

# Comparison of fetal and plasma fibronectin concentrations in diagnosis of Preterm: delivery A cross sectional, descriptive-analytical study

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## Research article

**Keywords:** Preterm delivery, Plasma fibronectin, Fetal fibronectin, Screening

**Posted Date:** March 25th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-19313/v1>

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# Abstract

## Background

Preterm delivery is amongst the main reasons for child mortality. Therefore, its prediction can reduce a large number of its complications. The present study aimed to compare fetal and plasma fibronectin concentrations in diagnosis of preterm delivery.

## Methods

Serum samples were obtained from 105 women at 24-36 weeks of gestation. However, only 40 women gave permission for taking vaginal samples. Fibronectin concentration was measured using ELISA technique. Then, plasma and fetal fibronectin levels were compared in term and preterm deliveries.

## Results

Out of the 105 participants, 28 experienced preterm delivery (26.7%). The mean gestational age at the time of sampling was 28.11 + 2.98 weeks. The mean plasma fibronectin level was 6226.43+7174.97 ng/ml among the mothers with term infants and 7724.01 + 1143.82 ng/ml among those with preterm infants. Although the mean plasma fibronectin level was higher in preterm delivery, the difference was not statistically significant ( $p=0.667$ ). The mean fetal fibronectin level was 156.61 + 126.42 ng/ml among the mothers with term infants and 127.71 + 43.14 ng/ml among those with preterm infants, but the difference was not statistically significant ( $p=0.241$ ). The cut-off point of plasma fibronectin level was 1750 ng/ml with the sensitivity of 80.25% and specificity of 85.17%. Additionally, the cut-off point of fetal fibronectin level was 158.98 ng/ml with the sensitivity of 94.62% and specificity of 22.08%.

## Conclusion

Plasma fibronectin testing had lower sensitivity and higher specificity compared to fetal fibronectin testing. This implies that plasma testing has lower false positive cases and can identify a larger number of true positive cases of preterm delivery.

## Background

Pregnancy is a complicated phenomenon accompanied with challenges, which can cause concerns for mothers. One of the most common problems that can disrupt the normal duration of pregnancy, particularly in the pregnancies that do not follow the natural process and are accompanied with problems, is preterm delivery that has been introduced as the main factor in the incidence of infant mortality and morbidity (1). Preterm delivery refers to delivery prior to 37 weeks of gestation or 259 days after the Last Menstrual Period (LMP) (2, 3), which has been identified as the cause of 75–80% of prenatal mortalities and 50% of neonatal neurological complications (4). Although new care techniques have improved these neonates' status to some extent, no stable and considerable reduction has been detected in the incidence of preterm deliveries. In fact, preterm delivery still accounts for 70% of

mortalities, neurological complications, and disabilities even years after birth, which imposes great costs to the health system (5).

Preterm delivery is accompanied with a wide range of health problems and developmental disabilities, including respiratory issues, gastrointestinal complications, central nervous system disorders, and cognitive, motor, and behavioral disorders (6).

In a previous quasi-experimental study, women were divided into two groups. The first group's physician was aware of the results of fetal fibronectin test, while the second group's physician was not. The study findings indicated that using the results of fetal fibronectin test in clinical management led to an increase in hospital costs, including hospitalization and utilization of tocolytics and steroids, compared to the control group. In other words, information about the results of fetal fibronectin test could not decrease the rate of preterm deliveries before 28, 32, 34, and 37 weeks of gestation. Therefore, it is not logical to continue using the results of fetal fibronectin test in the women who are at risk of preterm delivery (7). Consequently, attempts should be made to predict and prevent preterm delivery. In this context, identification of high-risk women using reliable tests can provide the ground for faster interventional care services for the mother, fetus, and infant (6).

Considering the importance of preterm delivery whose prediction can prevent prenatal problems, various biochemical measurements have been carried out to promote both maternal and fetal outcomes. These measurements include evaluation of cervical dilation, investigation of cervical activity using a tocodynamometer, performance of sonography for determination of gestational age, evaluation of cervical length through abdominal or vaginal ultrasound, assessment of salivary estriol level, and measurement of plasma and fetal fibronectin concentrations (8). Fibronectin is yet a stronger marker for diagnosis of preterm delivery. Fibronectin is a glycoprotein, which is produced by the chorion and acts as a glue between the placenta and the decidua. It is normally secreted in the vagina and the cervix within 16–20 weeks of gestation, but it is rarely found in the discharges after the 21<sup>st</sup> week. It increases again in the discharges prior to delivery. Hence, early presence of fibronectin in vaginal and cervical discharges can predict preterm delivery (9). In other words, fibronectin concentration follows a descending trend during pregnancy, but it may increase weeks or months prior to delivery under such conditions as preterm labor, preeclampsia, and Intrauterine Growth Restriction (IUGR) (8).

