OBSTETRICS

Fetal fraction of cell-free DNA in noninvasive prenatal testing and adverse pregnancy outcomes: a nationwide retrospective cohort study of 56,110 pregnant women

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BACKGROUND: Noninvasive prenatal testing by cell-free DNA analysis is offered to pregnant women worldwide to screen for fetal aneuploidies. In noninvasive prenatal testing, the fetal fraction of cell-free DNA in the maternal circulation is measured as a quality control parameter. Given that fetal cell-free DNA originates from the placenta, the fetal fraction might also reflect placental health and maternal pregnancy adaptation.

OBJECTIVE: This study aimed to assess the association between the fetal fraction and adverse pregnancy outcomes.

STUDY DESIGN: We performed a retrospective cohort study of women with singleton pregnancies opting for noninvasive prenatal testing between June 2018 and June 2019 within the Dutch nationwide implementation study (Trial by Dutch Laboratories for Evaluation of Non-Invasive Prenatal Testing [TRIDENT]-2). Multivariable logistic regression analysis was used to assess associations between fetal fraction and adverse pregnancy outcomes. Fetal fraction was assessed as a continuous variable and as <10th percentile, corresponding to a fetal fraction <2.5%.

RESULTS: The cohort comprised 56,110 pregnancies. In the analysis of fetal fraction as a continuous variable, a decrease in fetal fraction was associated with increased risk of hypertensive disorders of pregnancy (adjusted odds ratio, 2.27 [95% confidence interval, 1.89—2.78]), small for gestational age neonates <10th percentile (adjusted odds ratio, 1.37 [1.28—1.45]) and <2.3rd percentile (adjusted odds ratio, 2.63 [1.96—3.57]), and spontaneous preterm birth from 24 to 37 weeks of gestation (adjusted odds ratio, 1.02 [1.01—1.03]). No association was found for fetal congenital anomalies (adjusted odds ratio, 1.02 [1.00—1.04]), stillbirth (adjusted odds ratio, 1.02 [0.96—1.08]), or neonatal death (adjusted odds ratio, 1.02 [0.96—1.08]). Similar associations were found for adverse pregnancy outcomes when fetal fraction was <10th percentile.

CONCLUSION: In early pregnancy, a low fetal fraction is associated with increased risk of adverse pregnancy outcomes. These findings can be used to expand the potential of noninvasive prenatal testing in the future, enabling the prediction of pregnancy complications and facilitating tailored pregnancy management through intensified monitoring or preventive measures.

Key words: adverse pregnancy outcomes, cell-free DNA, cell-free DNA screening, cell-free fetal DNA, fetal fraction, gestational diabetes, hypertensive disorders of pregnancy, noninvasive prenatal testing, pregnancy complications, small for gestational age neonates, spontaneous preterm birth

Introduction

The presence of fetal cell-free DNA (cfDNA) in maternal blood allows for noninvasive prenatal testing (NIPT) for Down, Edwards, and Patau syndromes.[1] Since its introduction in clinical practice in 2011, NIPT has rapidly become available to pregnant women worldwide.[2] The accuracy of NIPT depends on a sufficient amount of fetal cfDNA in the maternal plasma. The ratio of fetal cfDNA to total cfDNA, known as the fetal fraction, is estimated in most cfDNA tests as a quality control parameter. It is known to vary because of biological factors such as maternal weight and gestational age (GA), but also depends on the bioinformatic method and molecular platform used for its estimation.[3,4]

Because the fetal cfDNA originates from apoptotic cytotrophoblast placental cells, the fetal fraction potentially reflects placental health and maternal pregnancy adaptation.[4] We hypothesize that impaired placentation with a relatively poor placental—maternal interface in early pregnancy leads to reduced release of fetal cfDNA in the maternal circulation, resulting in lower fetal fraction. To date, only a few studies with relatively small sample sizes have assessed the association between the fetal fraction and adverse pregnancy outcomes. Some of these studies reported an association between low fetal fraction and hypertensive disorders of pregnancy (HDP), small for GA neonates (SGA), spontaneous preterm birth (sPTB), and gestational diabetes mellitus (GDM).[5]

Herein, we assess the association between the fetal fraction in NIPT and adverse pregnancy outcomes in a large nationwide cohort of pregnant women who participated in the Dutch national implementation study on NIPT for fetal aneuploidies (the Trial by Dutch Laboratories for Evaluation of Non-Invasive Prenatal Testing [TRIDENT]-2 study[6]).

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AJOG at a Glance

Why was this study conducted?

Fetal fraction is universally measured as a quality parameter in noninvasive prenatal testing (NIPT) for fetal aneuploidies. Given that fetal cell-free DNA originates from the placenta, the fetal fraction could reflect placental health and maternal pregnancy adaptation. To date, the association between the fetal fraction and pregnancy outcomes has only been assessed in a few small-sized cohort studies, with conflicting results.

Key findings

This nationwide cohort study shows that a low fetal fraction in early pregnancy is associated with adverse pregnancy outcomes, including hypertensive disorders of pregnancy, small for gestational age neonates, and spontaneous preterm birth.

What does this add to what is known?

These findings demonstrate the potential of NIPT in the risk stratification of adverse pregnancy outcomes. This could broaden the scope of NIPT from enabling reproductive choices to also becoming a prevention-aimed test to improve pregnancy outcomes.

Materials and Methods

Study design

In the Netherlands, NIPT was introduced in April 2017 and offered as a first-tier screening test for Down, Edwards, and Patau syndromes to all pregnant women in the Netherlands within a nationwide implementation study, the TRIDENT-2 prospective cohort study, until April 2023.[6] In the current retrospective cohort study, we used data of women who participated in the TRIDENT-2 study between June 1, 2018 and June 1, 2019. During this period, NIPT analysis including fetal fraction measurement was uniformly performed at all 3 assigned NIPT laboratories in the Netherlands (ie, the laboratories of the VU University Medical Center [VUmc] [Amsterdam University Medical Centers], the Maastricht University Medical Center+, and the Erasmus University Medical Center). Within the TRIDENT-2 study, NIPT results and fetal fraction estimates were collected in the Dutch national prenatal screening registry (Peridos) that includes data regarding maternal characteristics, prenatal ultrasound findings, and prenatal testing results.[7] For the current study, the Peridos registry was linked to the Dutch national perinatal registry (Perined) that includes maternal characteristics and pregnancy outcomes of pregnant women in the Netherlands.[8] Linking the Peridos and Perined registries was performed by matching all pregnancies on a pseudonym based on maternal date of birth, postal code, and a 30-day GA range. This link was facilitated by a trusted third party (ZorgTTP, Houten, the Netherlands) to comply with the European General Data Protection Regulation. The structure and coherence of the registries are graphically presented in the Supplemental Figure, and the method and process of linking the registries are explained in Appendix 1.

Inclusion and exclusion criteria

Women with singleton pregnancies who opted for NIPT within the TRIDENT-2 study between June 1, 2018 and June 1, 2019 were eligible for inclusion. Women who had not given consent for use of their data in follow-up research beyond the TRIDENT-2 study were excluded.

Laboratory analysis

Peripheral blood samples were collected in Streck tubes (Streck, Inc., La Vista, NE) from 11 weeks of pregnancy and sent within 4 days to 1 of the participating laboratories. Genome-wide shallow sequencing was performed with the VeriSeq v1 NIPT Solution (Illumina, San Diego, CA), which involves 36-bp paired-end sequencing on a NextSeq 500 System (Illumina) according to the specifications of the supplier. Fetal fraction was estimated with the VeriSeq v1 NIPT Analysis Software (Illumina).

