Received: 15 March 2021 | Revised: 10 July 2021 | Accepted: 29 July 2021

DOI: 10.1002/pd.6028

REVIEW

PRENATAL DIAGNOSIS WILEY

Association between low fetal fraction in cell-free DNA testing and adverse pregnancy outcome: A systematic review

Peter G. Scheffer[1] | Soetinah A. M. Wirjosoekarto[1] | Ellis C. Becking[1] | Marjan M. Weiss[2] | Caroline J. Bax[3] | Dick Oepkes[4] | Erik A. Sistermans[5] | Lidewij Henneman[5] | Mireille N. Bekker[1]

[1]Department of Obstetrics, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands

[2]Department of Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

[3]Department of Obstetrics, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[4]Department of Obstetrics, Leiden University Medical Center, Leiden, The Netherlands

[5]Department of Clinical Genetics, Amsterdam Reproduction and Development Research Institute, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Correspondence

Mireille N. Bekker, Department of Obstetrics, Division Woman and Baby, Wilhelmina Children's Hospital, University Medical Center Utrecht, Huispostnummer KE.04.123.1, Postbus 85090, 3508 Utrecht, The Netherlands.
Email: m.n.bekker-3@umcutrecht.nl

Abstract

Objective: Low fetal fraction (LFF) in prenatal cell-free DNA (cfDNA) testing is an important cause of test failure and no-call results. LFF might reflect early abnormal placentation and therefore be associated with adverse pregnancy outcome. Here, we review the available literature on the relationship between LFF in cfDNA testing and adverse pregnancy outcome.

Method: A systematic literature search was conducted in MEDLINE and EMBASE up to November 1, 2020.

Results: Five studies met the criteria for inclusion; all were retrospective observational cohort studies. The cohort sizes ranged from 370 to 6375 pregnancies, with all tests performed in the first trimester or early second trimester. A 4% cutoff for LFF was used in two studies, two studies used the 5th and 25th percentiles, respectively, and one study used a variety of cutoff values for LFF. LFF in prenatal cfDNA testing was observed to be associated with hypertensive disease of pregnancy, small for gestational age neonates, and preterm birth. Conflicting results were found regarding the association between LFF and gestational diabetes mellitus.

Conclusions: LFF in cfDNA testing is associated with adverse pregnancy outcome, specifically pregnancy-related hypertensive disorders, preterm birth, and impaired fetal growth related to placental dysfunction. Since the available evidence is limited, a large prospective cohort study on the relationship between fetal fraction and pregnancy outcomes is needed.

Key points

What's already known about this topic?

- Low fetal fraction (LFF) in prenatal cell-free DNA (cfDNA) testing is an important cause of test failure and no-call results and has been associated with aneuploidy

Peter G. Scheffer and Soetinah A.M. Wirjosoekarto should be considered joint first author.

- LFF might also reflect early abnormal placentation and therefore be associated with adverse pregnancy outcome

What does this review add?

- This review summarizes the available data on LFF in prenatal cfDNA testing and its relation to adverse pregnancy outcomes
- LFF was found to be associated with pregnancy-related hypertensive disorders, preterm birth, and impaired fetal growth related to placental dysfunction
- Since the available evidence is limited, a large prospective cohort study on the relationship between fetal fraction and pregnancy outcomes is needed

1 | INTRODUCTION

The presence of cell-free fetal DNA in maternal plasma allows for prenatal screening for fetal aneuploidies.[1–3] In cell-free DNA (cfDNA) testing, the cfDNA in maternal blood is analyzed using high-throughput molecular technologies. The reliability of cfDNA testing, among other factors, depends on the amount of cfDNA derived from the trophoblast of the placenta in relation to the cfDNA of maternal origin. This is known as the fetal fraction and depending on the molecular platform and bioinformatics algorithm, a minimum threshold up to 4% is commonly required to provide a reliable test result.[4–8]

