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Do pregnancies complicated by preterm birth with a negative fetal fibronectin screen have different characteristics compared to those that have positive fetal fibronectin results?

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Thesis

DO PREGNANCIES COMPLICATED BY PRETERM BIRTH WITH A NEGATIVE FETAL FIBRONECTIN SCREEN HAVE DIFFERENT CHARACTERISTICS COMPARED TO THOSE THAT HAVE POSITIVE FETAL FIBRONECTIN RESULTS?

by

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DO PREGNANCIES COMPLICATED BY PRETERM BIRTH WITH A NEGATIVE FETAL FIBRONECTIN SCREEN HAVE DIFFERENT CHARACTERISTICS COMPARED TO THOSE THAT HAVE POSITIVE FETAL FIBRONECTIN RESULTS?

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ABSTRACT

Objective: The fetal fibronection (fFN) test is an excellent test to rule out preterm birth (PTB) as it has a negative predictive value of 98-99%. However, some patients in this group of women still deliver preterm, which puts the infant at risk for many chronic complications such as cerebral palsy and developmental delay. To date, there is limited data about PTB in the setting of a negative fFN. The purpose of this study is to look at maternal, fetal, and placental characteristics of women who had a spontaneous preterm delivery (PTD) with a negative fFN test and compare them with maternal, fetal, and placental characteristics of women who had a spontaneous PTD with a positive fFN test.

Methods: This retrospective chart review was conducted at Baystate Medical Center between 2006 and 2009. Women were included in the study if they delivered within 14 days of the fFN test at this institution. Demographic characteristics, clinical antecedents for delivery, and placenta pathology reports were compared between women with a positive fFN test and a negative fFN test. Clinical antecedents for delivery were either (a) Preterm premature rupture of membranes (PPROM) leading to preterm labor (PTL) (b) abruption leading to

PTL or (c) idiopathic PTL. Placenta histological reports were reviewed and organized into the following categories (a) normal placenta (b) evidence of inflammation (c) evidence of ischemic changes or (d) evidence of both inflammation and ischemic changes. Comparison between groups was conducted using Wilcoxon rank sum test and Fisher's exact test.

Results: A total of 82 women had fFN testing and delivered preterm. Of these, 58 women had spontaneous PTL and a placental pathology report. Demographic characteristics were found to be similar between the two groups. Women with a positive fFN test were found to deliver earlier (p=0.038). Overall, there was a trend toward an increase in placental ischemic changes in the fFN negative group (68.8% versus 23.8%) but this did not achieve statistical significance.

Conclusion: Women delivering spontaneously preterm after a positive fFN test may have different pregnancy characteristics than women delivering spontaneously preterm after a negative fFN test. Women in the positive fFN test group tended to deliver earlier than women in the negative fFN test group, suggesting that the mechanism contributing to PTB is likely to be different between the two groups. The data suggests that there is a trend toward women with a negative fFN having placental ischemic changes. More studies should be done to see if this trend continues. Clinically, this information would be helpful because these women may not be a good candidate for this test and may need to be hospitalized and given steroid treatment when showing signs of PTL. However, until further information is available, women with suspected ischemic

placentas should not yet be excluded from fFN testing. Clearly, further studies are needed to explore the relationship between placental ischemia and fFN testing.

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LIST OF ABBREVIATIONS

BMC ..... Baystate Medical Center

FDA ..... United States Food and Drug Administration

fFN ..... Fetal Fibronectin

g ..... gram

h ..... hour

IV ..... intravenous

mcg ..... microgram

mg ..... milligram

min ..... minute

mL ..... milliliter

n ..... number of subjects

ng ..... nanograms

No. ..... number

po ..... by mouth

PPROM ..... Preterm Premature Rupture of Membranes

PTB ..... Preterm Birth

PTD ..... Preterm Delivery

PTL ..... Preterm Labor

q ..... every

RDS ..... Respiratory Distress Syndrome

SC ..... Subcutaneous

Background

Preterm Birth (PTB)

A typical length of a pregnancy is about 40 weeks (280 days from the first day of the last menstrual period) or three trimesters (Simhan *et al*., 2012). This allows the fetus enough time to remain in the womb while the development of the brain, heart, skin, lungs, and more takes place (Simhan *et al*., 2012). Although, this is the average time period that is expected, each pregnancy is a unique experience and many women will give birth either before or after 40 weeks. A pregnancy reaches term when the fetus is delivered between 37 and 41 weeks gestation (Iams and Romero, 2007). A pregnancy that reaches term is associated with a low risk for adverse neonatal outcomes (Simhan *et al*., 2012). When a woman gives birth prior to 37 weeks gestation, this is defined as a preterm birth (PTB) (Simhan and Caritis, 2007). PTB refers to the birth of a baby who is born too early and, as a result, is at high risk for significant chronic medical complications which range from vision and hearing impairment, development delay, cerebral palsy, pulmonary insufficiency, to even death (Iams and Romero, 2007; Leveno *et al*., 2010).

Specifically, Simhan, Iams, and Romero (2012) state that there an inverse relationship between chronic medical complications and gestational age, meaning that as gestational age decreases, the risk for infant morbidity increases. Essentially, the less developmental progress made while in the womb, the more difficulty that infant will have (Figure 1) (Bender, 2001). These

difficulties are not only extremely emotionally taxing for families, but are also economically taxing. In the United States, the cost of PTB was estimated to exceed $26.2 billion or $51,600 per infant in the year 2005 (National Research Council, 2007).

According to the National Center for Health Statistics, the percentage of PTB's in the United States has continued to increase from 11.6% to 12.7% between 2000 and 2005 (MacDorman and Mathews, 2008). It is important to note that in 2005, 68.8% of all infants who did not survive to his or her first birthday were preterm infants (Table 1) (MacDorman and Mathews, 2008). In 2006, a study by Callaghan et al., assessed the contribution of PTB to infant mortality rates in the United States and showed that among all causes of death for infants, the leading cause was complications associated with prematurity (Callaghan *et al*., 2006; Simhan *et al*., 2012).