Definition of outcomes

Adverse pregnancy outcomes of interest included HDP, SGA neonates with GA-corrected birthweights <10th percentile (<p10) and <2.3rd percentile (<p2.3), sPTB, diabetes, congenital anomalies, stillbirth, and neonatal death. HDP comprised pregnancy-induced hypertension (PIH), preeclampsia (PE), and/or HELLP syndrome. PIH, PE, and HELLP syndrome were defined according to the International Society for the Study of Hypertension in Pregnancy classification.[9] SGA <p10 and <p2.3 was determined by the Hoftiezer birthweight charts.[10] sPTB was defined as a spontaneous birth between 24 and 37 weeks of GA and subdivided into moderate to late (32−37 weeks), very preterm (28−32 weeks), and extremely preterm birth (24−28 weeks).[11] Because of the nature of the Perined registry, diabetes included both GDM (determined by a 75-g 2-hour oral glucose tolerance test between 24 and 28 weeks of GA[12]) and preexisting diabetes mellitus. Congenital anomalies were classified as major anomalies according to the guidelines of EUROCAT (European network of population-based registries for the epidemiological surveillance of congenital anomalies).[13] Stillbirth was defined as an intrauterine fetal demise occurring after 24 weeks of GA. Neonatal death was defined as death occurring from the 1st until the 28th day postnatally.

Statistical analysis

To assess if missing data in the database were missing completely at random, we compared characteristics between pregnant women without missing data and pregnant women with missing data in at least 1 variable (Supplemental Table 1). Because statistically significant differences were found across multiple characteristics, we concluded that data were not missing completely at random and were

therefore assumed to be missing at random. Given that a complete case analysis can result in imprecision and bias in association estimates in the presence of missing data that are missing at random, missing data in the data set were imputed using multiple imputation by chained equations.[14] All outcomes and explanatory variables were used in the imputation model and 20 imputations were performed. The amount of missing data for each variable, including the baseline characteristics of the study cohort before and after multiple imputation, is provided in Supplemental Table 2. Descriptive analyses were performed to describe the study cohort. The association between the fetal fraction and the outcomes was assessed by univariable logistic regression analysis, resulting in an unadjusted odds ratio (OR). Multivariable logistic regression analysis was performed to correct for possible confounding (adjusted OR). Relevant confounders were selected from previous literature and clinical practice and were included in the multivariable model. Associations between all continuous covariables and all outcomes were assessed graphically by spline plots for potential nonlinearity, and if needed, continuous covariables were transformed. An overview of the confounders, transformations, and exclusions used in the multivariable analysis by outcome is provided in Supplemental Table 3. Fetal fraction was primarily analyzed as a continuous variable to assess whether an association between fetal fraction and adverse pregnancy outcomes was present. In addition, a low fetal fraction cutoff <p10 was used, corresponding to a fetal fraction of <2.5%. Analyses were performed in each imputed data set separately and results were pooled using Rubin's rules.[15] Statistical analyses were performed in R, version 4.2.1 (R Core Team, Vienna, Austria).

Ethical approval

The TRIDENT-2 study was approved by the Dutch Ministry of Health, Welfare and Sport (license 1017420-153371-PG) and the Medical Ethical Committee of VUmc (No.2017.165). The Medical Ethical Committee VUmc declared that the Medical Research Involving Human Subjects Act (WMO) did not apply to the present study (No.2020.10).

Results

The flowchart of the study population is displayed in Figure 1. The Peridos registry contained 77,478 records of all women with singleton pregnancies who opted for NIPT from June 1, 2018 to June 1, 2019. After exclusion of pregnancies of women who had not given consent for the use of their data in follow-up research beyond the TRIDENT-2 study (n=5965), 71,513 pregnancies were eligible to be linked to the Perined registry. For 15,403 pregnancies, no match with the Perined registry could be accomplished. This resulted in a final linked database of 56,110 women with singleton pregnancies. This was 72.4% (56,110/77,478) of the total cohort of pregnant women with singleton pregnancies who opted for NIPT within the study period.

The baseline characteristics of the study cohort (after imputation) are presented in Table 1. The distribution of the fetal fraction estimates is displayed in Figure 2. On first blood sampling, 1028 of 56,110 participants (1.8%) did not receive a result because of test failure. Test failure was caused by a too low fetal fraction in 782 of 56,110 participants (1.4%). On second blood sampling, no test result was issued for the remaining 287 participants. Of these 287 test failures, 225 were caused by a too low fetal fraction. Supplemental Table 4 shows the comparison of baseline characteristics between the study cohort and the total population of women opting for NIPT. Baseline characteristics of the study cohort were comparable to those of the total NIPT population during the study period.[7] Compared with the general Dutch obstetrical population, women in the study population were more often nulliparous and of White ethnicity.[8]

Association between fetal fraction and adverse pregnancy outcomes

Table 2 shows the unadjusted and confounder-adjusted ORs of the analysis of fetal fraction as a continuous variable. The values of the ORs are not comparable between outcomes in this analysis because for some outcomes (HDP and SGA), a transformation was used for fetal fraction. An overview of the possible transformation used for fetal fraction by outcome is provided in Supplemental Table 3. The unadjusted and confounder-adjusted ORs for fetal fraction <p10 are shown in Table 3. The adjusted OR is displayed graphically in Figure 3. Results of the adjusted analyses for each individual adverse pregnancy outcome are reported below.

Hypertensive disorders of pregnancy

A decrease in fetal fraction was associated with a higher risk of HDP (OR, 2.27 [95% confidence interval, 1.89−2.78]). A higher risk of HDP was also found when fetal fraction was <p10 (OR, 1.30 [1.14−1.49]).

Small for gestational age neonates <p10 and <p2.3

A decrease in fetal fraction was associated with a higher risk of SGA <p10 (OR, 1.37 [1.28−1.45]) and <p2.3 (OR, 2.63 [1.96−3.57]). The risks of both SGA <p10 (OR, 1.49 [1.33−1.68]) and SGA <p2.3 (OR, 1.75 [1.42−2.16]) were higher when fetal fraction was <p10.