Low fetal fraction (LFF) is an important cause of test failure in cfDNA testing. It has been reported to be responsible for test failure rates up to 6.1%.[9] Obese pregnant women are at higher risk of test failure due to LFF, probably due to dilution because of an increased circulating volume or by higher release of maternal cfDNA into the systemic circulation through apoptosis of adipose cells.[6,10,11] Other maternal factors such as ethnicity and smoking have too been found to influence fetal fraction.[6,12]

Fetal aneuploidy has also been associated with LFF.[13] The NEXT study reported a 4.7% aneuploidy rate in women with a fetal fraction of less than 4% compared to 0.4% in their overall cohort.[14] Several other studies have shown a high rate of test failure due to LFF in aneuploid pregnancies.[15,16] For this reason, genetic counseling, ultrasound evaluation, and invasive testing are offered to women with a repeated test failure due to LFF.[4]

As the "fetal" cfDNA finds its origin in the placenta, it has been hypothesized that the amount of "fetal" cfDNA released in the maternal circulation reflects placental health and function and that an LFF might reflect a smaller placental mass or even placental dysfunction.[17,18] Indeed, several mostly small-scaled and case-oriented studies have shown associations between the level of fetal cfDNA and adverse pregnancy outcomes, such as pregnancy-induced hypertension, preeclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), intrauterine growth restriction, preterm birth (PTB), gestational diabetes mellitus (GDM), and invasive placentation.[19–29] Most of these studies were performed in the early days of cfDNA exploration, using data from conventional DNA analysis techniques and not high-throughput technologies. In this review, we aim to summarize the available data on LFF in prenatal cfDNA testing and its relation to adverse pregnancy outcomes.

2 | MATERIAL AND METHODS

We followed the statement on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.[30]

2.1 | Data sources and searches

A literature search was conducted in the MEDLINE and EMBASE database up to November 1, 2020. Variations of entry terms for "fetal fraction," "cell-free (fetal) DNA," and "NIPT" were combined with the entry term "outcome" or the MeSH term "pregnancy outcome." The full detailed search entry is presented in Supporting Information S1.

2.2 | Study selection and eligibility criteria

Studies were eligible for inclusion if the fetal fraction was reported as "low" or below the commonly used cutoff of 4% and the clinical outcome of the pregnancy was reported for adverse events. Adverse pregnancy outcome was defined as the presence of hypertensive disease of pregnancy (HDP; including pregnancy-induced hypertension, preeclampsia, and HELLP syndrome), small for gestational age (SGA) neonates, PTB, and GDM. We included studies with >100 subjects with a singleton pregnancy at either low or high risk for aneuploidy and with testing performed on high-throughput platforms. Publication language was restricted to English and Dutch. Reviews, letters to the editor, case reports, and case series were excluded as well as studies reporting on cfDNA testing for other than the common aneuploidies or in multiple pregnancies. Four authors (PS, SW, EB, and MB) screened titles and abstracts and performed final selection of the eligible studies based on full-text reviewing. Eligible studies were cross-referenced for publications not identified in the literature search. Disagreements were resolved by discussion. Quality assessment of the included studies was performed using the Newcastle–Ottawa Scale for cohort studies.[31]