Fetal Development and Prospective Survival

Developmental progress	Chance of survival outside womb and related difficulties
FIRST TRIMESTER	
Body segments appear Primitive external heart beats and lung buds develop First bone cells develop, body features become apparent Some organs function Lungs begin primitive breathing motion	**None**
SECOND TRIMESTER	
Blood vessels develop, organs and structures form Hair grows, eyelids blink Skins loses transparency Skeleton forms rapidly Eyes form, fetus can swallow Fetus reaches 1–1½ pounds	**Minimal**, though improving with medical advances 1 in 5 infants who do survive suffer serious disabilities, including blindness, deafness or cerebral palsy **50%** survive with intensive care Disabilities are likely, including neuromotor impairment and blindness
THIRD TRIMESTER	
Surfactant forms to prepare lungs for breathing Fetus reaches 3 pounds, can suck thumb Fat deposits are stored in preparation for birth Digestive track and lungs mature Body begins to grow plump Brain grows rapidly Lungs mature, mother's antibodies are transferred	**Good** with intensive care Infant remains at risk for subnormal or deficient intelligence and respiratory distress syndrome Respiratory difficulties are common Respiratory difficulties at birth but generally good long-term outcomes **Excellent** Full term

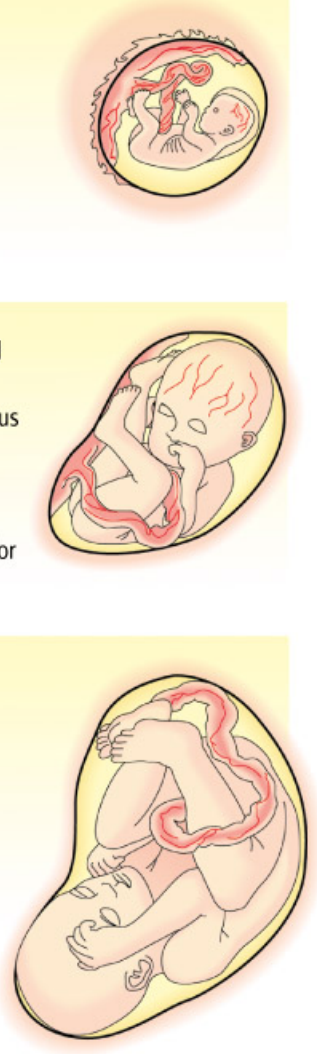

Figure 1 Fetal development and corresponding prospective survival. The average length of pregnancy is 40 weeks, divided into three trimesters. During this time, the fetus remains in the womb while making developmental progress. If expelled too early, the fetus is a risk for significant health complications depending on the time point within the pregnancy. Figure taken from Bender, 2001 at http://www.hawaii.edu/malamalama/2001/01/Pretermbirth.html.

Table 1 Infant mortality rates by gestational age. The number of infant deaths varies by gestational age showing the highest mortality for infants born preterm. Infants born preterm that do survive are at risk for significant complications such as developmental delay, cerebral palsy, and pulmonary insufficiency. Figure taken from Leveno *et al*. (2010) in *Williams Obstetrics*.

Infant Mortality Rates in the United States in 2005

	Live Birth No. (%)	Infant Deaths No. (%)
Total infants	4,138,573 (100)	28,384 (100)
Gestational age at Birth		
<32 weeks	83,428 (2)	15,287 (54)
32-33 weeks	65, 853 (1.6)	1099 (4)
34-36 weeks	373,663 (9)	1727 (10)
37-41 weeks	3,346,237 (81)	8116 (29)
≥42 weeks	239,850 (6)	637 (2)
Unknown	29,542 (0.7)	516 (2)

Medically Indicated PTB versus Spontaneous PTB

PTBs have been classified into two main overarching categories: medically indicated PTBs and spontaneous PTBs (Iams, 2002). Twenty percent of PTBs are medically indicated (Norwitz, 2013). This occurs when the physician deems delivery of the fetus necessary because of medical complications that compromise maternal well-being or fetal well-being (Iams and Romero, 2007). These issues include, but are not limited to, intrauterine growth restriction where the fetus is not growing appropriately for gestational age, non-reassuring fetal testing where the fetus is thought to be at risk for fetal death, and preeclampsia (hypertensive disorders of pregnancy) (Iams and Romero, 2007; Norwitz, 2013).

The majority of PTBs (80%) occur spontaneously (Iams, 2002). Women with signs and symptoms of PTL often have one or more of the demographic characteristics listed in Table 2 (Simhan *et al*., 2012). These characteristics include African American race, low socioeconomic status, poor prenatal care, history of a prior PTB, cigarette smoking, substance abuse, and cervical/uterine abnormalities (Simhan *et al*., 2012; Norwitz, 2013). In a perfect world, identifying women with these characteristics may help to prevent PTB, however; many PTB's occur in women who have no apparent risk factors (Simhan *et al*., 2012; Norwitz, 2013).

Table 2 Demographic profile of women with spontaneous preterm birth
Women with a spontaneous PTB may have these characteristics. However, half of women with a spontaneous PTB have no known risk factors. Table adapted from Simhan *et al*., 2012.

Demographic Profile of Women with Spontaneous Preterm Birth
• History of genital tract colonization, infection, or instrumentation
○ Urinary tract infection and bacteriuria
○ Sexually transmitted infections such as *Chlamydia*, gonorrhea, human papillomavirus, or *Trichomonas*
○ Bacterial vaginosis
○ Cervical dysplasia and treatment for same
○ Spontaneous or induced abortion
• African American
• Bleeding of uncertain origin in pregnancy
• History of a previous spontaneous preterm birth
• Uterine anomaly
• Assisted fertility care
• Multifetal gestation
• Cigarette smoking, substance abuse
• Poor nutrition and low prepregnancy weight (body mass index <19.6)
• Periodontal disease
• Limited education, low income, and low social status
• Late registration for prenatal care
• High levels of personal stress in one or more domains of life

Preterm Labor (PTL) Syndrome

In 1994, Romero et al. proposed that labor after 37 weeks gestation (term labor) and labor prior to 37 weeks gestation (preterm labor) share a common endpoint, which first begins with uterine contractility, cervical changes, and ends with separation and rupture of fetal membranes (Romero *et al*., 1994). However, activation of labor prior to 37 weeks is thought to be inherently different. The initiation of PTL was proposed to be the result of a pathological process rather than a physiological process (Romero *et al.*, 1994). It was also proposed that PTL may be in response to multiple inciting factors and thus the term “Preterm Labor Syndrome” was coined (Romero *et al.*, 1994). These factors include PTL due to microorganisms producing chemicals that can induce the process, disruption of the blood supply to the placenta, overdistension and stretching of the uterus (due to multiple gestations, too much fluid in the amniotic sac, etc.), uterine abnormalities, allergic mechanisms (due to chemicals released by mast cells), and fetal development and growth disorders (Romero *et al.*, 1994; Iams and Romero, 2007; Simhan *et al*., 2012) (Figure 2).