Fetal fibronectin test is a standard method for predicting preterm delivery, which is carried out through the vagina or blood plasma. Nonetheless, blood sampling is more accepted. Moreover, no studies have been conducted on the comparison of fetal and plasma fibronectin levels to determine which test has a higher predictive value and can help predict preterm delivery sooner. Therefore, the present study aims to compare these two techniques.

## Methods

### Study design and patients

This cross-sectional, descriptive-analytical study was extracted from a research proposal (93104) approved by Fasa University of Medical Sciences. The inclusion criteria were aging 18–35 years, singleton pregnancy, gestational age of 24–36 weeks, and not suffering from chronic hypertension, diabetes, renal problems, and inflammatory disorders such as lupus. The exclusion criterion was having the history of receiving tocolytic agents (terbutaline, ritodrine, magnesium sulfate, salbutamol, and isoxsuprine). Gestational age was determined according to the LMP. In case of doubt about the LMP, the first trimester sonography was taken into consideration. The demographic data (parents' ages, occupations, and education levels) and obstetric information were collected for each participant.

## **Diagnostic criteria for chronic hypertension**

Chronic hypertension is diagnosed in women whose hypertension ( $BP \geq 140/90$ ) is established before pregnancy or in those who have been diagnosed with hypertension before the 20th week of pregnancy(2).

## **Diagnostic criteria diabetes**

criteria for diabetes, One or more of the following criteria must be met:

Fasting plasma glucose (7.0 mmol / l) (126 dl / mg)

2 hours after administration of 75 g oral glucose > 11.1 mmol / l

(200 mg / dl)(2).

## **Assessment of fetal and plasma fibronectin**

Then, venous blood samples were collected and the sera were separated and kept at  $-70\text{ }^{\circ}\text{C}$ . All the samples were preceded for the assessment of fibronectin level using 96-well ELISA kits.

Blood samples were taken from all 105 participants. However, only 40 women gave permission for taking vaginal samples. Vaginal samples were obtained from the posterior fornix using a cotton swab. In doing so, after inserting a speculum, a cotton swab was put in the posterior fornix for 10 seconds. The sample was then put in a tube containing normal saline at  $-70\text{ }^{\circ}\text{C}$ . The criteria observed for taking vaginal samples included not having had sexual activities during the past 24–48 hours and not having vaginal bleeding at the time of sampling.

All participants were followed until delivery and information about infant's weight, type of delivery, and gestational age at delivery was collected and recorded. Plasma and vaginal fibronectin concentration was assessed using ELISA kits (BE59341 IBL international GMBH). The results of the biomarker analysis were not available until the end of study and did not, therefore, influence management decisions.

# Participant/sample selection

The pregnant women who had referred to the gynecology clinic in Vali-e-Asr hospital, Fasa, Iran for routine prenatal care between 2014 and 2016 were informed about the study. The 105 pregnant women after considering the inclusion and exclusion criteria, who were interested in cooperation were given a written informed consent form to sign.

## Statistical analyses

All data were analyzed using the SPSS software, version 22. Descriptive statistics, such as mean, Standard Deviation (SD), and percentage, were used. Independent and paired t-test and logistic regression analysis were employed, as well. Besides, Receiver Operating Characteristic (ROC) curve was used to assess the cut-off points. Significance level was set at  $\alpha < 0.05$ .

## Results

### Baseline characteristics

The mothers' mean age was  $28.81 \pm 6.257$  years. The results showed no significant differences between the women with term and preterm deliveries regarding mother's age, father's age, and interval between marriage and the first pregnancy ( $p > 0.05$ ). The results also showed no significant differences between the two groups with respect to occupation, education level, parity, history of miscarriage, husband's cigarette smoking status, family history of preterm delivery, contraception method, and utilization of Assisted Reproductive Techniques (ART) ( $p > 0.05$ ) (Table 1).