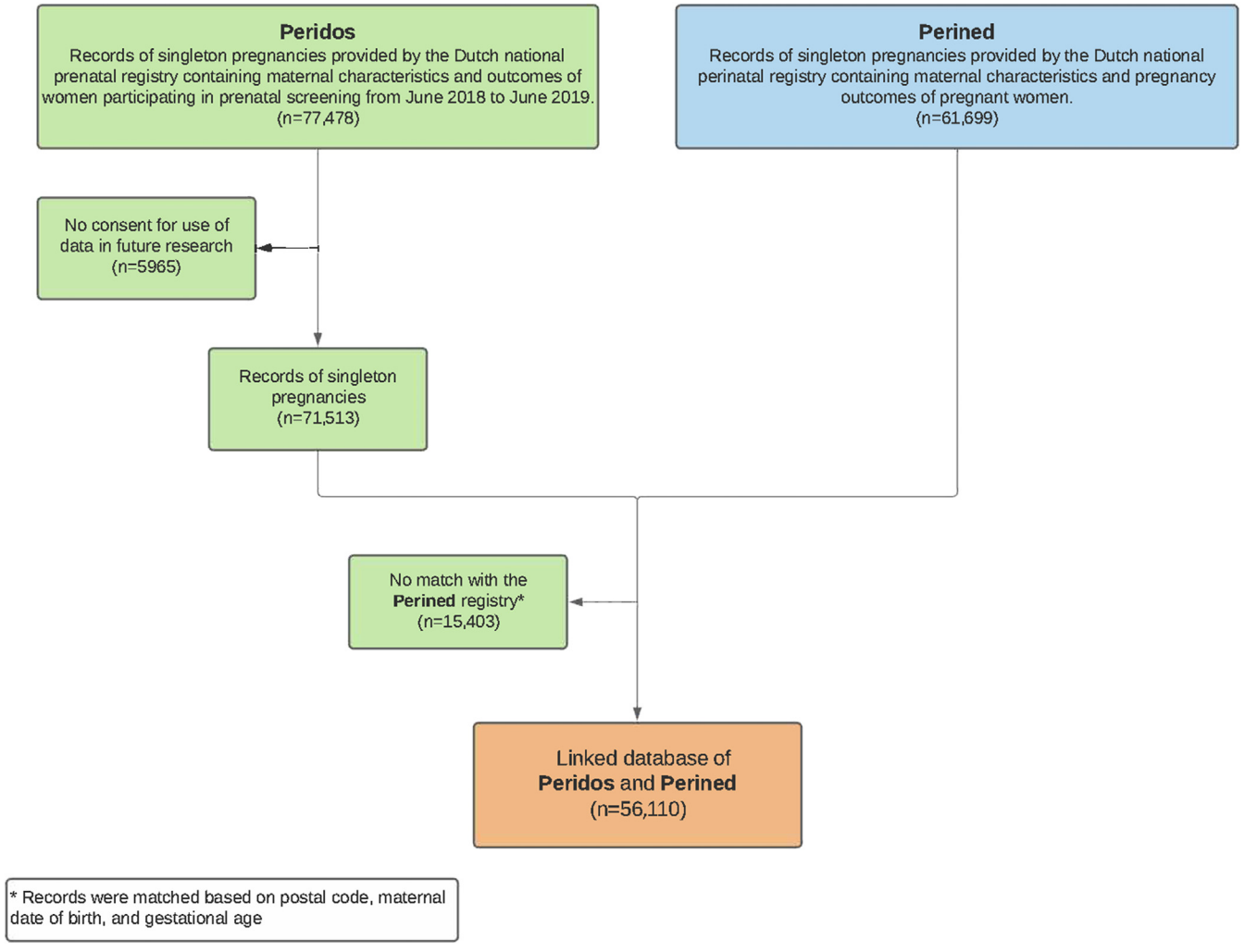
Spontaneous preterm birth

A decrease in fetal fraction was associated with a higher risk of all sPTB (OR, 1.02 [1.01−1.03]) and moderate to late sPTB (OR, 1.02 [1.01−1.04]) specifically, but no association was found for spontaneous very preterm birth (OR, 1.02 [0.97−1.06]) or spontaneous extremely preterm birth (OR, 0.98 [0.93−1.05]). Higher risks of all sPTB (OR, 1.25 [1.04−1.50]) and moderate to late sPTB (OR, 1.25 [1.02−1.51]) and spontaneous very preterm birth (OR, 1.89 [1.08−3.32]) specifically, were found for a fetal fraction <p10. No association was found for spontaneous extremely preterm birth when fetal fraction was <p10 (OR, 0.45 [0.10−2.07]).

Diabetes (both preexisting and gestational)

A decrease in fetal fraction was associated with a higher risk of diabetes (OR, 1.03 [1.02−1.04]). No higher risk of

FIGURE 1
Flowchart of the study population



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diabetes was found when fetal fraction was <p10 (OR, 1.05 [0.89−1.25]).

Congenital anomalies

Fetal fraction was not associated with congenital anomalies in the analysis of the fetal fraction as a continuous variable (OR, 1.02 [1.00−1.04]) or when fetal fraction was <p10 (OR, 1.27 [0.96−1.68]).

Stillbirth and neonatal death

Fetal fraction was not associated with stillbirth or neonatal death in the analysis of fetal fraction as a continuous variable (OR, 1.02 [0.96−1.08] and OR, 1.02 [0.96−1.08], respectively). A higher risk of neonatal death was found when fetal fraction was <p10 (OR, 2.07 [1.01−4.21]). No association was found between fetal fraction <p10 and stillbirth (OR, 1.37 [0.64−2.93]).

Comment

Principal findings

This nationwide retrospective cohort study of 56,110 women opting for NIPT in the Netherlands shows that pregnant women with a lower fetal fraction in early pregnancy are at increased risk of adverse pregnancy outcomes. The introduction of NIPT to screen for fetal aneuploidies in clinical practice in 2011 has rapidly revolutionized the field of prenatal screening. NIPT is now available to pregnant women worldwide, with a global market value that soared to $4.11 billion in 2021 and is expected to grow to $13.2 billion in 2028.[2,16] Our findings show not only that NIPT reveals the fetal chromosomal constitution, but also that the fetal fraction in NIPT is related to placental health and consequently to both maternal pregnancy adaptation and fetal development and maturation. This implies that NIPT has the potential to be used in obstetrical care in the prediction and monitoring of placenta-related adverse pregnancy outcomes in addition to screening for fetal aneuploidies.

Results in the context of what is known

In uncomplicated pregnancies, the placenta remodels the maternal uterine vasculature by the invasion of placental trophoblast cells into the uterine wall and maternal spiral arteries, transforming them into low-resistance, high-flow vessels that facilitate efficient nutrient and oxygen exchange at the maternal–fetal interface.[17] Abnormal placental development, which is characterized by impaired trophoblast invasion and failed spiral artery transformation in the first trimester and subsequent maternal vascular malperfusion and placental dysfunction, generally leads to adverse pregnancy outcomes.[17] Given that fetal cfDNA originates from cytotrophoblast cells of placental chorionic villi undergoing apoptosis, the release of fetal cfDNA could be closely related to placental function.[4] Our findings suggest that in the case of early abnormal placental development with a disturbed maternal-fetal interface, less fetal cfDNA is released in the maternal circulation, resulting in a lower fetal fraction. Conversely, in third-trimester samples of pregnant women diagnosed with PE, considerably higher levels of fetal cfDNA have been reported.[18] Intriguingly, fetal cells have also been found to be increased in cases of PE.[19,20] This might be explained by placental lesions in early pregnancy, which facilitate increased passage of fetal cells into the maternal circulation. Whether these cells, or even fetal cfDNA, cause a maternal inflammatory response and play a causal role in the manifestation of PE remains to be elucidated.