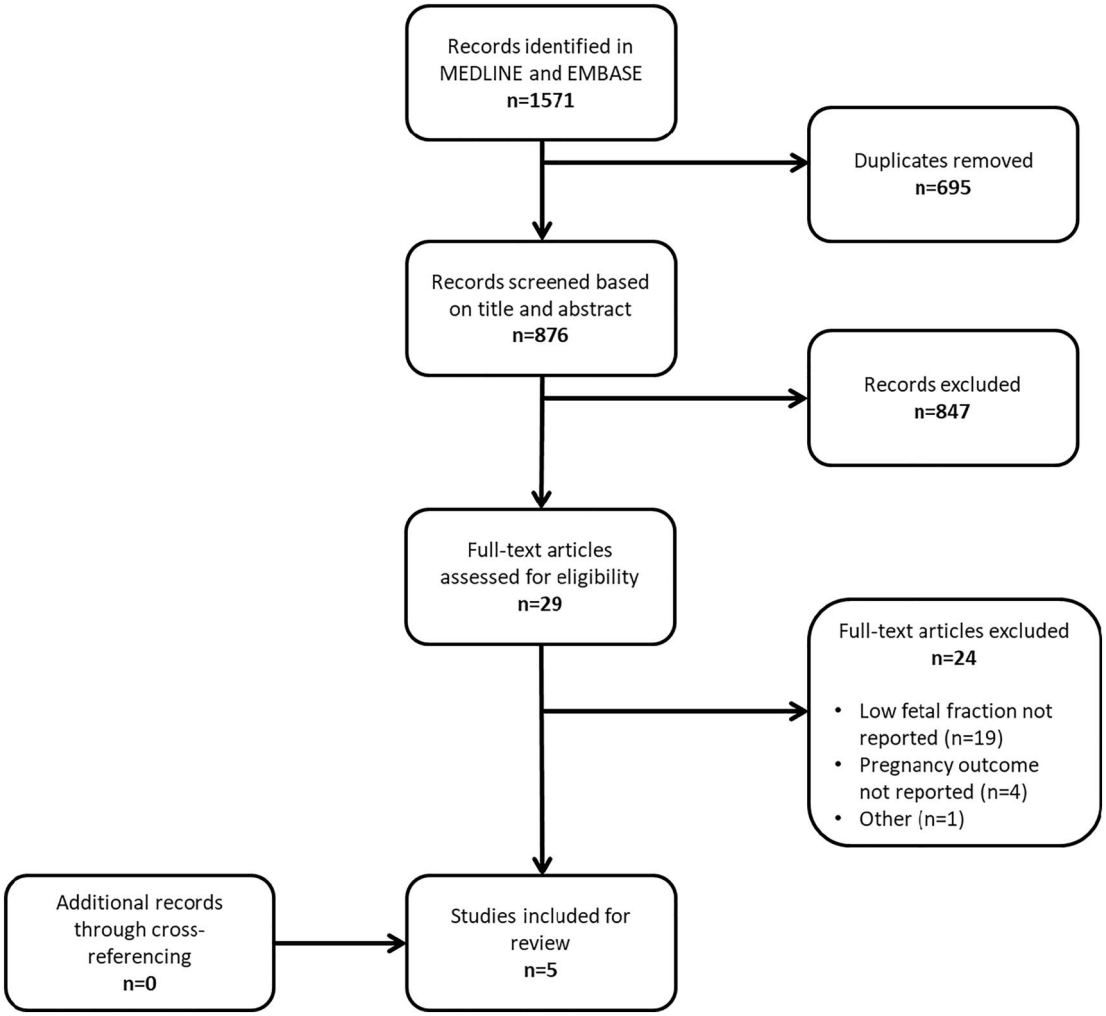

FIGURE 1 Flowchart summarizing selection of studies for inclusion for review

2.3 | Data extraction

From the eligible studies, details on study design and number of cases were extracted as well as the type of the molecular platform or method used, the fetal fraction cutoff, body mass index (BMI), the gestational age at sampling, and the reported pregnancy outcome.

2.4 | Ethical approval

No ethical approval was needed for this study.

3 | RESULTS

The search identified a total of 1571 publications (Figure 1), including publications on other applications of cfDNA testing outside the field of prenatal testing, predominantly related to malignancies outside pregnancy. After duplicate removal, 876 records were screened on title and abstract, resulting in 29 potential eligible studies. A total of five studies met the inclusion criteria and were included for review.[32–36] No additional articles were found after cross-referencing the included studies. All five studies were retrospective observational cohort studies and were published between 2016 and 2020. The cohort sizes ranged from 370 to 6375 pregnancies. All tests were performed in the first trimester or early second trimester. Quality assessment of the included studies is shown in Table 1. The main study characteristics are summarized in Table 2.