Among the 105 participants, 28 (26.7%) had preterm delivery and 77 (73.3%) had term delivery. Out of the 40 women who gave vaginal samples, seven had preterm delivery. It should be noted that the mean gestational age at the time of sampling was  $28.11 \pm 2.98$  weeks (Table 1).

### Biomarker performance

In this study, logistic regression analysis was used to explore the effective factors in preterm delivery. At first, the variables were entered into the univariate model. In this model, Body Mass Index (BMI), family history of preterm delivery, planned pregnancy, and suffering from disorders such as hypertension and Gestational Diabetes Mellitus (GDM) during the pregnancy period were significant. Accordingly, increase in BMI was accompanied with a 15% increase in the odds of preterm delivery. Additionally, the odds of preterm delivery was 2.57 folds higher among the individuals who had the family history of this disorder compared to those who did not. Besides, the odds of preterm delivery was 84% lower among the individuals with planned pregnancy in comparison to those with unplanned pregnancy. Finally, the odds of preterm delivery were respectively 7.33 and 14.66 folds higher among the individuals who had the

history of hypertension and GDM in comparison to those who did not. After adjustment, the results revealed that BMI and pregnancy intention were associated with preterm delivery. Accordingly, increase in BMI caused an 18% increase in the odds of preterm delivery [CI (1.04–1.35),  $p = 0.009$ ]. In addition, the odds of preterm delivery was 87.7% lower among the individuals with planned pregnancy compared to those with unplanned pregnancy [CI (0.02–0.59),  $p = 0.009$ ] (Table 2).

The mean plasma fibronectin level was  $6226.43 \pm 7174.97$  ng/ml among the mothers with term infants and  $7724.01 \pm 1143.82$  ng/ml among those with preterm infants. Although the mean plasma fibronectin level was higher in preterm delivery, the difference was not statistically significant ( $p = 0.667$ ). The mean fetal fibronectin level was  $156.61 \pm 126.42$  ng/ml among the mothers with term infants and  $127.71 \pm 43.14$  ng/ml among those with preterm infants, but the difference was not statistically significant ( $p = 0.241$ ) (Table 3).

The plasma and fetal fibronectin concentrations in diagnosis of preterm delivery have been presented in ROC curves. Accordingly, the cut-off point of plasma fibronectin level was 1750 ng/ml with the sensitivity of 80.25% and specificity of 85.17%. Additionally, the cut-off point of fetal fibronectin level was 158.98 ng/ml with the sensitivity of 94.62% and specificity of 22.08%. Thus, plasma fibronectin testing had lower sensitivity and higher specificity compared to fetal fibronectin testing. This implies that plasma testing had lower false positive cases and could identify a larger number of true positive cases of preterm delivery (Figure 1). Furthermore, the Kappa coefficient was 40% for fetal fibronectin testing and 45.19% for plasma fibronectin testing. Thus, the Kappa coefficient was higher for plasma testing compared to fetal testing. Although the Kappa coefficient was lower than 0.4 for both types of testing, the rate of getting a positive result by chance was lower for plasma testing compared to fetal testing (Table 4).

## Discussion

### Principal findings

The study results indicated that plasma fibronectin testing had lower sensitivity and higher specificity compared to fetal fibronectin testing. This implies that plasma testing had lower false positive cases and could identify a larger number of true positive cases of preterm delivery.

In the research carried out by Forouhari et al., three groups of pregnant women (with the symptoms and risk factors of preterm delivery, with the symptoms and without the risk factors of preterm delivery, and healthy pregnant women) were compared regarding plasma fibronectin concentration. The results indicated significantly higher plasma fibronectin levels among the women with preterm delivery compared to those with term delivery (8). Zygmunt et al. also conducted a 10-month study in an obstetrics and gynecology hospital affiliated to Gibbon University in Germany in 1996 to investigate plasma fibronectin concentration as a predictive marker of preterm delivery. In that study, 80 pregnant women with the symptoms of preterm delivery were enrolled into the case group and 64 healthy pregnant women were allocated to the control group. All pregnant women were at 22–36 weeks of gestation.