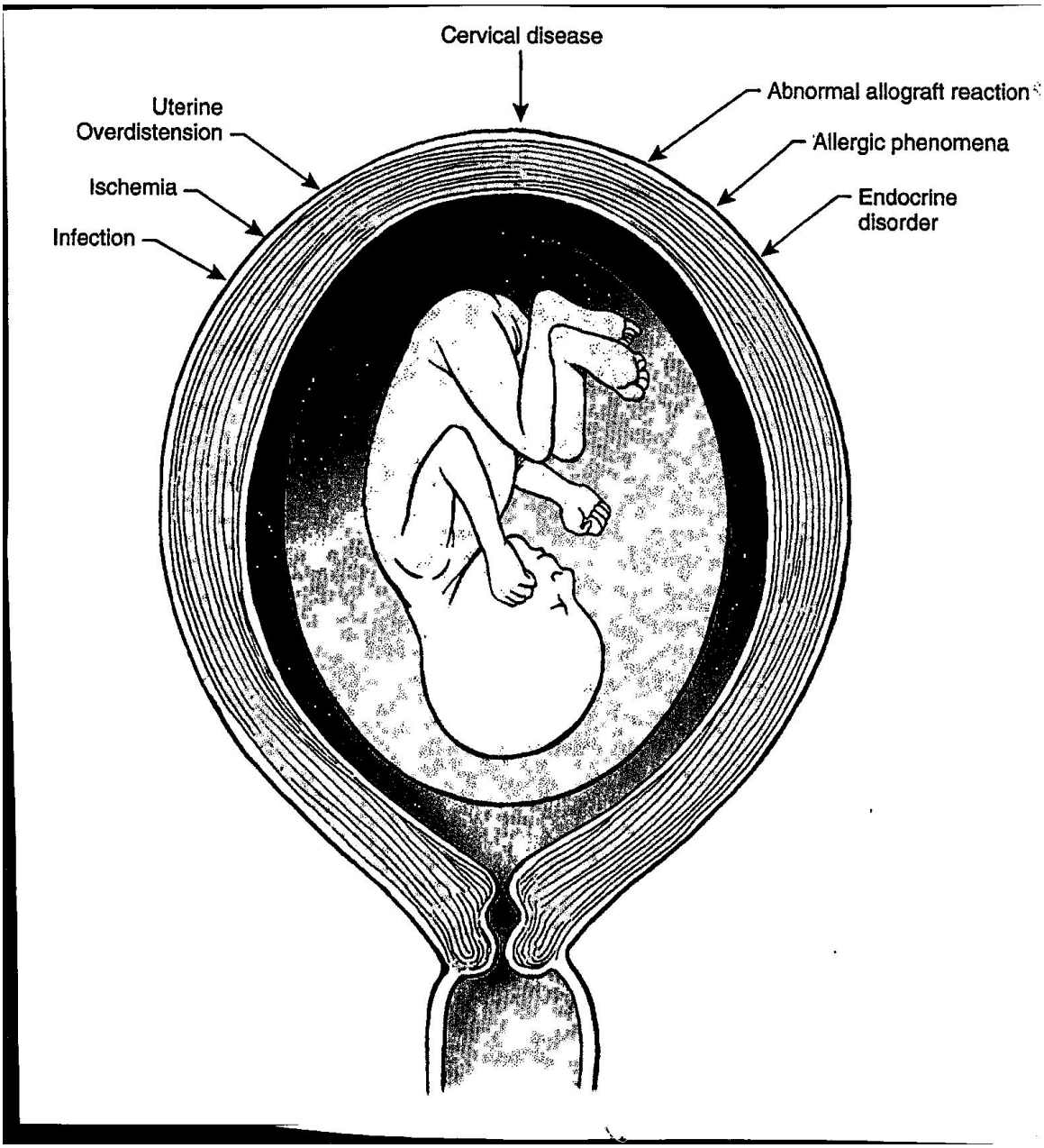

Figure 2 Causes of preterm labor syndrome. Figure taken from Iams and Romero, 2007. The causes of PTL are multiple and include infection such as chorioamnionitis, ischemia, growth and development (endocrine) disorders, overdistension of the uterus, uterine abnormalities, cervical insufficiency and allergies. To date, some factors that cause PTL syndrome remain unknown (Romero *et al*., 1994).

Tocolysis and Corticosteroids for Treatment of PTL

PTL is defined as cervical dilation with maternal contractions before 37 weeks gestation is reached (American College of Obstetricians and Gynecologists, 2003; Simhan *et al*., 2012). Not all patients that present to the hospital with PTL will go on to deliver preterm (King *et al*., 1988). It is important to distinguish those patients with PTL that will lead to PTB, so that they receive treatment to avoid the newborn complications of prematurity (Simhan *et al*., 2012). In order to avoid the complications associated with prematurity, patients with symptoms of PTL are admitted to the hospital for prolonged observation, tocolysis, and corticosteroid treatment (King et al., 1988; Simhan and Caritis, 2007; Simhan *et al*., 2012).

Tocolytics are medications that are given to stop contractions of the uterus long enough to administer corticosteroids (Leveno *et al*., 2010). Corticosteroids, act on type 1 and type 2 alveolar cells, which are cells in the lungs that work to improve gas exchange and produce surfactant, respectively (Lee and Guinn, 2013). This is important because surfactant reduces resistance and surface tension in the lungs, allowing the fetus to breathe much easier. Ultimately, steroids reduce the incidence of respiratory distress syndrome (RDS) (Figure 3) (Lee and Guinn, 2013). Additional studies have found that steroids have been associated with a reduction in systemic infection in the first 48 hours of life, intraventricular hemorrhage, necrotizing entercolitis, and neonatal mortality (Lee and Guinn, 2013). This is possibly due to improvement of fetal respiratory status

but may also be due to the effects of steroid regulation on additional genes in the genome (Lee and Guinn, 2013).

