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Low fetal fraction in cell-free DNA testing is associated with adverse pregnancy outcome: Analysis of a subcohort of the TRIDENT-2 study

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Abstract

Objectives: To assess the association between low fetal fraction (FF) in prenatal cell-free DNA (cfDNA) testing and adverse pregnancy outcomes.

Methods: We conducted a retrospective cohort study of participants of the TRIDENT-2 study (Dutch nationwide government-supported study offering cfDNA screening for fetal aneuploidies) who received a failed test result due to low FF (<4%) between April 2017 until February 2018. Outcome measures included pregnancy-induced hypertension (PIH), pre-eclampsia (PE), small for gestational age neonates (SGA), spontaneous preterm birth (sPTB), gestational diabetes mellitus (GDM), chromosomal aberrations, and congenital structural anomalies.

Results: Test failure due to low FF occurred in 295 women (1.12% of tests performed). Information regarding pregnancy outcomes was available for 96.3% of these women. The incidence of PIH, PE, SGA, sPTB, and GDM was 11.2%, 4.1%, 7.3%, 5.1%, and 14.8%, respectively. The prevalence of chromosomal aberrations and congenital structural anomalies was 1.4% and 4.1%, respectively. Incidences of PIH, PE ≥ 34 weeks of gestation, GDM, and prevalence of aneuploidy and congenital structural anomalies were higher in women with low FF compared to the general Dutch obstetric population.

Conclusion: Low FF is associated with adverse pregnancy outcomes. The value of FF in the prediction of these outcomes needs to be further established.

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1 | INTRODUCTION

The analysis of cell-free DNA (cfDNA) in maternal plasma is a highly accurate screening method for the detection of chromosomal aneuploidies of the fetus, with sensitivities ranging between 91% and 100% for trisomy 18, 13, and 21.[1,2] No-call results and test failure due to low fetal fraction (FF), however, have been reported to occur in up to 2.2% of tests performed.[2] FF is the amount of fetal cfDNA, originating from apoptotic trophoblastic placental cells, relative to the amount of maternal cfDNA in the maternal circulation. FF is influenced by various biological factors and increases as gestation advances.[3–5] In women with high BMI, FF tends to be decreased, possibly due to higher amounts of maternal cfDNA resulting from apoptosis of maternal adipose tissue.[6–8] As fetal cfDNA originates from the placenta, the amount of fetal cfDNA found in the maternal circulation could reflect placental health and/or maternal pregnancy adaptation.[9,10] Reasoning that the placenta is the prime culprit for the development of pregnancy-related complications, FF has the potential to be an important parameter in the prediction of adverse pregnancy outcomes. Some small-scaled retrospective cohort studies have shown an association between low FF and placenta-related adverse pregnancy outcomes, such as pregnancy-induced hypertension (PIH) and pre-eclampsia (PE), fetal growth restriction, preterm birth, and gestational diabetes mellitus (GDM).[11–14] In addition, low FF has been associated with aneuploid pregnancies[15–19] and the rate of some fetal aneuploidies, including trisomy 13 and 18, monosomy X and triploid pregnancies, but not trisomy 21, has been reported to be 2.7%–23.3% in pregnancies with low FF.[15,19,20]

In the Netherlands, prenatal cfDNA testing is offered as first-tier test within the nationwide government-supported screening program for the detection of fetal aneuploidies (TRIDENT-2 study).[1] Here, we report on a subcohort of the TRIDENT-2 study population in which we assess the relationship between low FF and adverse pregnancy outcomes.

Practitioner notes

What is already known about this topic?

- Low fetal fraction in prenatal cell-free DNA testing is a cause of test failure or no-call results in ~2% of tests performed
- Low fetal fraction may reflect abnormal placentation and has been associated with placenta-related adverse pregnancy outcomes

What are the novel findings of this work?