Plasma fibronectin concentration was measured in both groups and the mean values were determined. The results revealed a significant difference between the two groups concerning the mean plasma fibronectin level. In the control group also, the mean plasma fibronectin level was higher among the women with preterm labor compared to their peers. Hence, it was concluded that ascending plasma fibronectin concentrations could be influential in prediction of preterm delivery ( $p < 0.05$ ) (10). In the same line, Moradi et al. performed a study to compare plasma fibronectin concentrations at two stages of delivery in 2020. They reported that plasma fibronectin testing after the 30<sup>th</sup> week of gestation could be effective in prediction of preterm delivery, thereby providing the mothers as well as the medical team to carry out care interventions at due time and have access to intensive care services for infants (11). Furthermore, Gredmark et al. demonstrated that in comparison to the overall assessment of fibronectin concentration at a particular age, longitudinal measurement of plasma fibronectin concentration at 26, 30, and 34 weeks of gestation was a better predictor of the incidence of preeclampsia (12). Consistently, Juha Rasanen indicated that plasma fibronectin testing could be effective in prediction of preeclampsia (13). On the contrary, Michelle Gates conducted a systematic review in 2018 and showed the unsatisfactory results of using plasma fibronectin testing in identification of the pregnant women who required interventions. Additionally, physicians' information about fetal fibronectin concentration was not influential in reduction of the rate of preterm delivery and had no benefits for either the mother or the fetus (14). Similarly, Soo et al. carried out a research in 2019 and demonstrated that fetal fibronectin testing could not improve the prognosis of preterm delivery. In symptomatic women, however, this test could help identify at-risk cases prior to 34 and 37 weeks of gestation (15).

Based on what was mentioned above, plasma fibronectin testing has been used as a screening test for preeclampsia. Considering the two studies performed by Michelle Gates et al. (14) and Soo et al. (15) and that blood sampling is easier than vaginal sampling during pregnancy, it is time to change the type of sampling for prediction of preterm delivery.

Considering the cut-off points computed for diagnosis of preterm delivery, infants' actual status as the gold standard, and Kappa coefficient  $< 0.4$  in both plasma and vaginal samples, this criterion was very weak and, as a result, fetal and plasma fibronectin levels at this gestational age could not predict preterm delivery properly. Nonetheless, this test might be useful at higher gestational ages. Moradi et al. also disclosed that sampling after the 30<sup>th</sup> week of gestation could have a better predictive value.

## **Strengths and weaknesses**

Since no studies have been conducted on the comparison of fetal and plasma fibronectin testing, the findings of the present study cannot be compared to those of other investigations. Future studies in this field can help achieve more definite results.

## **Conclusion**

Fetal fibronectin testing has no preventive role and is usually useful in diagnosis of preterm delivery when the delivery process has begun. Nevertheless, plasma fibronectin testing can have a better prognostic value compared to fetal fibronectin testing. Since plasma fibronectin testing is more easily performed and more accepted by mothers, it is recommended to be used as a screening test for prediction of preterm delivery after 30 weeks of gestation.

## **Abbreviations**

NVD: Natural vaginal delivery; GDM: Gestational diabetes mellitus; ART: Assisted reproductive technology; UTI: Urinary Tract Infection; PROM: Premature rupture of membranes; BMI: Body mass index; BP: Blood pressure; CI: Confidence intervals; c/s: Caesarean section; EIA: Enzyme immunoassay; Fn: fibronectin; IUGR: Intrauterine growth restriction; PEC: Preeclampsia; ROC: Receiver-operating characteristic; SD: Standard deviation; N: Number; OR: Odds ratio. NI: normal. BP: Blood pressure.

## **Declarations**

### **Acknowledgments**

This article was extracted from a research proposal (93104) approved by Fasa University of Medical Sciences. Hereby, the authors would like to thank the Fasa University of Medical Sciences for their financial assistance for this paper, the Clinical Trial Research Center of Vali-e-Asr Hospital, the Dean of the hospital, and all hospital personnel for their cooperation. We are also thankful to the women for their kind participation in the study.

### **Ethical approval and consent to participate**

Women were informed about the study and those who were interested to join, provided written informed consents. This article was extracted from a research proposal (93104) approved by Fasa University of Medical Sciences.

### **Authors' contributions**

MZ drafted this manuscript together with JS. MM Performed the tests according to the protocol, and revised the manuscript. MZ was principal investigator and revised the manuscript. MP and KA helped with recruitment, data acquisition and revised the manuscript. DA performed the statistical analysis. All authors have given final approval of the version to be published

### **Funding**

Hereby, the authors would like to thank the Fasa University of Medical Sciences for their financial assistance for this paper.