Like every other medication, tocolytics and corticosteroids are not without risk. Magnesium sulfate, terbutaline, nifedipine, and indomethacin are common agents used to prevent PTB (Gibson, 2011). Side effects of tocolytics include, but are not limited to, complications such as maternal tachycardia, hyperkalemia, hyperglycemia, neonatal hypoglycemia and ileus (Simhan *et al*., 2012). Common dosage and additional side effects are noted in Table 3 (Gibson, 2011). While a single course of corticosteroids may be considered if a woman is likely to deliver within the next week, multiple courses are not recommended due to the increase in risk for reduced fetal growth and adverse brain effects (Simhan *et al*., 2012; Lee and Guinn, 2013).

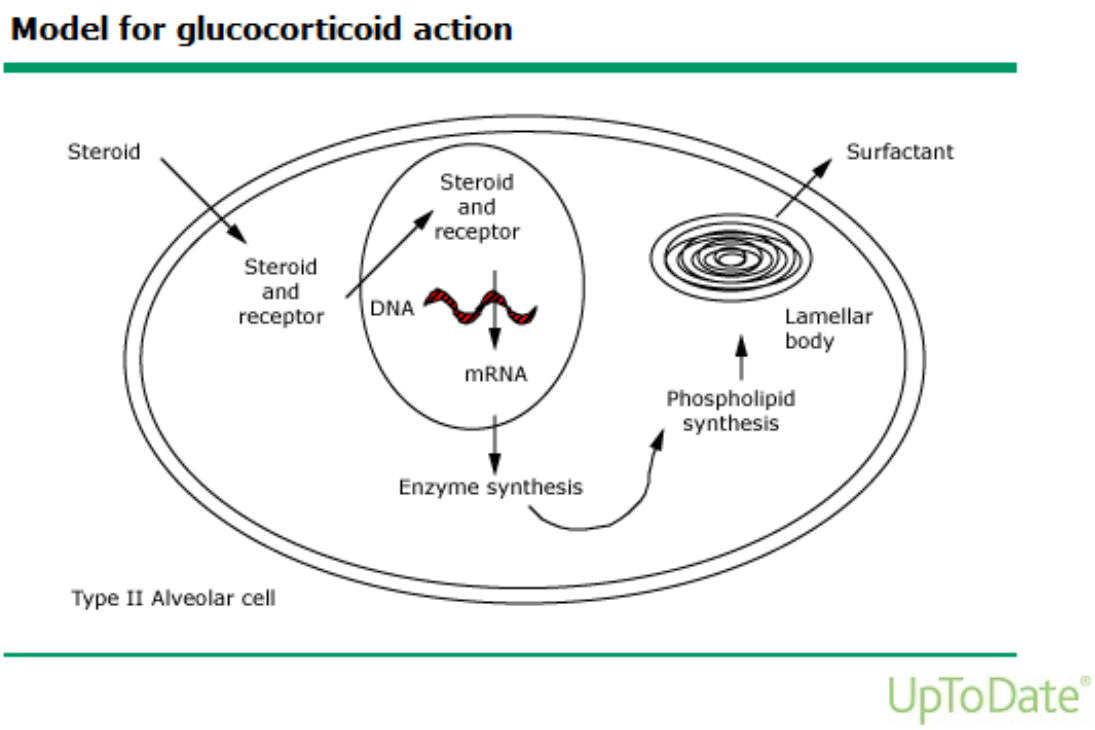

Figure 3 Model for corticosteroid action on type II alveolar cells. Glucocorticoids are corticosteroids that act on surfactant producing cells in the lungs. Surfactant reduces resistance and surface tension in the lungs. Glucocorticoids also help type 1 alveolar cells, which help with gas exchange. Overall, glucocorticoids help reduce the incidence of RDS (Simhan *et al*., 2012; Lee and Guinn 2013). Figure taken from Lee and Guinn, UpToDate, 2013.

Table 3 Common tocolytic agents. Tocolytic agents are given to inhibit uterine contractions long enough for clinicians to administer corticosteroids. These agents are known to have life-threatening side effects and should only be used when needed. Many women who present with signs and symptoms of PTL are given tocolytics but end up delivering at term (Simhan *et al*., 2012). Table modified from Gibson, 2011 in U.S. Pharmacist.

Common Tocolytic Agents		
Agent	Common Dosage	Side Effects
Magnesium Sulfate	4 to 6 g IV bolus followed by 2 to 3 g per hour IV infusion	May cause maternal flushing, lethargy, headache, weakness, dry mouth, pulmonary edema, cardiac arrest, may cause neonatal lethargy, hypotonia, respiratory depression
Terbutaline	0.25 mg SC every 20 min to 3 h or 2.5 to 10 mcg per min continuous infusion, gradually increased to 17.5 to 30 mcg per min	May cause maternal cardiac arrhythmias, pulmonary edema, myocardial ischemia, hypotension, tachycardia, metabolic abnormalities, nausea, vomiting, fever, hallucination, may cause neonatal tachycardia, hypoglycemia, hypocalcemia, hyperbilirubinemia, hypotension, intraventricular hemorrhage
Nifedipine	30 mg po followed by 10 to 20 mg q 4 to 6 h	May cause maternal flushing, headache, dizziness, nausea, transient hypotension
Indomethacin	50 mg rectally or 50 to 100 mg po followed by 25 to 50 mg po q 4 to 6 h for 48 h	May cause maternal nausea, heartburn; may cause neonatal constriction of ductus arteriosus, pulmonary hypertension, decreased renal function, intraventicular hemorrhage, hyperbilirubinemia, necrotizing entercolitis

Introduction

Limitations in Predicting PTB

To date, a clinician's ability to determine whether PTL will result in PTB is limited (King *et al*., 1988). Up to 50% of patients in controlled clinical trials admitted for tocolysis who received placebo went on to deliver at term (King *et al.*, 1988). Thus, a large numbers of obstetrical patients who are destined to deliver at term, but present with symptoms of PTL, potentially receive treatments with possible life-threatening side effects (King *et al*., 1988). In addition to maternal and fetal health effects of these treatments, hospital visits are very expensive (King *et al*.,1988; National Research Council, 2007). Therefore, methods to detect women who are truly at risk for delivering early would be beneficial (King *et al*., 1988; Peaceman *et al*., 1997; National Research Council, 2007; Lockwood and Barss, 2013).

