a role in cell differentiation and growth.[2] One of these fibronectins, fFN, is an oncofetal antigen present in some malignant cell lines. Similar to other oncofetal antigens, it is a normal protein in fetal life, present in amniotic fluid and placental tissue. In fetal life, it exists in the extracellular matrix where the implanted ovum and placental membranes come in contact with the uterine wall. It most likely functions as an adhesion protein, connecting the placenta to the uterus. When this extracellular matrix is broken down because of stress, infection, or hemorrhage, fFN leaks into cervicovaginal secretions.[2,4,5,6,7]

fFN concentrations in vaginal and cervical secretions in pregnancy follow a pattern that correlate with its role in implantation and adhesion. In the early weeks of pregnancy before 20 weeks gesta-

tion, fFN is measurable in significant concentrations. In a normal pregnancy, after 20 to 22 weeks gestation when the gestational sac would be attached to the endometrium, fFN decreases to <50 ng/mL, an undetectable level by routine assays.[8] Therefore, its presence in detectable concentrations after 20 weeks should indicate some type of premature rupture in the attachment of fetal membranes in the uterus. A rupture of these membranes places a woman at high risk for premature delivery (delivery before 37 weeks).

Symptoms of premature delivery, most often uterine contractions before 37 weeks, do not always result in premature birth. Digital cervical examination and other procedures such as transvaginal ultrasound to evaluate the cervix, are performed to help determine risk of PTD. The patient may be treated with tocolytic agents to arrest contractions and/or antibiotics, if a bacterial infection places patient at risk for early delivery. Researchers have been seeking a biochemical marker or markers for PTD measurable in blood or secretions. Utilization of biochemical marker(s) in conjunction with tocolytic therapy and antibiotics may increase fetal survival rates. In 1995, the FDA approved the fFN enzymatic immunoassay as a biochemical marker for preterm labor.[3,4] It has been approved for the diagnosis of PTD in symptomatic women and as a screening assay for premature labor in asymptomatic women who are at risk for PTD.

To perform a fFN assay, cervicovaginal secretions are collected with a Dacron swab and placed in a tube of buffer provided in manufacturer's specimen collection kit (Adeza Biomedical Corporation, Sunnyvale CA). The qualitative assay is performed on a solid-phase immunosorbent cassette containing a monoclonal antifetal fibronectin antibody. The specimen is extracted, filtered, and dispensed into a sample well and resulting color intensities are interpreted by the instrument in 20 minutes. Color intensity is compared to a reference calibrator of 50 ng/mL; a positive reaction indicates a concentration of fFN greater than or equal to the calibrator and a negative indicates a concentration of less than 50 ng/mL. A quantitative assay that uses antibody coated micro titer wells is also available.[9,10]

If a patient is to have a digital examination or vaginal cultures collected, the fFN sample should be collected first. These procedures are disruptive to the membranes and may cause leakage of fFN into vaginal secretions. Moderate and gross vaginal bleeding also interfere with result interpretation. Since fFN is normally present in amniotic fluid and fetal membranes, patients with advanced cervical dilatation and rupture of amniotic membranes are unsuitable for the test. The manufacturer also recommends not collecting samples on patients who have had sexual intercourse in the past 24 hours; test results on these patients are also difficult to interpret.[9,10,11,12,13]

There have been numerous studies evaluating fFN measurement and preterm delivery prediction.[5,7,11,14-20] Several of these studies compared fFN to other biochemical markers of PTD and other researchers included cervical dilatation, transvaginal ultrasound, or presence of bacterial vaginosis. Most studies included symptomatic and asymptomatic patients. Some researchers have compiled the data and published meta-analysis of results, reporting fFN sensitivities and specificities overall, and for specific weeks' gestation. In a meta-analysis published in May 1999, Leitich reviewed 27 studies published in English.[21] Table 1 lists sensitivity and specificity for all patients for delivery at <37 and <34 weeks' gestation. Table 2 depicts overall sensitivity and specificity rates for delivery within 7, 14, 21, and 28 days of sample collection for all patients and Table 3 for symptomatic patients. This data supports their conclusion that fFN is an effective predictor of PTD in symptomatic women.[21] Another earlier meta-analysis by Revah in 1998, reviewed 24 studies and found similar sensitivities and specificities.[22] Their overall specificity was 80% for all outcomes, very close to 84% and 83% on Table 1. Their sensitivities and specificities were grouped differently than those on Table 2 and Table 3 but were also lower for asymptomatic women. For a patient with symptoms of PTD, a negative test for fFN is useful in ruling out delivery in the next seven to ten days. These authors concluded that testing for fFN is not as useful in asymptomatic women as in symptomatic individuals.[22]