- Women with low fetal fraction have a higher incidence of pregnancy-induced hypertension, preeclampsia ≥34 weeks of gestation, and gestational diabetes as compared to a general obstetric population
- Additionally, a higher prevalence of aneuploidy and congenital structural anomalies is observed in women with low fetal fraction
- Further large-scaled studies are needed to establish the value of fetal fraction in the prediction of pregnancy complications

2 | METHODS

2.1 | Setting, study design and participants

Prenatal screening for aneuploidies by cfDNA testing has been introduced in the Netherlands in 2017 and is offered within the TRIDENT-2 study to all pregnant women.[1] Women can choose to receive a report only for the common aneuploidies trisomy 13, 18, and 21, or to include chromosomal aberrations on the other autosomes (size resolution of 10–20 Mb) as well. The sex chromosomes are not analyzed. We conducted a retrospective cohort study of participants of the TRIDENT-2 study who received a failed cfDNA test result due to low FF from the 1st of April, 2017 until the 1st of February, 2018. During this time frame, routine measurement of FF was performed in only one of the three clinical genetic laboratories in which cfDNA testing is performed within the TRIDENT-2 study (i.e., the laboratory of the VU University Medical Centre [VUMC], Amsterdam), so only participants tested in this center were candidates for inclusion.

2.2 | Inclusion criteria

Women with a singleton pregnancy and a failed cfDNA test due to low FF between April 2017 until February 2018, were included.

2.3 | Exclusion criteria

Women were excluded if they were lost to follow-up, when informed consent was withdrawn for this substudy (as part of the TRIDENT-2 study), or if essential data on gestational age (GA) at time of delivery or information concerning pregnancy outcomes was not available.

2.4 | Laboratory analysis

Blood sample handling and cfDNA isolation were performed as previously reported.[1] The Illumina HiSeq4000 was used for genome-wide shallow sequencing and WISECONDOR (v2.0.1) algorithm was used for bioinformatic analysis.[21] The Y chromosome-based FF measuring method DEFRAG was used to measure FF.[22] As a result, only male-bearing pregnancies were included for analysis. Blood redraw was requested when DEFRAG did not provide a result due to low FF (below 4%) or was classified as "bad cluster" on two consecutive analyses of the first sample.[1,22]

2.5 | Definition of low FF

Low FF was defined as a FF <4%, as this was the cut-off value used in the laboratory protocol at the time.[22]

2.6 | Data collection

Laboratory results, patient characteristics (age, BMI, parity, smoking, medical and obstetric history), and data regarding the pregnancy outcome (both maternal and neonatal) were extracted from the laboratory database and the Dutch national registry for antenatal screening (Peridos), as well as from the obstetric records of the hospitals and midwifery practices.

2.7 | Outcome measures

Primary outcome measures were PIH, PE < 34 weeks of gestation and ≥34 weeks, small for gestational age neonates (SGA), spontaneous preterm birth (sPTB), and GDM. PIH was defined as a blood pressure >140 mmHg systolic or >90 mmHg diastolic after 20 weeks of gestation and measured twice with an interval of 4 h; PE was defined as PIH plus proteinuria (≥300 mg/24 h or a protein/creatinine ratio of ≥30). Women who developed PE after PIH were classified as PE. SGA was defined as a birthweight below the 10th percentile on the Visser Dutch birthweight curve.[23] sPTB was defined as a spontaneous birth before 37 weeks of gestation and divided in birth <32 weeks and birth between 32 and 37 weeks. GDM was defined as a fasting venous blood glucose ≥7.0 mmol/L or ≥7.8 mmol/L after 2 h on a 75 g 2-h oral glucose tolerance test between 24 and 28 weeks of gestation.

Secondary outcomes were chromosomal aberrations, and congenital structural anomalies.

2.8 | Statistical analysis and comparison with the general obstetric population

The incidence of adverse pregnancy outcomes was reported in absolute frequencies and percentages. Distributions of continuous variables, including maternal age, BMI, GA at time of cfDNA testing, GA at time of delivery, and birthweight were assessed using histograms and the Shapiro–Wilks test. Subgroup analyses were performed between women with one versus those with consecutive failed cfDNA tests due to low FF, to assess whether there were differences in baseline characteristics and the occurrence of adverse pregnancy outcomes.

