

## Introduction

Since 2011, cell-free DNA (cfDNA) screening has been widely validated and as a highly accurate screening test with high sensitivity (92-99%) and specificity (>99%) for determining the risk of trisomies 13, 18, and 21, and sex chromosome aneuploidies. The majority of cfDNA originates from the mother with the fetal component (fetal fraction; FF) making up 10-20% of the total. While FF appears to be correlated with risk of trisomies, little is known about how FF is related to pregnancy complications and fetal outcomes.

## Objective

This study aims to evaluate the association of low and high FF on cfDNA with pregnancy outcomes.

## Methods

- Retrospective live birth cohort study (N=1,774)
- Single institution between Jan 2021 - Dec 2023
- 3 comparison groups: low (FF≤4%), normal (4%<FF<16%), and high (FF≥16%)
- Demographics, clinical characteristics, maternal and neonatal outcomes were compared
- Chi-square, Fisher's exact, and multivariable logistic regression, with a statistical significance considered at p-value <0.05
- Analyses were conducted using SPSS

## Results (Table 1)

- 181 (10.2%) had low FF; 92 (5.2%) had high FF; and 1501 (84.6%) had normal FF
- Those with high FF were on average two years younger than normal and low FF ( $p=0.002$ )
- Having government insurance was associated with higher rates of high FF (7.3%) ( $p=0.03$ )
- Obesity was associated with higher rates of low FF (13.8%) and lower rates of high FF (3.5%) ( $p<0.001$ )
- CHTN was associated with higher rates of low FF (12.7%,  $p<0.001$ )

**Table 1. Maternal Demographics and Pregnancy Complications**

\* Data represented as n(%) or mean (SD)

Characteristics	Full Sample n=1774 n (%)	Low FF n=181 n (%)	Normal FF n=1501 n (%)	High FF n=92 n (%)	p-value
Age	32.1 (5.1)	32.6 (5.0)	32.1 (5.0)	30.3 (5.8)	<b>0.002</b>
BMI kg/m <sup>2</sup>	32.9 (6.2)	38.1 (7.8)	32.4 (5.7)	29.6 (5.0)	<b>&lt;0.001</b>
Obesity	1129 (63.6)	156 (86.2)	931 (62.0)	40 (43.5)	<b>&lt;0.001</b>
Race / Ethnicity					0.16
Caucasian	1277 (72.0)	138 (76.2)	1076 (71.7)	63 (68.5)	
African-American	112 (6.3)	14 (7.7)	97 (6.5)	1 (1.1)	
Hispanic	160 (9.0)	13 (7.2)	132 (8.8)	15 (16.3)	
Insurance status					<b>0.02</b>
Public	560 (31.6)	50 (27.6)	469 (31.2)	41 (44.6)	
Private	1197 (67.5)	131 (72.4)	1015 (67.6)	51 (55.4)	
Tobacco Use	33 (1.9)	1 (0.6)	29 (1.9)	3 (3.3)	0.26
Gravidity	2.6 (1.6)	2.8 (1.8)	2.6 (1.6)	2.4 (1.5)	0.14
Parity	0.9 (1.0)	0.9 (0.9)	0.9 (1.0)	0.9 (0.9)	0.90
IVF pregnancy	90 (5.1)	9 (5.0)	77 (5.1)	4 (4.3)	0.94
LMWH use during pregnancy	37 (2.1)	8 (4.4)	28 (1.9)	1 (1.1)	0.06
Abnormal amniocentesis	4 (16.7)	0 (0.0)	3 (21.4)	1 (33.3)	0.33
GA at delivery	38.8 (1.7)	38.4 (1.9)	38.8 (1.6)	39.0 (1.4)	<b>0.002</b>
Chronic hypertension	74 (4.2)	23 (12.7)	49 (3.3)	2 (2.2)	<b>&lt;0.001</b>
GHTN/Preeclampsia without SF	143 (8.1)	18 (9.9)	121 (8.1)	4 (4.3)	0.28
Preeclampsia with SF	82 (4.6)	13 (7.2)	69 (4.6)	0 (0.0)	<b>0.03</b>
Subchorionic hemorrhage	9 (0.5)	0 (0.0)	8 (0.5)	1 (1.1)	0.46
Placenta previa	6 (0.3)	1 (0.6)	5 (0.3)	0 (0.0)	0.76
Placenta accreta	7 (0.4)	0 (0.0)	7 (0.5)	0 (0.0)	0.53
Placental abruption	14 (1.0)	2 (1.3)	11 (0.9)	1 (1.6)	0.79
Fetal growth restriction	56 (3.2)	6 (3.3)	48 (3.2)	2 (2.2)	0.86
Small for gestational age	111 (6.3)	11 (6.1)	91 (6.1)	9 (9.8)	0.60
Large for gestational age	163 (9.2)	18 (9.9)	139 (9.3)	6 (6.5)	0.60
Premature delivery	112 (6.3)	19 (10.5)	85 (5.7)	5 (5.4)	<b>0.04</b>
NICU admission	240 (13.5)	28 (15.5)	197 (13.1)	15 (16.3)	0.50

## Results (Table 2)

- In a multinomial logistic regression controlling for age and obesity, CHTN was associated with a higher odds of low FF (aOR 2.55, 95% CI 1.11-5.89)
- Low FF was associated with higher rates of preterm birth (PTB) (10.5%) compared to normal (5.7%) and high FF (5.4%) ( $p=0.04$ )
- Binary logistic regression indicated that low FF was associated with 78% higher odds of PTB (aOR 1.78, 95% CI 1.04-3.04) after controlling for clinical covariates

**Table 2. Multivariate Analysis for Preterm Birth**

Variables	OR	95% CI	p-value
Normal FF (reference)	---	---	---
Low FF	1.78	1.04 – 3.04	<b>0.04</b>
High FF	0.83	0.35 – 2.32	0.90
Nulliparous	1.60	1.08 – 2.39	<b>0.02</b>
Any hypertension	2.63	1.71 – 4.06	<b>&lt;0.001</b>
Active smoking	3.00	1.16 – 7.77	<b>0.02</b>
Private health insurance (reference)	---	---	---
Public insurance	1.78	1.18 – 2.69	<b>0.01</b>
No insurance	0.78	0.17 – 10.36	0.78

## Conclusions

There is a risk of low FF in participants with CHTN and obesity, which may aid in counseling participants with additional risk factors for no-call results. Low FF is also associated with PTB, whereas high FF is not. High FF may be reassuring to participants when discussing the risk of PTB. However, further studies are needed to determine the utility of antenatal surveillance for participants with low FF.

## References

- Rafi, Hill, Hayward, Chitty, I. R. M. H. J. H. L. C. (2017). Non-invasive prenatal testing: use of cell-free fetal DNA in Down syndrome screening. *British Journal of General Practice*, 67, 1. <<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5565870/>>
- ARUP Laboratory (2014, March 19). Panorama Prenatal Screen: Patient Guide to Results. Arup Lab. <<https://www.aruplab.com/files/resources/genetics/panorama/Patient%20Guide%20to%20Results.pdf>>