Fetal Fibronectin Testing

Traditional methods to predict whether a woman was destined to deliver preterm relied on clinical indicators such as obstetrical history or symptoms (Lockwood and Barss, 2010). However, neither is very reliable. A number of biological markers in serum, amniotic fluid, and cervical secretions have been evaluated for their potential to predict PTB (Leitich *et al*., 1999; Lockwood and Barss, 2013). One excellent biochemical approach for predicting PTB is the measurement of fetal fibronectin (fFN) in the cervico-vaginal secretions (Leitich *et*

al., 1999).

fFN is a glycoprotein that acts as a glue to help adhere the placenta to the uterine lining (Peaceman *et al*., 1997; Hologic, 2010; Lockwood and Barss 2013) (Figure 3). fFN appears in cervico-vaginal secretions normally until about 20 weeks gestation, is absent from 22 to 35 weeks, and reappears as a woman approaches her 40th week of pregnancy (Hologic, 2010; Peaceman *et al*., 1997) (Figure 4). The presence of fFN in the cervix or the vagina after the 20th week of pregnancy is abnormal and may indicate a disruption of the attachment of membranes (Peaceman *et al*., 1997; Hologic, 2010).

Clinical evidence supports the use of fFN as a marker for PTD. Peaceman et al. found that patients who had both symptoms of PTL and the presence of fFN in vaginal secretions were at an increased risk for PTD within 14 days (Peaceman *et al*., 1997). Peaceman *et al* also found that women who tested fFN negative were not likely to deliver within 7 days (negative predictive value=99%) (Peaceman *et al*., 1997). In this way, fFN is known as a biochemical marker that allows us to differentiate those who are at high risk for PTD and those who are not (Peaceman *et al*., 1997; Hologic, 2010; Lockwood and Barss, 2013).

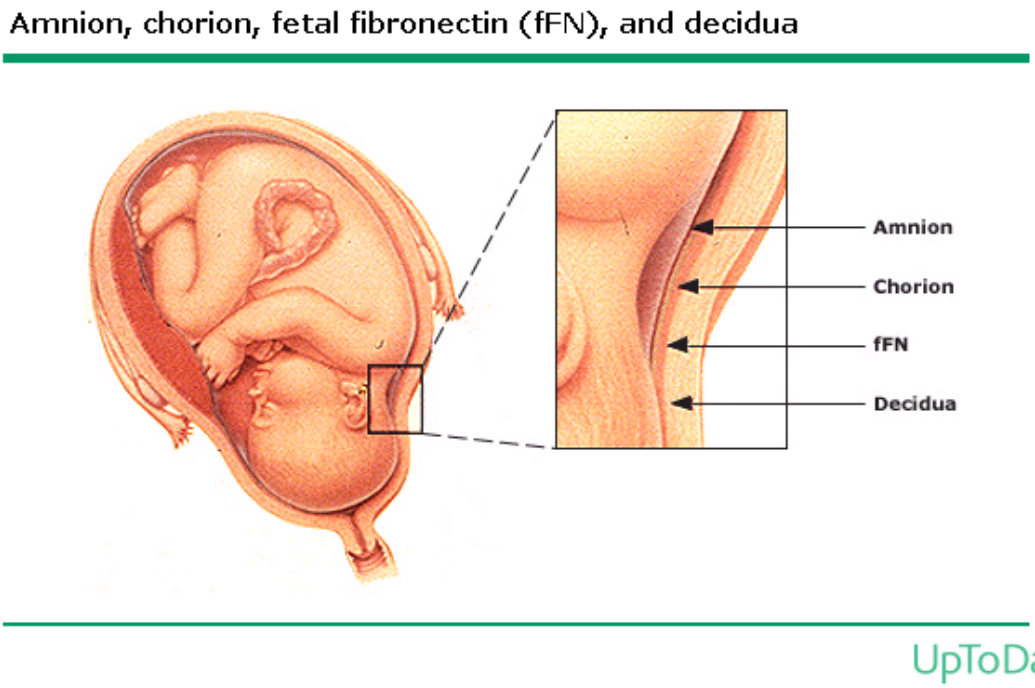

Figure 4 Connection of the placenta to the uterine lining by fetal fibronectin. fFN is the glue between the fetal membranes (which include the amnion and the chorion) and the uterine wall (known as the decidua). Between 22 to 35 weeks gestation, this glycoprotein should be absent in cervico-vaginal secretions. When fFN is found during this time, a woman is at an increased risk for early delivery (Peaceman *et al*., 1997). Figure taken from Lockwood and Barss, 2013, UpToDate.