To compare the baseline characteristics and incidence of adverse pregnancy outcomes in women with low FF to the general Dutch obstetric population, previously published data sets on the latter were used.[1,24–28] For comparison of continuous variables, the Student's *t*-test or Mann–Whitney *U*-test (depending on the distribution) were applied. Categorical data were compared using the Pearson's Chi-squared test or Fisher's Exact test, depending on outcome frequency. A *p*-value of <0.05 was considered statistically significant. All statistical analyses were performed in RStudio (version 1.3.1093).

3 | RESULTS

Between April 2017 until February 2018, a total of 26,226 cfDNA tests were performed in the genetic laboratory of the VUMC. On initial testing, 295 women (1.12%) received a failed cfDNA test result due to low FF (Figure 1). After application of the exclusion criteria, 284 women (96.3%) were included for analysis. Out of these 284 women, 276 opted for a second cfDNA test (97.2%), of which a consecutive test failure due to low FF occurred in 90 women (32.6%). During the study period, 10 women were lost-to-follow-up, and one woman withdrew informed consent. Baseline characteristics of the study cohort are shown in Table 1.

The occurrence of adverse pregnancy outcomes is displayed in Table 2. There were two miscarriages, two immature deliveries before 24 weeks of gestation, one fetal demise at 32 weeks of gestation, and four terminations of pregnancy because of fetal aneuploidy ($n = 3$) or severe congenital anomalies ($n = 1$) in the study cohort. With regard to chromosomal aberrations, there were two cases of trisomy 18, one case of trisomy 13, and one case of an XXY fetal karyotype, the latter detected following amniocentesis for fetal abnormalities. There were 13 cases of congenital structural anomalies in the study cohort (Table 3). One pregnancy (Case 1) resulted in a termination of pregnancy, because of severe congenital anomalies. In another pregnancy (Case 2), there were multiple anomalies on ultrasound and this pregnancy resulted in an intrauterine fetal demise. All other pregnancies (cases 3–13) resulted in live births.

There were no differences in baseline characteristics or the incidence of adverse pregnancy outcomes between women with a valid test result upon second blood draw and those with consecutive test failure (Tables S1 and S2).

Compared to the general Dutch obstetric population, women with low FF had a statistically significant higher BMI (28.7 vs. 23.7; $p < 0.01$), were more often nulliparous (64.8% vs. 44.5%; $p < 0.01$), and smoked more often (12.3% vs. 9%; $p < 0.05$) (data not shown). Women with a failed cfDNA test result due to low FF did not significantly differ from the general obstetric population with regards to the other reported baseline characteristics.

By univariate analysis, women with low FF had a significantly higher incidence of PIH (11.2% vs. 5.3%; $p < 0.01$), PE ≥ 34 weeks (3.7% vs. 1.9%; $p < 0.05$), and GDM (14.8% vs. 4.9%; $p < 0.01$) as compared to the general Dutch obstetric population (Table 4). No significant differences were found in the incidence of total PE (4.1% vs. 2.3%; $p = 0.07$), PE < 34 weeks (0.4% vs. 0.4%; $p = 1.00$), SGA (7.3% vs. 7.2%; $p = 0.98$), total sPTB (5.1% vs. 4.3%; $p = 0.54$), sPTB between 32 and 37 weeks (4.7% vs. 3.8%; $p = 0.44$), or sPTB <32 weeks (0.4% vs. 0.5%; $p = 1.00$). The