Some previous small retrospective cohort studies have already suggested an association between lower fetal fraction in early pregnancy and adverse pregnancy outcomes.[21–28] In these studies, the association with HDP has been described most consistently.[21–27] The findings of our study confirm this association. Lower fetal fraction was associated with SGA <p10 and <p2.3 in all analyses performed in this study. This is in line with the results of a previous

TABLE 1
Characteristics of the study cohort

Characteristics	Study cohort (n=56,110) Median (IQR) or n (%)
Baseline characteristics	
Maternal age (y)	31 (29–34)
Maternal BMI (kg/m^2)	23.2 (21.2–26.1)
Gestational age at NIPT blood draw (wk^{+d})	12^{+0} (11^{+4}–12^{+5})
Fetal fraction (%)	8 (6–11)
Ethnicity	
White	52,175 (93.0%)
Other	3935 (7.0%)
Method of conception	
Spontaneous	54,733 (97.5%)
Assisted (IVF/ICSI)	1377 (2.5%)
Smoking	
Yes	2523 (4.5%)
No	53,587 (95.5%)
Parity	
Nulliparous	29,044 (51.8%)
Para 1	20,223 (36.0%)
Para ≥2	6843 (12.2%)
Obstetrical history	
Previous preeclampsia[a]	160/27,066 (0.6%)
Previous preterm birth[a]	785/27,066 (2.9%)
Previous small for gestational age[a]	410/27,066 (1.5%)
Previous miscarriage/abortion	349 (0.6%)
Pregnancy outcomes	
Gestational age at delivery (wk)	39^{+5} (38^{+5}–40^{+5})
Mode of delivery	
Vaginal delivery	42,505 (75.7%)
Assisted vaginal delivery (vacuum/forceps)	4707 (8.4%)
Elective cesarean delivery	4148 (7.4%)
Emergency cesarean delivery	4750 (8.5%)
Birthweight (g)	3460 (3120–3785)
Hypertensive disorders of pregnancy	3207 (5.7%)
SGA, birthweight <p10	5726 (10.2%)
SGA, birthweight <p2.3	1796 (3.2%)
All sPTB (24–37 wk of GA)	1891 (3.4%)
Moderate to late sPTB (32–37 wk)	1675 (3.0%)
Spontaneous very PTB (28–32 wk)	140 (0.3%)
Spontaneous extremely PTB (24–28 wk)	76 (0.1%)

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(continued)

TABLE 1
Characteristics of the study cohort *(continued)*

Characteristics	Study cohort (n=56,110) Median (IQR) or n (%)
Diabetes[b]	1902 (3.4%)
Congenital anomalies[c]	741 (1.3%)
Stillbirth	88 (0.2%)
Neonatal death	82 (0.2%)

BMI, body mass index; *GA*, gestational age; *ICSI*, intracytoplasmic sperm injection; *IQR*, interquartile range; *IVF*, in vitro fertilization; *NIPT*, noninvasive prenatal testing; *<p2.3*, <2.3rd percentile; *<p10*, <10th percentile; *PTB*, preterm birth; *SGA*, small for gestational age neonates; *sPTB*, spontaneous preterm birth.

[a] Data of multiparous women only (n=27,066); [b] Composed of both preexisting diabetes mellitus and gestational diabetes mellitus; [c] Excluding pregnancies with confirmed Down, Edwards, or Patau syndrome.

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cohort study that reported higher rates of SGA below the fifth birthweight percentile when fetal fraction was below the fifth percentile (6.9% vs 3.2%; P=.04).[28] Other cohort studies that assessed this association did not report an association with SGA.[21−24,27] This is opposed to what would be expected according to our hypothesis of disturbed early placentation, and may be caused by the small sample size of these studies. The definition of SGA in our study was inevitably based on GA-corrected birthweights because fetal biometry ultrasound measurements were unavailable, and likely comprises both physiologically small fetuses and growth-restricted fetuses. Assessing fetal growth restriction specifically would be of high interest in future studies given its association with abnormal placentation and placental dysfunction.

In our analyses, we found an association with all sPTB (24−37 weeks) and specifically with moderate to late sPTB (32−37 weeks). This might be explained by the small sample size in these subgroups. Another possible explanation for the absence of a consistent association is that sPTB is also known to be caused by non−placenta-related biological mechanisms, such as cervical insufficiency, infection, or inflammation.[29] Although an association between low fetal fraction and preterm birth has been reported in 2 previous retrospective cohort studies, no distinction was made between spontaneous and medically indicated birth in these studies.[23,24] Studies that did specifically assess sPTB did not report an association.[21,22,27] Given the conflicting evidence, this association requires further research.

Our study showed that lower fetal fraction is associated with increased risk of diabetes in the analysis of fetal fraction as a continuous variable. Whether the same mechanism of impaired placentation, either by hyperglycemic status or other factors, can explain this association remains to be elucidated.[30] Unfortunately, because of the nature of the perinatal registration, we were not able to distinguish preexisting diabetes from GDM. Three previous studies reported significantly higher rates of GDM in women with low fetal fraction,[21−23] but results were not corrected for body mass index (BMI), which is one of the most important confounders in the etiology of diabetes. Other studies that did correct for confounders (including BMI) did not find an association for low fetal fraction.[24−26] Our results indicate that a decrease in fetal fraction does yield an additional risk for diabetes independent of BMI, but further research is needed on the association with GDM specifically.

Lower fetal fraction was not related to a significantly higher risk of congenital anomalies after confounder correction. Although the association between low fetal fraction and fetal aneuploidy has been described previously,[31] only 2 previous studies reported on fetal fraction and congenital anomalies unrelated to fetal aneuploidy, and with conflicting results.[27,32] In a cohort of women with a failed cfDNA test due to low fetal

FIGURE 2
Distribution of fetal fraction estimates in the study cohort

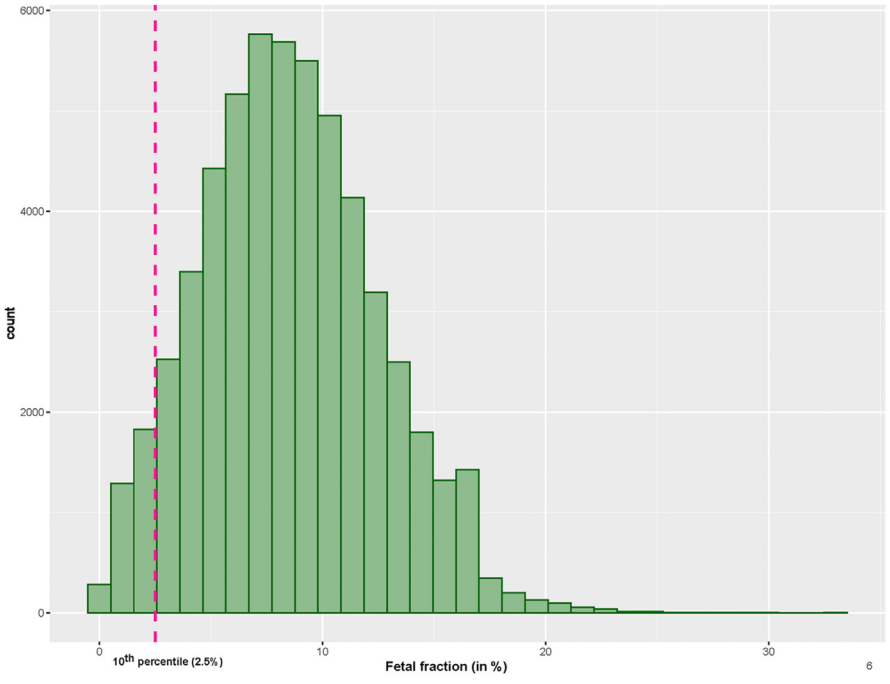

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TABLE 2
Association between fetal fraction and adverse pregnancy outcomes