TABLE 1 Quality assessment of the five studies included for review, according to the Newcastle–Ottawa Scale[31]

Study	Selection	Comparability	Outcome
Chan, 2018[32]	***	n/a	**
Clapp, 2020[33]	****	**	***
Gerson, 2019[34]	****	*	**
Krishna, 2016[35]	****	**	**
Yuan, 2020[36]	****	**	**

Note: The first author of each study is given. A maximum of one star can be awarded for each numbered item within selection (four items), comparability (two items), and outcome (three items) categories.
Abbreviation: n/a; not applicable.

TABLE 2 Main characteristics of studies reporting on low fetal fraction in prenatal cfDNA testing and adverse pregnancy outcomes

First author, year	Study description	Study objective	Molecular platform or *method*	LFF cutoff	LFF/cohort (*n*)	Mean BMI in LFF	Mean BMI in sufficient FF	Mean GA (weeks) in LFF	Mean GA (weeks) in sufficient FF
Chan, 2018[32]	Retrospective cohort	To investigate the pregnancy outcomes in women with cfDNA test failure	Harmony or *MPS*	4%	59/6375[a]	30.8[d]	23.3	11.3	11.4
Clapp, 2020[33]	Retrospective cohort	To determine the association between LFF and low birth weight in low-risk pregnancies	*MPS*	<p5 (i.e., 5.34%)	101/2035	29.8[b,d]	23.7[b]	12[b]	12[b]
Gerson, 2019[34]	Retrospective cohort	To investigate the association of LFF in asymptomatic women with adverse perinatal outcomes	Harmony or panorama	<p25 (i.e., 8.4%)	157/639	29	25	13	12
Krishna, 2016[35]	Retrospective cohort	To assess the risk of adverse perinatal outcome for women with LFF in cfDNA testing	*DANSR* or *SNP sequencing*	4%	22/370	36.5[d]	29.1	16.4	17.0
Yuan, 2020[36]	Retrospective cohort	To examine whether LFF of cfDNA is associated with risks of adverse pregnancy outcomes	NextSeq 500	<p25 (i.e., 8.11%)	548/2191	24.2	22.6[c]	17.3[b]	17.5[b,c]

Abbreviations: cfDNA, cell-free DNA; FF, fetal fraction; GA, gestational age; LFF, low fetal fraction; MPS, massive parallel sequencing.

[a]From a cohort of 12,033 cases, fetal fraction was measured in 6375 cases. Numbers are for comparison of total test failure group (n = 131/12,033), including those due to LFF, with a general obstetric population (no significant differences in adverse pregnancy outcomes between LFF group and the group that failed due to technical reasons).

[b]Median reported.

[c]Value for the second and third quartiles of fetal fraction.

[d]Significant difference between women with low and women with sufficient fetal fraction.

TABLE 3 Outcomes of studies reporting on low fetal fraction in prenatal cfDNA testing and adverse pregnancy outcomes