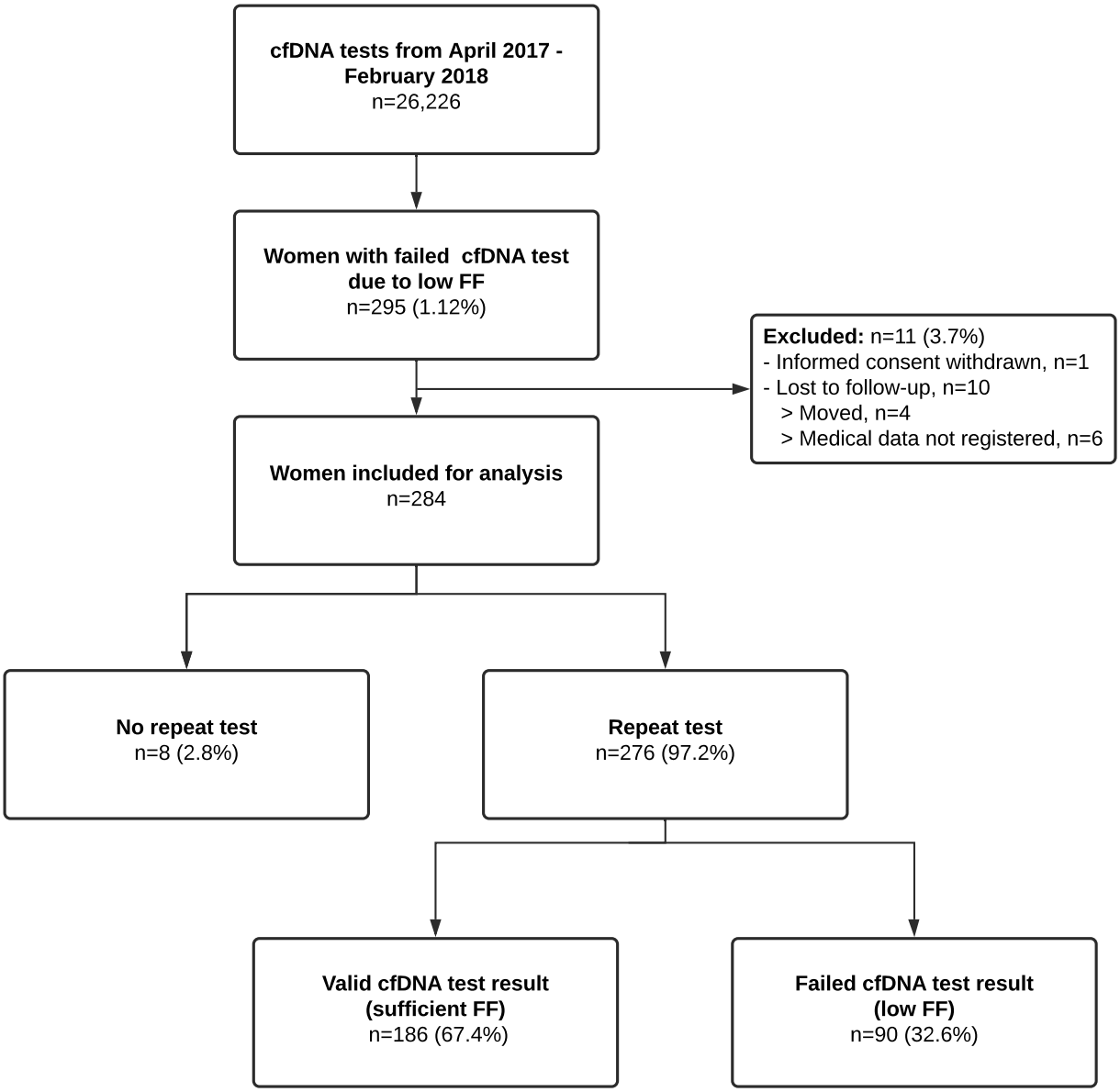

FIGURE 1 Flowchart of the study population. cfDNA, cell-free DNA; FF, fetal fraction

prevalence of aneuploidy was significantly higher in women with low FF compared to the general population (1.4% vs. 0.4%; $p < 0.05$), as was the occurrence of congenital structural anomalies (4.1% vs. 1.7%; $p < 0.05$).