Adverse pregnancy outcome (n=54,711)	Outcome/total (%)	Unadjusted OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Hypertensive disorders of pregnancy	3186/54,711 (5.8)	4.55 (3.85–5.26)	<.0001	2.27 (1.89–2.78)	<.0001
SGA, birthweight <p10	4784/54,711 (8.7)	1.25 (1.18–1.33)	<.0001	1.37 (1.28–1.45)	<.0001
SGA, birthweight <p2.3	1104/54,711 (2.0)	2.56 (1.92–3.45)	<.0001	2.63 (1.96–3.57)	<.0001
All sPTB (24–37 wk of GA)	1891/54,711 (3.5)	1.02 (1.01–1.04)	.00013	1.02 (1.01–1.03)	.0014
Moderate to late sPTB (32–37 wk)[a]	1675/54,345 (3.1)	1.03 (1.01–1.04)	<.0001	1.02 (1.01–1.04)	.00087
Spontaneous very PTB (28–32 wk)[a]	140/52,275 (0.3)	1.02 (0.97–1.06)	.39	1.02 (0.97–1.06)	.47
Spontaneous extremely PTB (24–28 wk)[a]	76/52,139 (0.1)	0.99 (0.93–1.05)	.78	0.98 (0.93–1.05)	.63
Diabetes[b]	1890/54,711 (3.5)	1.09 (1.08–1.10)	<.0001	1.03 (1.02–1.04)	<.0001
Congenital anomalies[c]	741/55,956 (1.3)	1.03 (1.01–1.05)	.0059	1.02 (1.00–1.04)	.13
Stillbirth	88/54,711 (0.2)	1.05 (0.98–1.11)	.090	1.02 (0.96–1.08)	.44
Neonatal death	82/54,711 (0.2)	1.01 (0.98–1.11)	.17	1.02 (0.96–1.08)	.51

Shown are the unadjusted OR and the confounder-adjusted OR. An OR of >1 indicates that lower fetal fraction is associated with higher odds of developing the outcome and vice versa. The values of the OR are not comparable between outcomes because for some outcomes, a transformation was used for fetal fraction. An overview of the variables used in the multivariable analysis and the transformation used for fetal fraction per outcome is provided in Supplemental Table 2. Only pregnancies with a GA at delivery ≥24 weeks were analyzed.

CI, confidence interval; *GA*, gestational age; *OR*, odds ratio; *<p2.3*, <2.3rd percentile; *<p10*, <10th percentile; *PTB*, preterm birth; *SGA*, small for gestational age neonates; *sPTB*, spontaneous preterm birth.

[a] Pregnancies with a sPTB within this GA range were compared with term pregnancies (GA ≥37 weeks); pregnancies outside of this range were excluded for this analysis; [b] Composed of both preexisting diabetes mellitus and gestational diabetes mellitus; [c]Excluding all pregnancies with confirmed Down, Edwards, or Patau syndrome.

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fraction, the prevalence of fetal congenital anomalies was reported to be higher compared with that of the general obstetrical population.[27] In contrast, Bardi et al[32] found that structural anomalies were not more prevalent in women who had an inconclusive NIPT result due to low fetal fraction twice compared with a general obstetrical population. However, both of these studies did not correct for confounders.

Fetal fraction <p10 was associated with increased risk of neonatal death, but no association was found in the analysis of fetal fraction as a continuous variable. For stillbirth, no association with fetal fraction was found. Given the low incidence of both outcomes, our study may have been underpowered to detect a consistent association. Whether the same mechanism of low fetal fraction and placental dysfunction would explain this association remains to be elucidated.

Clinical implications

Our study shows that the fetal fraction in NIPT is a potential biomarker of placental health. This implies that NIPT could be used in the risk stratification of adverse pregnancy outcomes. Early identification of pregnant women at risk for these outcomes is essential, allowing for timely preventive measures or intensified monitoring. For instance, the administration of aspirin starting at ≤16 weeks of pregnancy reduces the incidence of PE and fetal growth restriction in high-risk women and is available at low cost and low complication rate.[33] Women at risk for preterm birth could receive progesterone as a prevention strategy or be monitored more frequently by cervical length measurements through ultrasound.[11] In some health care settings, at-home telemonitoring could be used to effectively monitor blood pressure or other parameters of pregnant women at risk for adverse pregnancy outcomes.[34]

Research implications

This study aimed to assess the association between fetal fraction and adverse pregnancy outcomes. Having identified this association, further study into the clinical utility of fetal fraction as a screening parameter would be interesting. Whether fetal fraction provides additional prognostic value beyond the currently used prediction models needs to be established in further research. It should be noted that fetal fraction is currently only being measured for the purpose of prenatal screening for chromosomal aberrations and not as an obstetrical parameter. Ethical implications of broadening the scope of NIPT from enabling reproductive choices to also becoming a prevention-aimed screening test should also be explored. A "double-purpose" screening may lead to difficulties in counseling pregnant women. Conditions for responsibly offering such screening, including the perspectives of obstetrical professionals and pregnant women, should be examined.

Strengths and limitations

The Netherlands is one of the few countries worldwide that introduced NIPT in prenatal care within a government-supported national implementation study (TRIDENT-2).[6] A major strength of our study is that we were

TABLE 3
Association between a fetal fraction <10th percentile and adverse pregnancy outcomes

Adverse pregnancy outcome (n=54,711)	Fetal fraction <p10 outcome/total (%)	Fetal fraction ≥p10 outcome/total (%)	Unadjusted OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Hypertensive disorders of pregnancy	325/3298 (9.9)	2861/51,413 (5.6)	1.85 (1.64–2.10)	<.0001	1.30 (1.14–1.49)	<.0001
SGA, birthweight <p10	379/3298 (11.5)	4405/51,413 (8.5)	1.39 (1.24–1.55)	<.0001	1.49 (1.33–1.68)	<.0001
SGA, birthweight <p2.3	112/3298 (3.4)	992/51,413 (1.9)	1.78 (1.45–2.18)	<.0001	1.75 (1.42–2.16)	<.0001
All sPTB (24–37 wk of GA)	144/3298 (4.4)	1747/51,413 (3.4)	1.30 (1.09–1.55)	.0037	1.25 (1.04–1.50)	.016
Moderate to late sPTB (32–37 wk)[a]	127/3260 (3.9)	1548/51,085 (3.0)	1.29 (1.07–1.56)	.0079	1.25 (1.02–1.51)	.027
Spontaneous very PTB (28–32 wk)[a]	15/3100 (0.5)	125/49,175 (0.3)	1.93 (1.12–3.32)	.020	1.89 (1.08–3.32)	.026
Spontaneous extremely PTB (24–28 wk)[a]	2/3077 (0.06)	74/49,062 (0.15)	0.48 (0.11–2.22)	.35	.45 (.10–2.07)	.31
Diabetes[b]	186/3298 (5.6)	1704/51,413 (3.3)	1.74 (1.49–2.04)	<.0001	1.05 (.89–1.25)	.56
Congenital anomalies[c]	62/3398 (1.8)	679/52,558 (1.3)	1.42 (1.08–1.86)	.011	1.27 (.96–1.68)	.089
Stillbirth	9/3298 (0.3)	79/51,413 (0.2)	1.80 (0.85–3.67)	.12	1.37 (.64–2.93)	.41
Neonatal death	11/3298 (0.3)	71/51,413 (0.1)	2.24 (1.22–4.79)	.011	2.07 (1.01–4.21)	.046

Shown are the unadjusted OR and the confounder-adjusted OR. An OR of >1 indicates that a fetal fraction <p10 is associated with higher odds of developing the outcome. An overview of the variables used in the multivariable analysis per outcome is provided in Supplemental Table 1. Only pregnancies with a GA at delivery ≥24 weeks were analyzed.