			HDP			SGA			PTB			GDM		
First author, year	LFF cutoff	*Definition of HDP in individual study*	in LFF (%)	in sufficient FF (%)	*p*-Value	in LFF (%)	in sufficient FF (%)	*p*-Value	in LFF (%)	in sufficient FF (%)	*p*-value	in LFF (%)	in sufficient FF (%)	*p*-Value
Chan, 2018[32]	4%	*Preeclampsia: hypertension (not defined) and coexistence of at least one of proteinuria, uteroplacental dysfunction, or maternal organ dysfunction*	9.8	1.5	<0.0001[a]	15	10	0.1[a]	11	8.5	0.3[a]	20	7.5	<0.0001[a]
Clapp, 2020[33]	<p5 (i.e., 5.34%)	*Pregnancy-induced hypertension (not defined)*	2.0	0.7	0.16	6.9[b]	3.2[b]	0.04	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
						11.9	8.0	0.16						
Gerson, 2019[34]	<p25 (i.e., 8.4%)	*All of gestational hypertension, preeclampsia, preeclampsia with severe features, and HELLP syndrome combined (ISSHP classification)*	20	10	<0.001	10	13	0.29	10	9	0.94	n.r.	n.r.	n.r.
Krishna, 2016[35]	4%	*All of gestational hypertension, preeclampsia, HELLP syndrome, and eclampsia combined (not defined)*	59.1	26.4	0.001	20[c]	15.7[c]	0.539	40.9	15.6	0.002	22.7	8.9	0.051
Yuan, 2020[36]	<p25 (i.e., 8.11%)	*Separated reporting for preeclampsia and pregnancy-induced hypertension (ISSHP classification)*	OR 2.16[d]	-	0.009	OR 0.89	-	0.656	OR 1.19	-	0.495	OR 1.13	-	0.339
			OR 1.47[e]	-	0.179	OR 1.21[c]	-	0.558	OR 1.97[f]	-	0.118			
	<p10		OR 2.06[d]	-	0.031	OR 1.56	-	0.159	OR 1.71	-	0.095	OR 1.15	-	0.431
			OR 1.14[e]	-	0.742	OR 1.87[c]	-	0.108	OR 3.09[f]	-	0.018			
	<p5		OR 1.97[d]	-	0.107	OR 1.21	-	0.658	OR 2.02	-	0.073	OR 0.87	-	0.578
			OR 0.64[e]	-	0.472	OR 2.50[c]	-	0.047	OR 2.76[f]	-	0.083			

Note: SGA defined as a birthweight ≤10th percentile unless otherwise reported; PTB defined as delivery before 37 weeks of gestation unless otherwise reported.

Abbreviations: FF, fetal fraction; GDM, gestational diabetes mellitus; HDP, hypertensive disease of pregnancy; HELLP syndrome, hemolysis, elevated liver enzymes, and low platelets; ISSHP, International Society for the Study of Hypertension in Pregnancy; LFF, low fetal fraction; n.r., not reported; OR, odds ratio; PTB, preterm birth; SGA, small for gestational age neonates.

[a]Comparison between LFF and general obstetric population.

[b]Birthweight ≤5th percentile.

[c]Birthweight <2500 g.

[d]Preeclampsia.

[e]Pregnancy-induced hypertension.

[f]Delivery <34 weeks of gestation.

All five studies reported on LFF and HPD and SGA. Four studies reported on PTB and three studies reported on GDM. LFF was associated with HDP in four studies and with SGA in two studies. An association with PTB was reported in two studies. LFF was associated with GDM in one of the three studies in which this was an outcome measure. The outcomes of each study are presented in Table 3.