4 | DISCUSSION

We performed a study on the occurrence of adverse pregnancy outcomes in a cohort of women with low FF in prenatal cfDNA testing. Women with low FF had higher incidences of PIH, PE ≥ 34 weeks of gestation, and GDM compared to the general Dutch obstetric population. Additionally, a higher prevalence of aneuploidy and congenital structural anomalies was found in women with low FF. In our study cohort, women with one failed cfDNA test due to low FF and those with consecutive failed cfDNA tests did not differ in baseline characteristics or in the occurrence of adverse pregnancy outcomes.

The relationship between low FF and adverse pregnancy outcomes might be explained by abnormal placentation in early pregnancy.[29] Failed trophoblast invasion with abnormal spiral artery transformation and subsequent placental dysfunction later in pregnancy could lead to a disturbed placenta-maternal interface with smaller amounts of cfDNA molecules released in the maternal circulation. Retrospective cohort studies on the risk of developing PE and fetal growth restriction based on first-trimester markers and fetal cfDNA support this hypothesis.[10,30]

We found an increased rate of PIH and PE ≥ 34 weeks in women with low FF, but not for PE < 34 weeks. Several other studies support the findings of an increased incidence of PIH and PE in women with low FF, but no differentiation between late (≥34 weeks of gestation) and early (<34 weeks) onset PE was made.[11–13,30]

TABLE 1 Baseline characteristics of women with low FF

Characteristics	Women with low FF $n = 284$
Maternal age (years) at first cfDNA test	31 (27.5, 34.5)[a]
Maternal BMI (kg/m^2) at first cfDNA test	28.7 (24.1, 33.2)[a]
GA (weeks + days) at first cfDNA test	11 + 6 (11 + 1, 12 + 4)[a]
Parity, *n* (%)	
Nulliparous	184 (64.8)
Multiparous	100 (35.2)
Smoking, *n* (%)	
Yes	35 (12.3)
No	245 (86.3)
Unknown	4 (1.4)
Medical history, *n* (%)	
Hypertension	7 (2.5)
Diabetes mellitus	4 (1.4)
Auto-immune disease	0 (0)
No prior medical condition of interest	273 (96.1)
Obstetric history ($n = 100$)[b], *n* (%)	
Hypertensive disorders (i.e., PIH and/or PE)	13 (13.0)
Small for gestational age	7 (7.0)
sPTB <37 weeks GA	7 (7.0)
Gestational diabetes mellitus	6 (6.0)
Recurrent miscarriage[c]	13 (4.6)

Abbreviations: cfDNA, cell-free DNA; FF, fetal fraction; GA, gestational age; PE, pre-eclampsia; PIH, pregnancy-induced hypertension; sPTB, spontaneous preterm birth.

[a]Data are median (interquartile range).

[b]Data only of multiparous women.

[c]≥2 miscarriages, including data of nulliparous women.

We did not find an association between low FF and SGA. This is in accordance to several other retrospective cohort studies.[11,14,30] However, Clapp et al. reported a significantly higher incidence of a birthweight <fifth percentile in women with low FF. The use of a higher cut-off for low FF possibly led to a higher incidence of SGA in their study population.[13] In addition, Yuan et al. also found an association between low FF and low birthweight babies (<2500 g).[30] Our small sample size may explain the absence of a detected association between low FF and early PE or SGA, as opposed to what would be expected based on our hypotheses.

We did not find an association between low FF and sPTB. Two previous studies did report such an association, but no distinction between spontaneous and medically indicated (i.e., induced) PTB was made.[11,30] In studies specifically making this distinction, no association has been reported.[12,14] An explanation for this discrepancy could be that, as low FF is related to adverse outcomes, preterm births would have been more likely medically indicated.