### **Availability of data and materials**



The anonymised data supporting our results can be obtained on request to the corresponding author Moradi z.

## Conflicts of Interest

Not applicable.

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## Competing interests

All authors are faculty members of Fasa University of Medical Sciences. The authors report no conflict of interest.

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## Tables

Table1:Frequency distribution and comparison of demographic, labor and individual factors in two groups							
Personal and delivery details, and maternal factors		pretearm N=28(26.7%)		Tearm N=77(73.3%)		Total N=150(100%)	P-value
Mother's age (years) *		56/5±47/29		50/6±57/28		6.257±28.81	/518
Husband's age (years)*		28/7±39/35		03/6±45/33		6.417±33.97	/172
The average age of marriage for women (years) *		39/5±21/22		66/4±70/21		4.84±21.84	/634
Distance between the age of marriage and the first pregnancy (years) *		79/1±25/2		20/3±57/2		2.84±2.49	/634
Gestational age in the sampling (week) *		2.68±27.54		2.33±28.16		2.98±28.1	/87
Gestational age at birth (weeks) *		1.10±35.12		2.13±39.25		2.03±38.24	/012
Mother's weight at sampling (kg) *		56/10±3/65		74/10±42/60		10.867±61.72	/42
Maternal Weight at Delivery (Kg) *		33/12±25/74		24/12±54/67		12.566±69.33	/017
Maternal BMI before pregnancy *		46/3±24/26		88/3±18/24		3.87±24.73	/015
Neonatal Birth weight (g) *		89/573±2425		6/369±61/3229		559.59±3015.05	/000
Delivery type **	NVD	13	46.4	44	57.1		0.33
	C/S	15	53.6	37	42.9		
Mother's job **	Housewife	22	78.6	71	92.2		0.158
	Employed	6	21.4	6	7.8		
Husband's job**	The worker	2	7.1	16	20.8		0.346
	Employee	8	28.6	16	20.8		
	Free job	18	64.3	45	58.4		
Mother education **	Illiterate	2	7.1	1	1.3		0.372
	Under the diploma	7	15	27	35.1		
	Academic	19	67.8	49	63.7		
Husband's education **		-	-	-	-		0.085
	Illiterate	1	3.6	5	6.5		
	Under the diploma	15	53.6	53	68.8		
	Academic	12	42.8	19	24.7		

Gravida **	-	-	-	-	-	-	-
	1	13	6.4	27	35.1		0.703
	2	8	28.6	27	35.1		
	3≥	7	25	23	29.8		
Abortion history **	Yes	4	14.3	19	24.7		
	No	24	85.7	58	75.3		
Smoking by Husband **	Yes	6	21.4	14	18.2		0.708
	No	22	78.6	63	81.8		
Preterm labor history **	Yes	23	82.1	69	89.6		0.304
	No	5	17.9	8	10.4		
Use of fertility assisted methods **	Yes	2	7.1	3	3.9		0.49
	No	26	92.9	74	96.1		
Disease in the current pregnancy **	GDM	4	14.28	1	1.31		0.009
	PEC	4	14.28	2	2.63		
	Infection	7	25	19	25		
	Vaginal bleeding	1	3.57	10	13.15		

\* Mean  $\pm$  SD, \*\* Number and percentage, \*\*\* p, Qualitative variables were analyzed using independent and dependent t-test and quantitative variables were analyzed using logistic regression test.

Table 2: Crude and adjusted Odds Ratio (OR) estimates of different variables in the term

Variables	Crude			Adjusted		
	OR	95% CI	P-value	OR	95% CI	P-value
mother	1.042	.96 - 1.12	0.282	NI*		
	1.15	1.02-1.29	0.020	1.188	1.04-1.35	.009
of married	1.022	.93 -1.11	.630	NI*		
vidia	0.766	.48-1.21	0.257	NI*		
ily history				NI*		
	Reference					
	2.548	1.05-6.17	0.038			
				NI*		
	Reference	-				
	0.527	0.08-3.33	0.496			
nancy disease		=		NI*		
	Reference					
	0.56	0.23-1.34	0.197			
rtion	1.96	0.60-6.38	0.261	NI*		
ted prignancy						
	Reference					
	0.160	0.03-0.72	0.018	0.123	0.02-0.59	0.009
nancy disese				NI*		
	Reference					
ertension	7.33	1.19-44.96	0.031			
4	14.66	1.49-143.7	0.021			
nitise	2.61	0.70-9.73	0.151			
	0.611					
M	.00	-	1			
na	.40	0.04-3.54	0.416			
nalbleeding	0					