CI, confidence interval; *GA*, gestational age; *OR*, odds ratio; *<p2.3*, <2.3rd percentile; *<p10*, <10th percentile; *≥p10*, ≥10th percentile; *PTB*, preterm birth; *SGA*, small for gestational age neonates; *sPTB*, spontaneous preterm birth.

[a] Pregnancies with a sPTB within this GA range were compared with term pregnancies (GA ≥37 weeks); pregnancies outside of this range were excluded for this analysis; [b] Composed of both preexisting diabetes mellitus and gestational diabetes mellitus; [c]Excluding all pregnancies with confirmed Down, Edwards, or Patau syndrome.

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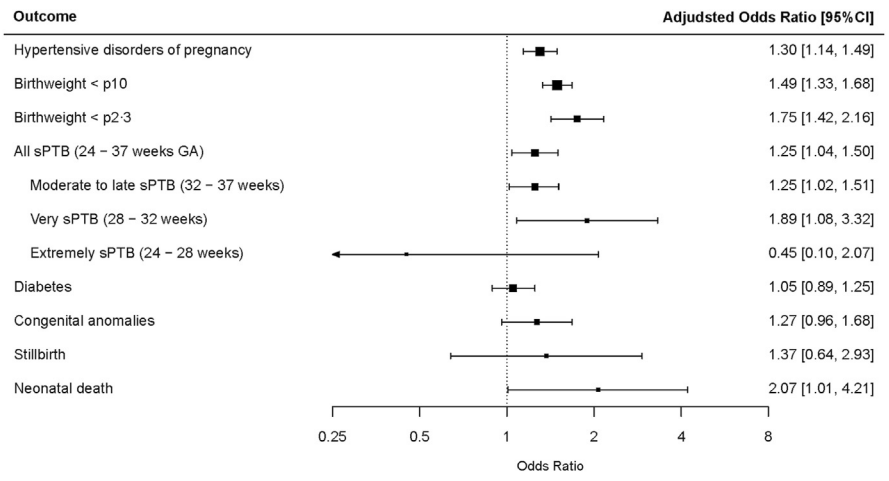
able to use data of this unique and large nationwide cohort of pregnant women opting for NIPT within a 1-year time period, and to link these data to the Dutch national perinatal registry of pregnancy outcomes. During the study period, fetal fraction was universally measured by the same whole genome sequencing–based methodology in all 3 assigned NIPT laboratories. The association between the fetal fraction and adverse pregnancy outcomes was assessed on a large national scale with adjustments for relevant confounders, and multiple imputation was used to address missing data.

A limitation of our study was that because of the use of a national perinatal registry, the quality of data registration depended on individual health care professionals, and some outcomes, including diabetes, were inevitably clustered. Our study cohort was representative of the total population of pregnant women opting for NIPT in the Netherlands during the study period (uptake of 44% of all pregnant women). Nevertheless, women opting for NIPT were more often nulliparous and of White ethnicity compared with the general Dutch obstetrical population (Supplemental Table 3). The incidence of adverse pregnancy outcomes in our cohort was also slightly lower compared with that of the general Dutch obstetrical population,[8] which means that associations found in this study may be different, or could even be more profound, in the general Dutch obstetrical population.

Conclusions

Pregnant women with a lower fetal fraction in early pregnancy are at increased risk of adverse pregnancy outcomes, specifically HDP, SGA <p10 and <p2.3, and sPTB. This implies that the traditional scope of NIPT as a screening tool to increase reproductive autonomy could be broadened, and that NIPT could additionally become a prevention-aimed test that provides pregnant women worldwide with the opportunity to improve their pregnancy outcome.

FIGURE 3
Fetal fraction <10th percentile and adverse pregnancy outcomes



CI, confidence interval; *GA*, gestational age; *<p2.3*, <2.3rd percentile; *<p10*, <10th percentile; *sPTB*, spontaneous preterm birth.

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Appendix

Appendix 1

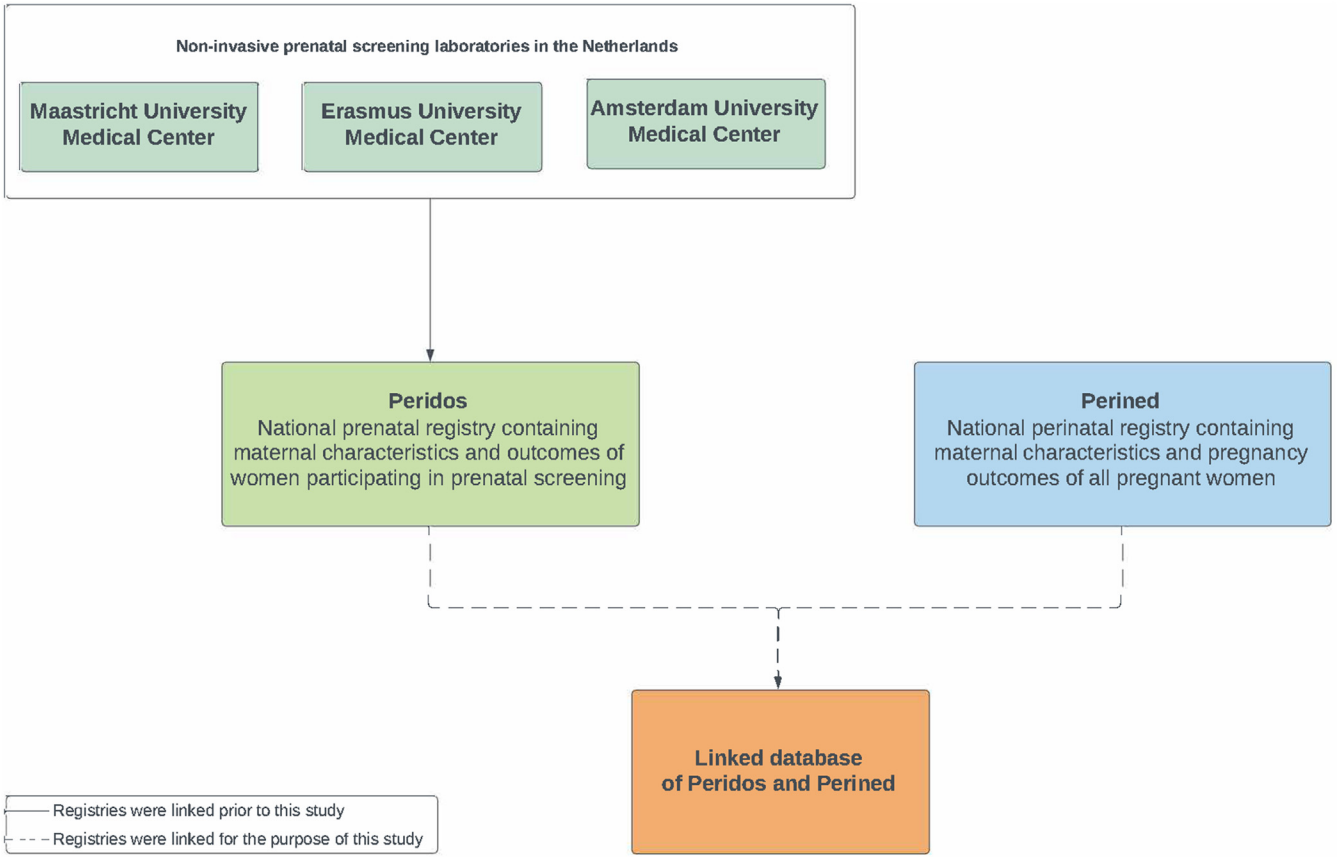
Method and process of linking national registries

Linking the Peridos and Perined registries was performed by matching pregnancies on a pseudonym based on maternal date of birth, postal code, and a 30-day gestational age range. A gestational age range was chosen because the exact registration of the gestational age could have differed slightly between registries while it concerned the same pregnancy. The pseudonymization process was carried out by a trusted third party specialized in secure transfers of personal data.