Chan et al. in 2018 described a group of 131 women with an initial failed test result of which 59 were due to a fetal fraction below 4% and found significantly higher rates of preeclampsia and GDM as compared to the general Australian obstetric population (9.8% vs. 1.5%; $p < 0.0001$ and 20% vs. 7.5%; $p < 0.0001$, respectively).[32] No significant association was found for SGA (birthweight <10th percentile) and PTB (15% vs. 10%; $p = 0.1$ and 11% vs. 8.5%; $p = 0.3$, respectively). Clapp et al. in 2020 described a group of 2035 women who had normal cfDNA testing and delivered a singleton.[33] When defining LFF as less than the 5th percentile (which amounted to a fetal fraction of 5.34% in their population), a birthweight ≤5th percentile was significantly higher in the LFF group compared to the group with a fetal fraction above the 5th percentile (6.9% vs. 3.2%; $p = 0.04$). For a birthweight ≤10th percentile, no significant difference was found (11.9% vs. 8.0%; $p = 0.16$). They did not find an association with pregnancy-induced hypertension, which was a secondary outcome in their study (2.0% vs. 0.7%; $p = 0.16$). In 2019, Gerson et al. reported an association between LFF and placental compromise, which was defined as HDP, fetal growth restriction, placental abruption, or oligohydramnios (29% vs. 17% for normal fetal fraction; $p < 0.001$).[34] Specifically, there was an association with HDP collectively (20% vs. 10%; $p < 0.001$) and preeclampsia specifically (7% vs. 3%; $p = 0.02$). LLF was defined as a fetal fraction less than the 25th percentile, which amounted to a fetal fraction cutoff of 8.4% in this cohort of 639 women. In another retrospective cohort study from 2016, comprising 370 women, Krishna et al. used adverse perinatal outcome (a composite of miscarriage, fetal demise, neonatal death, preterm delivery [<37 weeks], pregnancy-associated hypertensive disorder, placental abruption, and low birth weight) as their primary outcome.[35] Using a cutoff of 4% fetal fraction, they found 59.1% of the composite outcome in women with LFF compared to 29% in the group with sufficient fetal fraction. This association remained after adjusting for BMI and race with an adjusted OR of 2.5 (95% CI 1.01–6.2; $p = 0.049$). HDP and PTB were significantly more frequent (59.1% vs. 26.4%; $p = 0.001$ and 40.9% vs. 15.6%; $p = 0.002$, respectively), but SGA, defined as a birth weight <2500 g, and GDM were not (20% vs. 15.7%; $p = 0.539$ and 22.7% vs. 8.9%; $p = 0.051$, respectively). In 2020, Yuan et al. conducted a historical cohort study of 2191 women with a singleton pregnancy.[36] Outcome measures included PIH, PE, birthweight, GDM, and intrahepatic cholestasis of pregnancy. They described their population based on quartiles of fetal fraction (i.e., the 25th, 50th, and 75th percentiles, representing a fetal fraction of 8.11%, 10%, 61%, and 13.47%, respectively). A higher risk of PE was found for women in the first quartile as compared to women in the second and third quartile (OR 2.16 [1.21–3.86]; $p = 0.009$). In comparing women with a fetal fraction less than the 10th percentile to women with a fetal fraction between the 10th and 90th percentiles, an increased risk of PE (OR 2.06 [1.07–3.98]; $p = 0.031$), and PTB <34 weeks of gestation was found (OR 3.09 [1.21–7.92]; $p = 0.018$). In addition, a fetal fraction less than the 5th percentile was associated with an increased risk of a birthweight <2500 g (OR 2.50 [1.01–6.17]; $p = 0.047$). The results were adjusted for maternal age, gestational age, BMI, gravidity, and parity.

4 | DISCUSSION

We reviewed the available literature on the relationship between LFF in prenatal cfDNA testing and adverse pregnancy outcome. LFF was associated with HDP, SGA neonates, and PTB. Conflicting results were found for an association with GDM.

An association between LFF and HDP was found in four of the five included studies,[32,34–36] with the exception of the study by Clapp et al. in which it was a secondary outcome and a very low prevalence was found in both low and normal fetal fraction.[33] How HDP was defined in their study was not reported.

None of the studies found a significant difference in birthweight <10th percentile between women with LFF and those with normal fetal fraction. However, significant differences were found for the clinically far more relevant birthweight <5th percentile in the study by Clapp et al.[33] and for a birthweight <2500 g in the study by Yuan when using a cutoff for LFF <5th percentile.[36]

Four of the five studies reported on PTB (defined as delivery <37 weeks of gestation) and LFF.[32,34–36] A significant difference between the occurrence of PTB in women with LFF and women with normal fetal fraction was found in the study by Krishna et al., in which 40.9% of women with LFF gave birth prematurely.[35] This number seems rather high. Whether these PTBs included only those that occurred spontaneously or also those that were iatrogenic, was not reported. Yuan et al. reported an increased risk of PTB <34 weeks of gestation when using a cutoff for LFF <10th percentile in their adjusted analysis.[36] No significant difference in the prevalence of PTB between women with LFF and women with normal fetal fraction was found in the two other studies reporting on PTB.[32,34]

GDM was an outcome measure in three studies.[32,35,36] More GDM was seen in women with LFF in all studies, but only significantly so in the study by Chan et al.[32]

From the results of these studies, it can be concluded that LFF in cfDNA testing is associated with adverse pregnancy outcome, specifically with HDP, impaired fetal growth, and PTB. An association with GDM is less evident, as three studies reported on this as a primary outcome but with conflicting results.