We found considerably higher rates of GDM in the study cohort compared to the reference population. Previous studies have shown discrepant results on this association.[11,14,30] Chan et al. found a higher incidence of GDM in women with a failed cfDNA test compared to a general obstetric population, although their results were not adjusted for BMI.[14] Krishna et al. and Yuan et al. also found differences in the occurrence of GDM in women with low FF compared to women with normal FF, but after adjusting for BMI these differences were not statistically significant.[11,30] Since high BMI is both associated with low FF as well as the development of GDM,[6–8] it could possibly have influenced our results. To establish whether low FF indeed yields an additional higher risk for GDM or that this association is fully explained by high BMI, must be confirmed in further studies.

Altered placenta physiology may also explain why the prevalence of aneuploidy was higher in women with low FF. Some aneuploidies typically have lower placental masses and reported low FF.[16] In our study cohort there were two cases of trisomy 18 and one case of trisomy 13. These findings correspond to previous literature, in which

TABLE 2 Adverse pregnancy outcomes in women with low FF

Pregnancy outcome	Women with low FF (n = 284) *Percentage % (frequency[a])*
Pregnancy-induced hypertension	11.2 (30/268)
Total pre-eclampsia	4.1 (11/268)
≥34 weeks GA	3.7 (10/268)
<34 weeks GA	0.4 (1/268)
Small for gestational age neonates	7.3 (20/275)
Total spontaneous preterm birth	5.1 (14/275)
Spontaneous preterm birth (32–37 weeks)	4.7 (13/275)
Spontaneous preterm birth (<32 weeks)	0.4 (1/275)
Gestational diabetes mellitus	14.8 (40/271)
Chromosomal aberrations	1.4 (4/281)
Congenital structural anomalies	4.8 (13/269)

Abbreviations: FF, fetal fraction; GA, gestational age.

[a]Excluding cases with miscarriages, immature deliveries, fetal demise, terminations of pregnancy, and/or pre-existing conditions when applicable.

TABLE 3 Congenital structural anomalies in women with low FF

Case	Condition	Ultrasound finding	Postnatal finding	Outcome
1	Polycystic kidneys and lung hypoplasia[a]	Yes	Yes	TOP
2	Unilateral MCDK, suspected duodenal atresia, polyhydramnion[b]	Yes	No postmortem	IUFD
3	Ventricular septal defect	Yes	Yes	Live birth
4	Ventricular septal defect	Yes	Yes	Live birth
5	Unilateral MCDK	Yes	Yes	Live birth
6	Unilateral MCDK	Yes	Yes	Live birth
7	Unilateral UPJ stenosis	Yes	Yes	Live birth
8	Unilateral UPJ stenosis	Yes	Yes	Live birth
9	Hypospadias	No	Yes	Live birth
10	Hypospadias	No	Yes	Live birth
11	CCAM	Yes	Yes	Live birth
12	Unilateral clubfoot	Yes	Yes	Live birth
13	Bilateral post-axial polydactyly	No	Yes	Live birth

Abbreviations: CCAM, congenital cystic adenomatoid malformation; FF, fetal fraction; IUFD, intra-uterine fetal demise; MCKD, multicystic dysplastic kidney disease; TOP, termination of pregnancy; UPJ, ureteropelvic junction.

[a]Normal WES (whole exome sequencing) result.

[b]Normal SNP-array result.

an association between low FF and aneuploidies has been reported consistently.[15,19,20]

Irrespective of aneuploidy, we found higher frequencies of congenital structural anomalies in women with low FF. This study is to the best of our knowledge the first in reporting this association and should be assessed in more depth in future studies. Although the rates of GDM were higher in pregnancies with congenital anomalies (36.4%) compared to those without congenital anomalies (14.2%), this difference was not statistically significant.

4.1 | Strengths and limitations

The strength of our study is that it was performed within the TRIDENT-2 study: a nationwide study with standardized follow-up. All analyses were performed in the same laboratory according to standardized protocols and the GA at testing in our cohort of women with low FF was comparable to the total cohort of women opting for cfDNA testing.[1] The study population is representative for the Dutch obstetric population, as it includes women from both urban and rural areas with pregnancy follow-up in primary, secondary and tertiary healthcare.