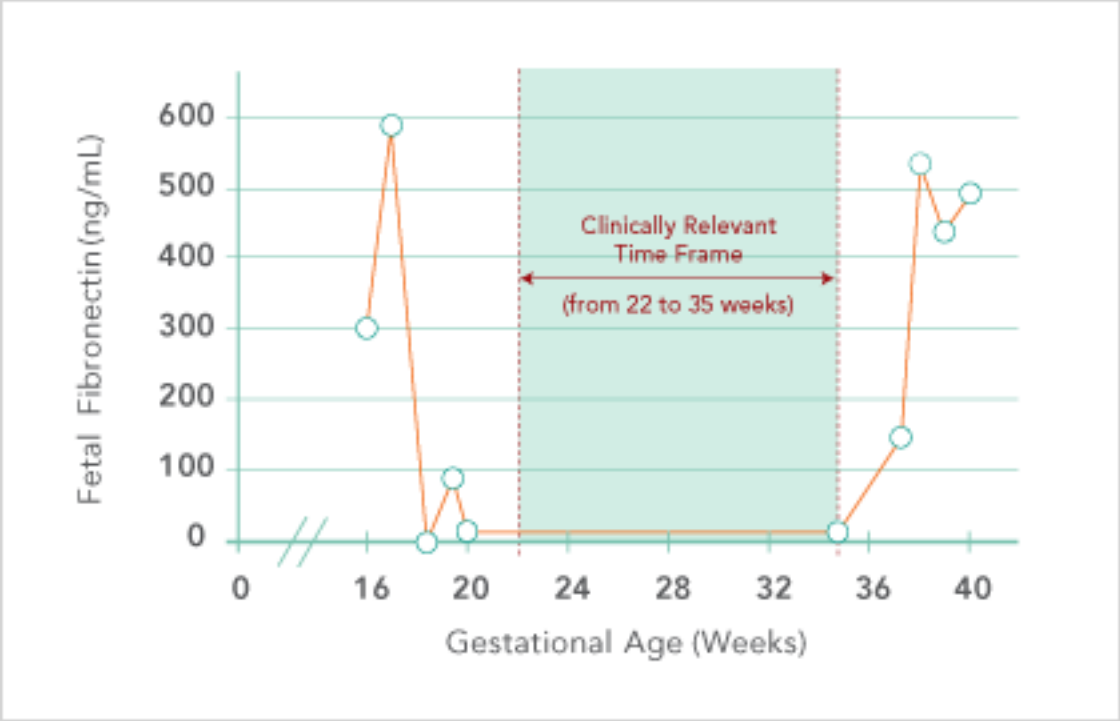

Figure 5 Fetal Fibronectin concentration in ng/mL by gestational age in weeks. Between 22 and 35 weeks gestational age, fFN should not be detected in cervico-vaginal secretions. However, a concentration of 50 ng/mL of fFN present in secretions is considered positive and indicates a woman is at an increased risk for early delivery. Figure taken from Hologic, 2010, www.ffntest.com/info/fetal_test/what_fetal.htm

Candidates for Fetal Fibronectin Testing

The fFN test is a United States Food and Drug Administration (FDA) approved test performed when women have clinical symptoms suggestive of PTL (Lockwood and Barss, 2013). The test is performed using a swab to collect a sample during a speculum examination (Lockwood and Barss, 2013). Candidates for this test should have intact fetal membranes because amniotic fluid does contain the fFN protein and could give a false positive result (Lockwood and Barss, 2013). The patient should also be between 22 and 35 weeks gestation because fFN should not be detected during this time period (Lockwood and Barss, 2013). Cervical dilation should be less than 3 centimeters as well (Lockwood and Barss, 2013). The test is considered positive when the concentration of fFN is greater than 50 ng/mL (Lockwood and Barss, 2013).

Utility of Fetal Fibronectin Testing

fFN testing can be a good tool for distinguishing the differences between those cases with false versus true PTL (Peaceman *et al*., 1997). If women test positive, they are more likely to deliver in the near future and should be admitted to the hospital for further observation (Peaceman *et al*., 1997).

However, a negative test, associated with a negative predictive value around 99%, highly suggests that women are not likely to deliver within 14 days (Peaceman *et al*., 1997). Therefore, when a woman tests negative and has other reassuring factors, obstetricians feel confident that PTD is not imminent and thus

they are comfortable in sending patients home with no intervention (Peaceman *et al*., 1997; Lockwood and Barss, 2013). This can prevent unnecessary interventions such as hospital admission or medications (Peaceman *et al*., 1997). However, despite the high negative predictive value of fFN screening, a negative fFN test does not eliminate the possibility of PTD as there are still occasional patients who deliver early regardless of test results (King *et al*., 1988). To date, little is known about false negatives. The advantage of knowing what type of pregnancies would be at the greatest risk for a false negative is important because false negatives in this clinical condition could result in suboptimal obstetrical care. The patient would not get the treatment that would help reduce the risks of prematurity (Iams and Romero, 2007).

Specific Aims/Objectives

Although the fFN test has a negative predictive value of 99%, there are still a small percentage of women who deliver early (Peaceman *et al*., 1997). To date, there is limited data about PTB in the setting of a negative fFN. This project aims to retrospectively look at singleton, spontaneous PTD's at Baystate Medical Center within 14 days of fFN testing.

Specifically,

1. This study intends to compare all maternal, fetal, and placental characteristics of women with a negative test and compare them with maternal, fetal, and placental characteristics of women with a positive test.

2. This study intends to assess clinical antecedents for delivery in the two groups.

Methods

This retrospective chart review was conducted at Baystate Medical Center (BMC) between 2006 and 2009. All charts for women who had an fFN assay drawn during the eligible time period were reviewed. Data was abstracted from the medical record and reviewed by a trained research assistant. Women were included in the study if they delivered an infant within 14 days of the fFN test at BMC. Women were excluded from the study if they had a fetus with fetal anomolies, fetal demise, or maternal and fetal medical indications for delivery such as preeclampsia or non-reassuring fetal status.

Of the 82 women who delivered a singleton at BMC less than 14 days after a fFN test, 24 were excluded: 1 woman was excluded because the neonate had Trisomy 21, 1 woman was excluded because she had a fetal demise, 1 woman did not have a placenta pathology report, 11 women delivered for maternal indications, and 10 women delivered for fetal indications. This left 58 women who delivered a preterm singleton sponanteously within 14 days of an fFN test.

This study was approved by the Institution Review Board at Baystate Medical Center.

Study Measures

Fetal Fibronectin Assays

Fetal fibronectin assays were performed using the Adeza Biomedical Rapid TLi system (Adeza Biomedical Corporation, Sunnyvale, California). The samples were collected as recommended by the manufacturer, that is, prior to a digital cervical exam, vaginal probe ultrasound, or the collection of other vaginal specimens. The last fFN result was used if a woman had multiple tests. Women who had contaminated results were excluded.