The relationship between low levels of fetal cfDNA in early pregnancy and adverse pregnancy outcome might be explained by abnormal placentation and subsequent placental dysfunction. Support for this hypothesis comes from a retrospective cohort study on the risk of developing preeclampsia and fetal growth restriction based on first-trimester markers and fetal fraction in which a negative correlation was found.[18] Interestingly, in third-trimester samples of pregnant women diagnosed with preeclampsia, several studies

have shown significantly higher levels of fetal cfDNA compared to healthy controls and higher levels were found in more severe disease.[37,38] Equally, high levels of fetal cfDNA have also been found to be associated with fetal growth restriction and PTB.[20,37–39] One mechanism that might explain the lower fetal fraction in the first trimester and a higher fetal fraction in the second or third trimester in pregnancies with adverse outcome could be the initial insufficient placentation with a relatively poor placenta–maternal interface in early pregnancy and the subsequent oxidative stress leading to increased trophoblast apoptosis and shedding of syncytiotrophoblast microparticles later in pregnancy.[17] Another mechanism that has been suggested is the reduced clearance of cfDNA from the maternal circulation due to impaired organ function in compromised pregnancies.[40] A longitudinal study measuring fetal fraction throughout gestation in both normal as well as compromised pregnancies and in combination with other, organ-specific biomarkers could help solve these questions.

Altered placenta physiology might also be the reason that LFF is more often encountered in aneuploid pregnancies. Various publications on the performance of prenatal cfDNA testing report high rates of aneuploidy in women with LFF,[14,41,42] some even up to 30%.[43,44]

A limitation of our review is that only five publications studied the relationship between LFF in prenatal cfDNA testing and adverse pregnancy outcome. The relatively small sample sizes of these studies restricts generalization to the pregnant population as a whole. Among the included studies, there was considerable heterogeneity in study design, population, molecular platform used, and definition of pregnancy outcomes. Women were of advanced maternal age (mean > 35 years) in four of the five studies,[32–35] potentially increasing the occurrence of adverse pregnancy outcomes in the study cohorts. Women with pre-gestational conditions, such as chronic hypertension and diabetes, were excluded in only two studies.[34,36] As demographic characteristics were collected variably between studies, important confounders for adverse pregnancy outcome (e.g., smoking, ethnicity, parity, or adverse outcomes in a previous pregnancy) might not have been identified. With regard to the fetal fraction cutoff, Chan et al. and Krishna et al. used a fetal fraction of 4% as a cutoff for LFF,[32,35] whereas Clapp et al. and Gerson et al. used the 5th and 25th percentiles, respectively.[33,34] Yuan et al. reported on various cutoff values.[36] The 25th percentile of fetal fraction in their cohort corresponded to a fetal fraction of 8.11%, not so different from that of Gerson et al., in which the 25th percentile corresponded to 8.4% fetal fraction. Using these higher cutoff values for LFF could possibly have led to higher rates of adverse events than would have been found if cutoffs had been 4% or one below which a reliable aneuploidy screening result would have been obtained. A further matter of concern in interpreting the association between LFF and adverse pregnancy outcome from all five studies was the increased maternal weight in all their cohorts. It has been well established that the no-call rate in cfDNA testing is increased in obese women[10] and that high BMI in itself is a risk factor for adverse pregnancy outcome. Nevertheless, in four of the five studies, BMI was adjusted for and the increased risk for adverse pregnancy outcomes remained.[33–36] So, high BMI and LFF could mean double trouble.