TABLE 4 Adverse pregnancy outcomes in women with low FF compared to the general Dutch obstetric population

Pregnancy outcome	Women with low FF (n = 284) *Percentage %*	General Dutch obstetric population *Percentage %*	*p*-value
Pregnancy-induced hypertension	11.2	5.3	<0.01
Total pre-eclampsia	4.1	2.3	0.07
≥34 weeks GA	3.7	1.9	<0.05
<34 weeks GA	0.4	0.4	1.00
Small for gestational age neonates	7.3	7.2	0.98
Total spontaneous preterm birth	5.1	4.3	0.54
Spontaneous preterm birth (32–37 weeks)	4.7	3.8	0.44
Spontaneous preterm birth (<32 weeks)	0.4	0.5	1.00
Gestational diabetes mellitus	14.8	4.9	<0.01
Chromosomal aberrations	1.4	0.4	<0.05
Congenital structural anomalies	4.1[a]	1.7	<0.05

Abbreviations: FF, fetal fraction; GA, gestational age.

[a]Excluding the two cases with hypospadias for comparison with general Dutch obstetric population.

Our study is limited by the fact that the study cohort contained male-bearing pregnancies only, and is therefore not entirely representative for the population of women with low FF. Another factor that may reduce representability is that, the uptake of cfDNA testing in the Netherlands was approximately 42% during the study period, and it is possible that reasons for declining cfDNA testing are related to maternal characteristics or adverse pregnancy outcomes. Although we established that the incidence of adverse pregnancy outcomes was higher in women with low FF compared to the general Dutch obstetric population in univariate analysis, it is uncertain if this difference is explained by low FF or by other factors influencing both the FF and adverse pregnancy outcomes, such as high BMI.[10,31] We recognize that a multivariate analysis regarding the role of BMI and other variables in relation to FF and adverse pregnancy outcomes would strengthen our study. Unfortunately, we were not able to perform a multivariate analysis since we did not have data on pregnancy outcomes for a control group of women with a normal FF. We therefore compared the perinatal outcomes of our large retrospective cohort with the incidence of adverse pregnancy outcomes in the general Dutch obstetric population, as previously published. The value of FF in the prediction of adverse pregnancy outcomes, additional to other factors, still needs to be established. Also, as some of the results in our study were borderline significant, they should be regarded with caution, as our sample size was relatively small to compare with a general obstetric population.

4.2 | Future research

To get a better understanding of the value of the FF in the prediction of adverse outcomes, future research should include large-scaled studies to establish the additional value of FF in prognostic models for pregnancy complications. In addition, although a cut-off value of 4% for low FF is commonly used,[3,6] in further studies it would be interesting to develop a golden standard for measuring FF in cfDNA testing and to investigate continuous values of FF in order to find an optimal cut-off value for "low" FF in relation to the pregnancy outcome.

5 | CONCLUSION

This study shows an association between a result of low FF in prenatal cfDNA testing and PIH and PE ≥ 34 weeks, GDM, aneuploidy and congenital structural anomalies. FF has the potential to be of predictive value in the early detection of adverse pregnancy outcomes. This may help identify pregnancies at risk and contribute to tailored pregnancy management through timely preventive measurements and monitoring. Further large-scaled studies are needed to establish the true value of FF in relation to pregnancy outcomes.

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CONFLICT OF INTEREST

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ETHICAL APPROVAL

The TRIDENT-2 study has been approved by the Dutch Ministry of Health, Welfare, and Sport (license 1017420-153371-PG) and the Medical Ethical Committee of the VUMC Amsterdam (No. 2017.165). An amendment to the TRIDENT-2 study protocol was approved for this retrospective study (No. 2017.165 [A2018.333]). Written informed consent was obtained from all women.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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