Clinical Antecedents for Delivery

Clinical antecedents for delivery among the two groups were among the following categories (a) Preterm premature rupture of membranes (PPROM) leading to PTL, (b) Abruption leading to PTL or (c) Idiopathic PTL. PPROM was defined as rupture of membranes before the onset of labor and before 37 weeks gestation. Histological evidence of abruption was confirmed by the pathologist and with the physician's clinical impression found in the patient's chart. Women who came to the hospital with symptoms of PTL with no known cause were categorized into the idiopathic PTL group.

Placental Analysis

Placentas were analyzed according to the institutional protocol for placenta histological analysis. All reports were reviewed by a single board certified pathologist. Placenta histological results were placed into the following

categories: (a) normal placenta, (b) evidence of inflammation, (c) evidence of ischemic changes or (d) evidence of both inflammation and ischemic changes. Inflammation was categorized as in Akers *et al*., (2007): acute chorioamnionitis, funisitis, or acute deciduitis. Acute chorioamnionitis was defined as presence of multiple neutrophilic infiltrates within and beneath the chorionic plate. Funisitis was defined as the presence of neutrophils in the other vessel layers, migrating out of an umbilical vessel and into Wharton's jelly. Acute deciduitis was defined as multifocal neutrophilic infiltrates within the maternal decidua (Akers *et al*., 2007). Cases were excluded that showed evidence of inflammation that was more likely to be the result of labor rather than the cause (i.e. acute chorioamnionitis that was found to be rising from the rupture site was more likely to be found as a result of labor rather than chorioamnionitis that was found over the entire membranes).

Placentas were put in the ischemic category if they showed signs of stress classified by any of the following: (a) infarcts, (b) nucleated red blood cells, (c) accelerated maturation, (d) uneven villous maturation, (e) villous edema, (f) syncytial knots or (g) abruption. Infarcts were defined as deposition of dense fibrinoid layer on the placental basal plate that acts to block normal maternal blood flow. Accelerated maturation of villi was defined as a preterm placenta having villous morphology approaching term, which was not consistent with gestational age. Syncytial knots are defined as aggregates of syncytial nuclei at the surface of terminal villi. Uneven maturation was defined as a preterm

placenta having certain areas of villous morphology approaching term, which was not consistent with gestational age. Villous edema was defined as diffuse multifocal edema affecting the immature intermediate villi of the placenta. Abruption was defined as histological basal plate disruption associated with retroplacental organized blood, placental villous compression, and focal increase in syncytialtrophoblastic knotting, focal trophoblastic necrosis, and villous agglutination without infarct. Normal placentas had neither inflammatory changes nor ischemic changes.

Statistical Analysis

The Fisher exact test was used to compare catagorical variables (clinical antecedents leading to PTD results and placenta pathology catagory results). The Student t test was used to calculate means for normally distributed continuous variables and the Wilcoxon rank-sum test was used for not normally distributed continuous variables. Significance was set a $p < 0.05$. Statistical analysis was performed using STATA.

Results

Table 4 shows the demographic characteristics such as maternal age, birthweight of the fetus, maternal race, parity, and duration between fFN test and delivery for all 58 subjects who met study inclusion criteria. The two groups of women (fFN positive versus fFN negative) had similar demographic characteristics. Women tended to be in their mid-twenties, were predominately white, and had given birth before (multiparous). As expected, women in the fFN positive group tended to deliver a few days earlier than women who test fFN negative (5 days versus 8 days), and this was statistically significant (p=0.038).

Table 4 Demographic characteristics for eligible women based on fFN results –The characteristics between the fFN positive and fFN negative groups are statistically similar except duration between fFN test and delivery.

Characteristics	Negative fFN n=16 n (%)	Positive fFN n=42 n (%)	p-value
Maternal Age	26.9± 6.1	26.2±6.5	0.72
Birthweight	2166.19±380.7	1838.54±592.9	0.13
Race			0.34
White	8 (50.0)	16 (38.1)	0.79
Black	3 (18.8)	13 (31.0)	
Hispanic	4 (25.0)	10 (23.8)	
Unknown	1 (6.2)	3 (7.1)	
Parity			0.77
Nulliparous	5 (31.2)	15 (36.6)	
Multiparous	11 (68.8)	26 (63.4)	
Duration between fFN test and delivery	7.88± 4.5	5.20±3.76	0.038

Clinical Antecedents for Delivery

There was no statistical difference in the cause for spontaneous PTB. Overall, clinical antecedents for delivery were largely due to idiopathic PTL between the two groups (52.4% of women with a positive fFN versus 62.5% of women with a negative fFN). PPROM accounted for 42.9% of women with a positive fFN versus 37.5% of women with a negative fFN. Placental abruption accounted for the smallest percentage of deliveries (4.8% of women with a positive fFN versus 0% of women with a negative fFN) (Table 5).

Table 5 Clinical antecedents leading to preterm delivery- Clinical antecedents leading to PTD were categorized into the following three categories: PPROM, Abruption, and Idiopathic PTL. Women who delivered for fetal or maternal medical indications were not included in this study.

Clinical antecedent	fFN negative n=16 n (%)	fFN positive n=42 n (%)	p-value
PPROM	6 (37.5)	18 (42.9)	0.88
Abruption	-	2 (4.8)	
Idiopathic PTL	10 (62.5)	22 (52.4)	

Placental Pathology

There was a trend toward an increase in evidence of placental ischemic changes in the negative fFN group. 68.8% of women in the fFN group had evidence of ischemia as compared with 28.6% of women in the positive fFN group, but this did not achieve statistical significance. 12.5% of women who had a negative fFN test had a normal placenta as compared to 23.8% of women who had a positive fFN test. 6.2% of women with a negative fFN had histological evidence of inflammation as compared to 11.9% of women who had a positive fFN test. 12.5% of women who had a negative fFN test showed both inflammation and ischemic changes upon histological analysis versus 35.7% women who had a positive fFN test. These results were not statistically significant (Table 6).

Table 6 Placenta pathology categories- Patient pathology reports were reviewed for histological evidence of inflammation, ischemia, both inflammation and ischemic changes, or neither inflammation or ischemia. Placentas with neither inflammatory nor ischemic changes were categorized as normal.