Table 1. Sensitivity and specificity by delivery for all patients

Delivery	<37 weeks	<34 weeks
Sensitivity	56%	61%
Specificity	84%	83%

Table 2. Sensitivity and specificity by sample collection date for all patients

Specimen collection within days of delivery

	7 days	14 days	21 days	28 days
Sensitivity	76%	68%	61%	43%
Specificity	88%	89%	91%	93%

Table 3. Sensitivity and specificity by sample collection date for symptomatic patients

Specimen collection within days of delivery

	7 days	14 days	21 days	28 days
Sensitivity	89%	78%	76%	71%
Specificity	86%	86%	88%	83%

Reductions in healthcare costs with the addition of fFN testing in preterm labor care and treatment have been evaluated. Decreased hospital admissions for preterm labor, reduced length of hospital stays, and fewer prescriptions administered without adverse consequences for these patients would justify routine addition of fFN assays. Joffe evaluated 243 subjects in a 12-month study and found significant reductions in healthcare costs.[12] They compared their study group costs that included fFN assays to a baseline group before the addition of fFN in patient care. There were no changes in neonatal intensive care admissions, neonatal intensive care length of stays, or days of ventilator support per patient in the two patient groups. They calculated a savings of $416,120 for the study group; this included the additional costs incurred for fFN assays on follow-up clinic visits.[12]

Though the fFN test is a useful marker in evaluating PTD, some researchers and practitioners are still hesitant to advocate its use.[21,23] The accurate prediction of PTD does not necessarily decrease its occurrence. Others are concerned that the test results may cause unnecessary anxiety for some patients. The successful use of tocolytic agents in preventing PTD needs further investigation. Also needed is documentation that antibiotic administration is effective when a patient has a positive fFN and follow-up cultures indicate a vaginal or cervical bacterial infection. More research is required to find interventions to prevent PTD when it is predicted to occur.[21]

Other biochemical markers of PTD are also being investigated.[1,3,24-26] Table 4 lists those found in literature on PTD. Interleukin-6 (IL-6), other cytokines, and C-reactive protein (CRP) indicate the presence of an inflammatory process or infection. Proteases such as collagenase, granulocyte elastase, and matrix metalloproteinases, increase in breakdown in the placental uterine protein interface. Increased levels of the hormones listed on Table 4 indicate maternal or fetal stress.

Table 4. Other biochemical markers of preterm delivery

Marker	Specimen
Cytokines:	
Interleukin-6 (IL-6)	Amniotic fluid, cervicovaginal fluid, and maternal plasma
Tumor necrosis factor (TNF)	Cervicovaginal fluid
Proteins:	
C-reactive protein (CRP)	Maternal plasma
Proteases:	
Collagenase	Maternal plasma
Granulocyte elastase	Cervicovaginal fluid and maternal plasma
Matrix metalloproteinases (MMPs):	Maternal plasma
Hormones:	
Human chorionic gonadotropin	Maternal plasma
Corticotropin releasing hormone	Maternal plasma
Estradiol-17β	Maternal plasma
Estriol	Maternal saliva, plasma, and amniotic fluid
Progesterone	Maternal plasma

CONCLUSION

The fFN test offers a rapid and easily performed assay to predict preterm delivery. fFN is an adhesion protein, part of the attachment of the gestational sac to the uterine membranes. Its presence in cervicovaginal fluid of pregnant women signals a rupture in this adhesion and pending fetal delivery. A qualitative assay has been developed to evaluate women symptomatic of or at risk for preterm delivery. Sensitivity and specificity studies have documented its usefulness as a predictor of preterm delivery.

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