Although for all the included studies in our review, the molecular platform or method by which the cfDNA testing was performed was reported (see Table 2), the bioinformatics tools by which the fetal fraction was measured, was generally not. There are many bioinformatics algorithms for measuring fetal fraction and these are not necessarily directly comparable. For instance, in a publication by Hestand et al., an on-average difference of 2.34% fetal fraction was found in favor of a Y chromosome-based method compared to a sequence read count method to measure fetal fraction.[7] Since Y chromosome-based methods can only be used in male-bearing pregnancies, this is problematic. As fetal fraction measurement is platform dependent, as is the use of a cutoff value for reporting a reliable result, there is a need for a golden standard to determine the fetal fraction. Moreover, as fetal fraction tends to increase with gestational age, using multiple of the median (MoM) values relative to gestational age rather than a fixed cutoff could be preferred to further understand the association between fetal fraction and adverse pregnancy outcome.

Additionally, cfDNA testing techniques should be further developed to accurately detect aneuploidy, despite fetal fraction. For now, if gestational age allows, women confronted with cfDNA test failure due to LFF should be advised to opt for repeat testing from a second blood draw. Factors related to a successful redraw in women with LFF were investigated by White et al. who found a higher probability for test success with a longer interval between blood sampling (+4% per day) and a lower probability for test success with a higher maternal weight (−1.2% per kilogram).[45] Overall, a success rate of 53% on the second draw and a comparable rate on a third draw were found, similar to previous publications on redraw success rates.[46,47] After repeated failure, women should be counseled by their obstetric caregiver and offered invasive testing.

This is the first literature review on the association between LFF in prenatal cfDNA testing and a variety of adverse pregnancy outcomes. It is of importance given the fact that the uptake of cfDNA testing is increasing worldwide and many pregnant women and healthcare professionals will be confronted with a no-call result due to LFF. From the results of our review, we believe that further prospective research is required, ideally with continuous values of fetal fraction. If true continuous values of fetal fraction are to be obtained, it would allow for determining cutoff values for increased risk of adverse pregnancy outcomes. As a single marker for adverse pregnancy outcomes in its own right, however, LFF could have unsatisfactory predictive value, as the majority of such outcomes seem to occur in women with normal fetal fraction. This is not unlike the properties of other first-trimester markers, such as PAPP-A.[48] More likely, adding fetal fraction to existing multivariate risk-stratification models, could help to better identify pregnancies at risk for adverse outcomes at an early gestational age. This would allow for timely interventions and tailored pregnancy care to prevent adverse outcomes, be it by the administration of aspirin or calcium and meticulous monitoring of maternal blood pressure and timely commencement of antihypertensive drugs, strict ultrasound evaluation of fetal growth and cervical length, or dietary and lifestyle modifications and glucose monitoring.

In conclusion, next to being associated with aneuploidy, LFF in cfDNA testing is associated with adverse pregnancy outcome, specifically pregnancy-related hypertensive disorders, PTB, and impaired fetal growth related to placental dysfunction. Since the available evidence is limited, a large prospective cohort study on the relationship between fetal fraction and pregnancy outcomes is needed.

CONFLICT OF INTEREST STATEMENT

Marjan M. Weiss, Caroline J. Bax, Erik A. Sistermans, Lidewij Henneman, and Mireille N. Bekker are all involved in the TRIDENT-2 study (Dutch NIPT Consortium) supported by a grant from the Netherlands Organization for Health Research and Development (ZonMw, No. 543002001).

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Peter G. Scheffer https://orcid.org/0000-0001-9253-5341
Ellis C. Becking https://orcid.org/0000-0003-0418-7880
Lidewij Henneman https://orcid.org/0000-0003-3531-0597
Mireille N. Bekker https://orcid.org/0000-0002-7372-4291

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Scheffer PG, Wirjosoekarto SAM, Becking EC, et al. Association between low fetal fraction in cell-free DNA testing and adverse pregnancy outcome: a systematic review. *Prenat Diagn*. 2021;41(10):1287-1295. doi:10.1002/pd.6028