The Peridos registry contained 77,478 records of all women with singleton pregnancies who opted for noninvasive prenatal testing from June 1, 2018 to June 1, 2019. After exclusion of pregnancies of women who had not given consent for the use of their data in follow-up research beyond the TRIDENT-2 study (n=5965) and the removal of duplicate records within the Peridos registry (ie, records with identical pseudonyms; n=920), 70,593 pregnancies were eligible to be linked to the Perined registry. The Perined registry provided information of 61,699 singleton pregnancies with a possible match to the Peridos registry. After removal of duplicate records within the Perined registry (n=1241), records of 60,458 pregnancies could be linked to the Peridos registry, resulting in a match for 55,624 pregnancies. An additional step was taken by linking the duplicate records based on additional information by use of the exact gestational age (n=486). This resulted in a final linked database of 56,110 women with singleton pregnancies.

SUPPLEMENTAL FIGURE

Structure and coherence of national registries and the linked database



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SUPPLEMENTAL TABLE 1

Characteristics of pregnant women without missing data and pregnant women with missing data in ≥1 variable

Characteristics	Pregnant women without missing data	Pregnant women with missing data	*P* value
n	34,873	21,237	
Fetal fraction, mean (SD)	8.35 (3.86)	8.43 (3.90)	.028
Gestational age, mean (SD)	277.11 (11.36)	272.68 (26.26)	<.001
Gravidity, mean (SD)	2.03 (1.19)	2.04 (1.23)	.334
Parity, mean (SD)	0.64 (0.78)	0.62 (0.78)	.001
Maternal length, mean (SD)	169.48 (6.67)	169.49 (6.90)	.881
Maternal weight, mean (SD)	69.30 (13.09)	69.85 (13.88)	<.001
Maternal age, mean (SD)	31.53 (4.07)	31.79 (4.22)	<.001
Socioeconomic status score, mean (SD)	0.08 (1.14)	0.05 (1.18)	<.001
Previous abortion/miscarriage (%)	143 (0.4)	206 (1.0)	<.001
Previous hypertensive disorder of pregnancy (%)	97 (0.3)	63 (0.3)	.751
Previous preterm birth (%)	407 (1.2)	378 (1.8)	<.001
Previous SGA (%)	253 (0.7)	157 (0.7)	.893
Level of urbanization (%)			<.001
>2500 inhabitants/m^2	16,578 (47.5)	10,736 (51.1)	—
1500–2500 inhabitants/m^2	3542 (10.2)	1921 (9.1)	—
1000–1500 inhabitants/m^2	2561 (7.3)	1421 (6.8)	—
500–1000 inhabitants/m^2	4345 (12.5)	2771 (13.2)	—
<500 inhabitants/m^2	7847 (22.5)	4146 (19.7)	—
Method of conception=IVF/ICSI (%)	1051 (3.0)	326 (1.5)	<.001
Ethnicity=White (%)	32,789 (94.0)	18,693 (91.3)	<.001
Smoking=no (%)	33,370 (95.7)	4479 (94.8)	.005
Deprived area of living=yes (%)	3266 (9.4)	2375 (11.3)	<.001
Diabetes=yes (%)	1243 (3.6)	659 (3.1)	.004
Hypertensive disorders of pregnancy (%)	2035 (5.8)	1049 (4.9)	<.001
Preeclampsia/HELLP (%)	133 (0.4)	65 (0.3)	.166
Hoftiezer percentile, mean (SD)	50.96 (28.61)	50.55 (29.05)	.103
Start of birth (%)			<.001
Spontaneous	24,693 (70.8)	12,604 (67.2)	—
Induced: amniotomy	2674 (7.7)	1590 (8.5)	—
Induced: prostaglandins	786 (2.3)	753 (4.0)	—
Induced: oxytocin	558 (1.6)	433 (2.3)	—
Induced: prostaglandins+oxytocin	48 (0.1)	38 (0.2)	—
Primary cesarean delivery	2602 (7.5)	1434 (7.6)	—
Foley catheter	3512 (10.1)	1894 (10.1)	—
Congenital anomaly=yes (%)	398 (1.1)	441 (2.1)	<.001
Neonatal mortality (%)			<.001
Alive	34,828 (99.9)	19,700 (92.8)	—
Death before birth	0 (0.0)	124 (0.6)	—
Not viable	0 (0.0)	1262 (5.9)	—

Becking. Fetal fraction in noninvasive prenatal testing and adverse pregnancy outcomes. Am J Obstet Gynecol 2023. *(continued)*

SUPPLEMENTAL TABLE 1

Characteristics of pregnant women without missing data and pregnant women with missing data in ≥1 variable

(continued)

Characteristics	Pregnant women without missing data	Pregnant women with missing data	*P* value
Death through birth	0 (0.0)	64 (0.3)	—
Death at <24 h after birth	20 (0.1)	57 (0.3)	—
Death at 2nd–7th d after birth	12 (0.0)	18 (0.1)	—
Death at 8th–28th d after birth	7 (0.0)	8 (0.0)	—
Death at >28 d after birth	6 (0.0)	4 (0.0)	—
missing=TRUE (%)	0 (0.0)	21,237 (100.0)	<.001

ICSI, intracytoplasmic sperm injection; *IVF*, in vitro fertilization; *NIPT*, noninvasive prenatal testing; *SGA*, small for gestational age neonates.

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SUPPLEMENTAL TABLE 2

Characteristics of the study cohort before and after imputation and amount of missing data