Variable	Term	Preterm	P-value
	Mean ±SD	Mean ±SD	
Fetal fibronectin	156.61±126.42	127.71±43.14	0.199
Plasma fibronectin	7727.50 ± 11506.734	6726.43 ± 7174.916	0.668

Table 3: Comparison of plasma concentration with fetal concentration of fibronectin

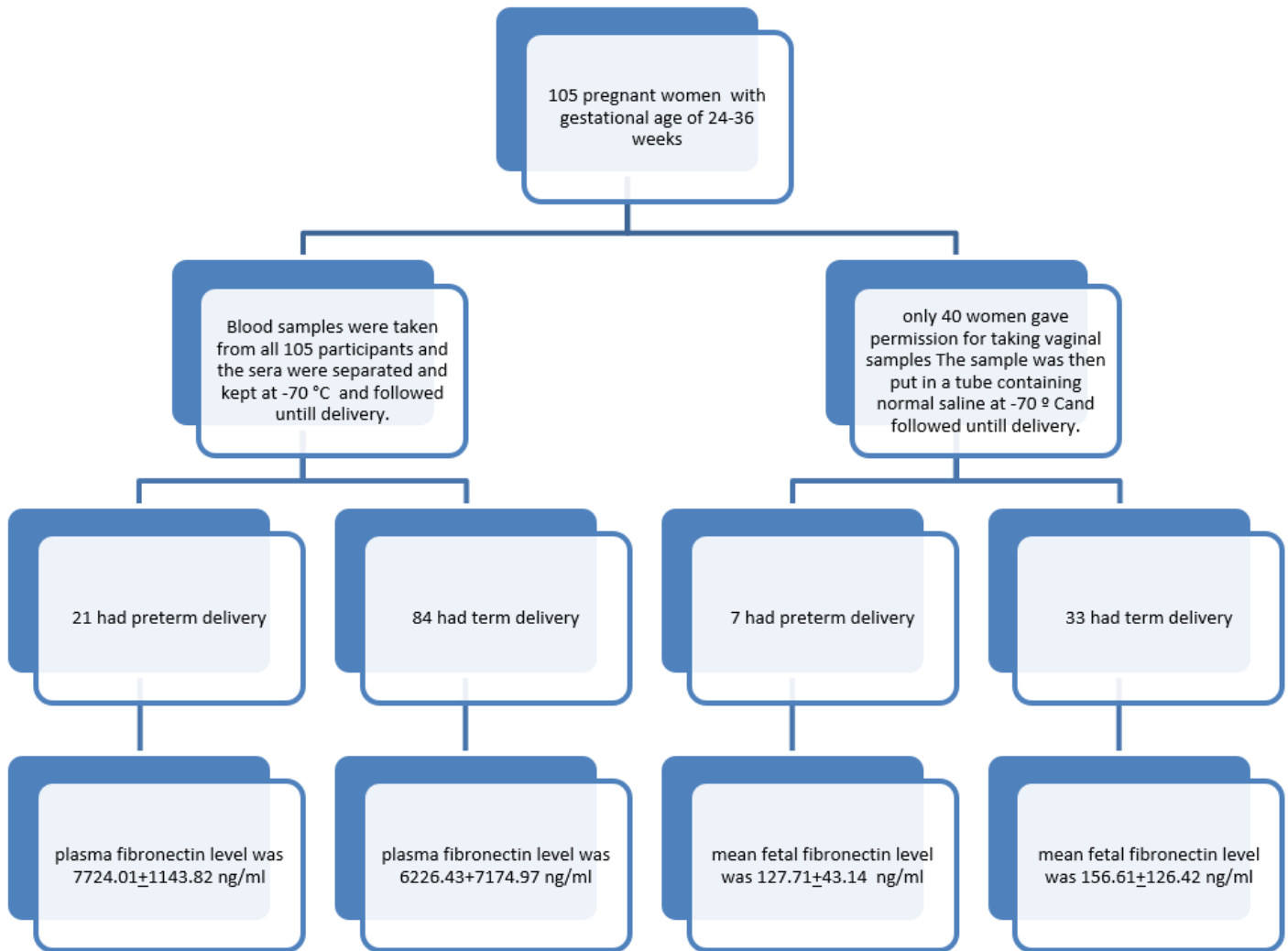
Table 4: Comparison of diagnosis of preterm delivery based on tests performed with the actual condition of the newborn