Placenta pathology	fFN negative n=16 n (%)	fFN positive n=42 n (%)	P-value
Normal	2 (12.5)	10 (23.8)	0.062
Inflammation	1 (6.2)	5 (11.9)	
Ischemia	11 (68.8)	12 (28.6)	
Ischemia and Inflammation	2 (12.5)	15 (35.7)	

Discussion

This study was initiated with the specific aim of comparing pregnancy characteristics of women with a positive fFN test and a negative fFN test within 14 days of a spontaneous PTD. Clinically, this would be useful because there may be some additional factors that would exclude women from being a candidate for this type of testing. Clinical evidence suggests that a fFN test should be given to women with signs and symptoms of PTL (Peaceman *et al*., 1997; Leitich *et al*., 1999; Lockwood and Barss, 2013) fFN is considered positive when the concentration is greater than 50 ng/mL in the cervicovaginal fluid. Women with a positive test should be hospitalized for observation and would be candidates for tocolytics and corticosteroids, in order to decrease the complications associated with PTB (Simhan *et al*., 2012). In the setting of a negative fFN test, PTB is unlikely and there is no need for hospitalization and medical intervention (Simhan *et al*., 2012). Although very unlikely, a negative fFN test does not eliminate any possibility of delivery and some women will go on to deliver preterm. This may have an unfortunate impact on the infant since corticosteroids may not have been administered which would reduce complications associated with prematurity. Due to the very low incidence of PTB after a negative fFN, data regarding specific characteristics of this population are limited.

In this study, the demographic characteristics of the women in the two groups were statistically similar with age, race, and infant birth weight. However,

going along with what would be expected, the timing of delivery after an fFN test was different between the two groups. Women in the positive fFN test group tended to deliver earlier than women in the negative fFN test group. This suggests that the mechanism contributing to PTB may be different between the two groups.

Past researchers have tried to understand the mechanism behind fFN release. In the literature, it was previously thought that histological evidence of placental inflammation at delivery was a separate finding from a positive fFN test result (Goldenberg *et al.,* 1996; Rizzo *et al.,* 1997; Yoon *et al.,* 2001; Akers *et al.,* 2007). One group of researchers thought that the two findings were not a coincidence—that is, they thought that the inflammation may have disrupted the fFN and allowed it to be detected in cervico-vaginal secretions (Akers *et al.,* 2007). Therefore, Akers *et al* performed a study to determine if women with placental inflammation were more likely to have a positive fFN result (Akers *et al.,* 2007). In this study, Akers *et al*. categorized placentas as having inflammation if they showed any signs of the following: chorioamnionitis, funisitis, deciduitis, and microabcess formation (Akers *et al.,* 2007). The authors ultimately concluded that women with a positive fFN were not more likely to have placental inflammation as noted by histological evidence at delivery (Akers *et al.,* 2007). In this study, the criteria of Akers *et al* were used to categorize placentas as having inflammation.

No studies have been done specifically looking at histological evidence of placental ischemia and fFN result. However, based on other studies that looked at placental ischemic changes, the following were used for this category (a) infarcts, (b) nucleated red blood cells, (c) accelerated maturation of villi, (d) uneven villous maturation, (e) villous edema, (f) syncytial knots, and (g) abruption. Several studies have been done looking at placental ischemic changes and fetal outcomes which all utilized the same markers of placental ischemic changes as identified above by our pathologist (Salafia *et al.,* 1995).

The choices for markers of placental ischemia were made based on the following known scientific data: (a) Infarcts are associated with PTD and act to block normal maternal blood flow (Leveno et al., 2010). (b) Nucleated red blood cells are found due the exposure of the bone marrow and other sites of fetal hematopoiesis (the liver) in response to persistent and significantly decreased levels oxygen, which stimulate increased production and premature release of red blood cells into the periphery (Redline, 2013). (c) Accelerated maturation of villi within the placenta could be a marker of poor perfusion because the placenta has certain villous morphology approaching term rather than villous morphology of a preterm placenta (Benirschke and Kaufmann, 1990). (d) Uneven villous maturation within the placenta is similar to accelerated villous maturation within the placenta where the whole placenta shows villous morphology approaching term rather than the morphology of a preterm placenta. This is a marker of poor perfusion (Benirschke and Kaufmann, 1990). (e) Villous edema is seen as a

placental response to fetal stress and is associated with fetal hypoxia due to compression of the blood vessels within the edematous villous (Naeye, 1992). (f) Syncytial knots are increased in amount within the placenta as oxygen tension decreases (Benirschke and Kaufmann, 1990) (g) Abruption refers to bleeding at the decidual-placental interface which causes partial to total placental detachment prior to delivery of the fetus, which inhibits the exchange of gases and nutrients (Ananth and Kinzler, 2013).

The sample size in this study presents a limitation to the interpretation of the results obtained. Peaceman *et al* found that the negative predictive value for fFN testing is 98-99%, meaning that women who test fFN negative tend not to deliver within 14 days of testing (Peaceman *et al*., 1997). In translation, it is a rare event for a woman to test fFN negative and still deliver preterm (it represents about 1% of women). Due to the nature of this event, it is difficult to increase the sample size and look at women in this category. With three years of data, we found 16 women who tested negative yet still delivered. However, a strength of this study is for all of those women included in the study only one did not have data for placental analysis.

From this dataset, there is a trend suggesting that women delivering preterm after a negative fFN test are more likely to have placental ischemic changes. Although this relationship was not found to be statistically significant, due to the limited numbers, we may not have been able to have enough power in order to achieve statistical significance. More studies should be done to see if

this trend continues. Clinically, this information would be helpful because these women may not be a good candidate for this test and may need to be hospitalized and given steroid treatment when showing signs of PTL. However, until further information is available, women with suspected ischemic placentas should not yet be excluded from fFN testing. Clearly, further studies are needed to explore the relationship between placental ischemia and fFN testing.

Conclusion and Future Directions

Overall, this study of 58 women suggests that women with a negative fFN who still deliver within 14 days of testing tend to have histological evidence of ischemic changes within the placenta (68.8%). This was a pilot study done to compare specific pregnancy characteristics in women with a positive or negative fFN test. Since a trend was found with women who had a PTD after a negative fFN test having ischemic changes within the placenta, the study will be expanded to include births from January 2010- December 2013 at BMC.

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