Characteristics	Study cohort after imputation Median (IQR) or n (%)	Study cohort before imputation Median (IQR) or n (%)	Amount of missing data n (% of total cohort of 56,110)
Baseline characteristics	(n=56,110)		
Maternal age (y)	31 (29–34)	31 (29–34)	0 (0%)
Maternal BMI (kg/m^2)	23.2 (21.2–26.1)	23.2 (21.2–26.1)	17 (0.03%)
Gestational age at NIPT blood draw (wk$^+$ ^{d})	12^{+0} (11^{+4}–12^{+5})	12^{+0} (11^{+4}–12^{+5})	101 (0.18%)
Fetal fraction (%)	8 (6–11)	8 (6–11)	2752 (4.9%)
Ethnicity			752 (1.3%)
White	52,175 (93.0%)	51,482 (93.0%)	—
Other	3935 (7.0%)	3876 (7.0%)	—
Method of conception			0 (0%)
Spontaneous	54,733 (97.5%)	54,733 (97.5%)	—
Assisted (IVF/ICSI)	1377 (2.5%)	1377 (2.5%)	—
Smoking			16,511 (29.4%)
Yes	2523 (4.5%)	1750 (4.4%)	—
No	53,587 (95.5%)	37,849 (95.6%)	—
Parity			117 (0.2%)
Nulliparous	29,044 (51.8%)	28,982 (51.8%)	—
Para 1	20,223 (36.0%)	20,182 (36.0%)	—
Para ≥2	6843 (12.2%)	6829 (12.2%)	—
Obstetrical history			0 (0%)
Previous preeclampsia[a]	160/27,066 (0.6%)	160/27,066 (0.6%)	—
Previous preterm birth[a]	785/27,066 (2.9%)	785/27,066 (2.9%)	—
Previous small for gestational age[a]	410/27,066 (1.5%)	410/27,066 (1.5%)	—
Previous miscarriage/abortion	349 (0.6%)	349 (0.6%)	—
Pregnancy outcomes			
Gestational age at delivery (wk)	39^{+5} (38^{+5}–40^{+5})	39^{+6} (38^{+6}–40^{+5})	855 (1.5%)
Mode of delivery			2771 (4.9%)
Vaginal delivery	42,505 (75.7%)	40,157 (75.3%)	—
Assisted vaginal delivery (vacuum/forceps)	4707 (8.4%)	4544 (8.5%)	—
Elective cesarean delivery	4148 (7.4%)	4036 (7.6%)	—
Emergency cesarean delivery	4750 (8.5%)	4602 (8.6%)	—
Birthweight (g)	3460 (3120–3785)	3472 (3142–3792)	1046 (1.9%)
Hypertensive disorders of pregnancy	3207 (5.7%)	3207 (5.7%)	0 (0%)
Birthweight <p10	5726 (10.2%)	4782 (8.8%)	1713 (3.1%)
Birthweight <p2.3	1796 (3.2%)	1114 (2.0%)	1713 (3.1%)
All sPTB (24–37 wk)	1891 (3.4%)	1747 (3.2%)	1069 (1.9%)
Spontaneous extremely PTB (24–28 wk)	76 (0.1%)	61 (0.1%)	—
Spontaneous very PTB (28–32 wk)	140 (0.3%)	104 (0.2%)	—
Moderate to late sPTB (32–37 wk)	1675 (3.0%)	1582 (2.8%)	—

Becking. Fetal fraction in noninvasive prenatal testing and adverse pregnancy outcomes. Am J Obstet Gynecol 2023. *(continued)*

SUPPLEMENTAL TABLE 2

Characteristics of the study cohort before and after imputation and amount of missing data *(continued)*

Characteristics	Study cohort after imputation Median (IQR) or n (%)	Study cohort before imputation Median (IQR) or n (%)	Amount of missing data n (% of total cohort of 56,110)
Diabetes[b]	1902 (3.4%)	1902 (3.4%)	0 (0%)
Congenital anomalies[c]	741 (1.3%)	741 (1.3%)	0 (0%)
Stillbirth	88 (0.2%)	88 (0.2%)	0 (0%)
Neonatal death	82 (0.2%)	82 (0.2%)	0 (0%)

BMI, body mass index; *ICSI*, intracytoplasmic sperm injection; *IQR*, interquartile range; *IVF*, in vitro fertilization; *NIPT*, noninvasive prenatal testing; *<p2.3*, <2.3rd percentile; *<p10*, <10th percentile; *PTB*, preterm birth; *sPTB*, spontaneous preterm birth.

[a] Data of multiparous women only (n=27,066); [b] Composed of both preexisting diabetes mellitus and gestational diabetes mellitus; [c] Excluding pregnancies with confirmed Down, Edwards, or Patau syndrome.

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SUPPLEMENTAL TABLE 3

Overview of adverse pregnancy outcomes, exclusions, transformations used for fetal fraction, and variables adjusted for in multivariable analyses

Adverse pregnancy outcome	Exclusions	N (remaining/total)	Transformation fetal fraction	Covariables multivariable analysis
Hypertensive disorders of pregnancy	Gestational age <24 wk	54,711/56,110	(fetal fraction+1/10)^-0.5	BMI, maternal age, ethnicity, parity, smoking, method of conception, previous small for gestational age, previous miscarriage, previous preeclampsia, socio economic status
SGA, birthweight<p10	Gestational age <24 wk	54,711/56,110	log(fetal fraction+1/10)	BMI, maternal age, ethnicity, parity, method of conception, smoking, previous preeclampsia, previous small for gestational age, socio economic status
SGA, birthweight<p2·3	Gestational age <24 wk	54,711/56,110	(fetal fraction+1/10)^-0.5	BMI, maternal age, ethnicity, parity, method of conception, smoking, previous preeclampsia, previous small for gestational age, socio economic status
Diabetes[a]	Gestational age <24 wk	54,711/56,110	No transformation	Gravidity, parity, BMI, maternal age, ethnicity, method of conception, smoking, previous preeclampsia, socio economic status
All sPTB (24–37 wk GA)	Gestational age <24 wk	54,711/56,110	No transformation	BMI, maternal age, ethnicity, parity, gravidity, Method of conception, smoking, previous preterm birth, socio economic status
Moderate to late sPTB (32–37 wk)	Gestational age < 32 wk	54,345/56,110	No transformation	BMI, maternal age, ethnicity, parity, gravidity, Method of conception, smoking, previous preterm birth, socio economic status
Very sPTB (28–32 wk)	Gestational age <28 wk and between 32–37 wk	52,275/56,110	No transformation	BMI, maternal age, ethnicity, parity, gravidity, Method of conception, smoking, previous preterm birth, socio economic status
Extremely sPTB (24–28 wk)	Gestational age <24 wk and between 28–37	52,139/56,110	No transformation	BMI, maternal age, ethnicity, parity, gravidity, Method of conception, smoking, previous preterm birth, socio economic status
Congenital anomalies[b]	Cases with confirmed trisomy 21, 13, or 18	55,956/56,110	No transformation	BMI, maternal age, parity, socio economic status, fetal fraction
Stillbirth	Gestational age <24 wk	54,711/56,110	No transformation	BMI, maternal age, parity, socio economic status, fetal fraction
Neonatal death	Gestational age <24 wk	54,711/56,110	No transformation	BMI, maternal age, parity, socio economic status, fetal fraction

BMI, body mass index; GA, gestational age; SGA, small for gestational age neonates; sPTB, spontaneous preterm birth.

[a] Composed of both pre-existing diabetes mellitus and gestational diabetes mellitus; [b] Excluding pregnancies with confirmed Down, Edwards, or Patau syndrome.

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SUPPLEMENTAL TABLE 4

Maternal characteristics of the study cohort, the total population of women opting for noninvasive prenatal testing, and the general Dutch obstetrical population

Characteristic	Study cohort (n=56,110)	Total NIPT population (n=77,478)[7]	General Dutch obstetrical population[8]
Maternal age (mean, SD)	31.6 (4)	31.6 (4.2)	31.3[a]
Fetal fraction (median, IQR)	8 (6–11)	8 (6–11)	Not known
BMI (median, IQR)	23.2 (21.2–26.1)	23.1 (20.6–25.6)	Not known
Gestational age at time of NIPT (mean, SD)	12^{+4} wk (1^{+1} wk)	11^{+6} wk (1^{+3} wk)	Not applicable
Ethnicity	93% White 7% other	Not known	87% White 13% other
Parity	51.8% nulliparous	Not known	43.6% nulliparous

BMI, body mass index; *IQR*, interquartile range; *NIPT*, noninvasive prenatal testing.

[a] Source: Statistics Netherlands.

Becking. Fetal fraction in noninvasive prenatal testing and adverse pregnancy outcomes. Am J Obstet Gynecol 2023.