		Baby age at delivery		P- Value	Agreement	Kappa	
		Preterm	Term				
Fetal fibronectin	Preterm	28(100)	57(75)	0.002*	%40	-0.015	
	Term	0	19(25)				
Plasma fibronectin	Preterm	19(67.9)	54(70.01)	0.823**	45.19%	0.152	
	Term	9(32.1)	23(29.9)				

\*Fisher exact test

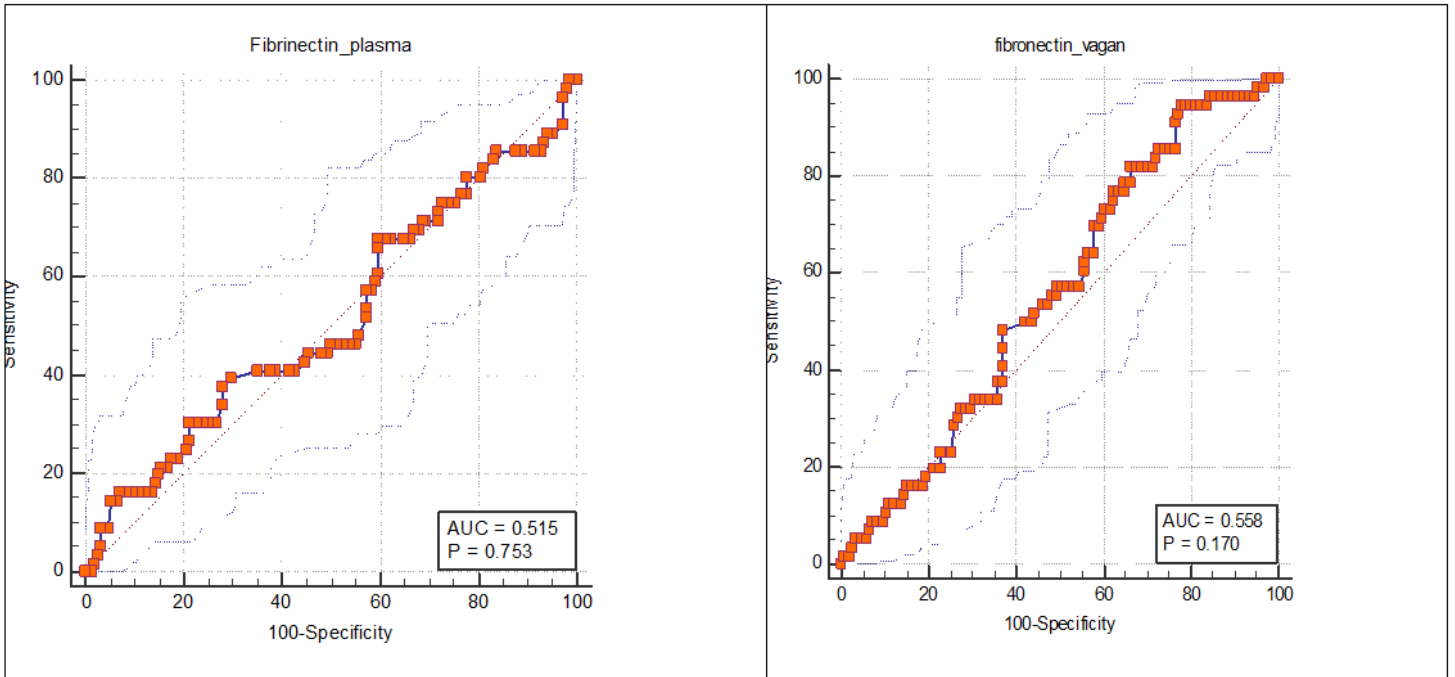
\*\*Chi-square test

## Figures



**Figure 1**

The number and type of settings for data collection



**Figure 2**

Chart 1: Rock curve for comparison of plasma and vaginal fibronectin in predicting preterm labor

## Supplementary Files

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