Added prognostic value of the fetal fraction in non-invasive prenatal testing in the prediction of adverse pregnancy outcomes: analysis of a nationwide cohort of 56 110 pregnant women participating in the TRIDENT-2 study.

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Layman summary (461/500 words)

All pregnant women in the Netherlands are offered a screening test in early pregnancy to determine if their baby is affected by chromosomal abnormalities including Down's, Edward's, and Patausyndrome. This test is called the non-invasive prenatal test (NIPT). The NIPT is a test in which a blood sample of the mother is drawn and analysed around 12 weeks of pregnancy. In the blood circulation of the mother, small particles of DNA (cell-free DNA) of the baby are present. This cell-free DNA is analysed and will indicate whether the baby is affected by Down's, Edward's, or Patausyndrome. Apart from this, and because the cell-free DNA of the baby actually originates from the placenta, the amount of cell-free DNA of the baby present in the blood circulation of the mother (i.e. the fetal fraction) could give an indication of the functioning the placenta. Previous studies have found that the fetal fraction is lower if the development of the placenta is impaired. In case of impaired placental development risks of pregnancy complications such as high blood pressure or diabetes of the mother, or a baby born too small or too early are increased. This means that the fetal fraction could potentially be used in predicting the risks of these complications. If risks of pregnancy complications are known in early pregnancy, a high-risk mother can receive medication or be monitored more closely in order to attempt to prevent the complication. In this study we assessed if the fetal fraction is useful in predicting the risk of pregnancy complications in pregnant women that chose the NIPT in the Netherlands from June 2018 to June 2019. First, we selected factors that are known to be useful in predicting risks of pregnancy complications, including for instance Body Mass Index and age of the mother. These factors were included in a statistical base model to predict risks of pregnancy complications. Next, the fetal fraction was added to this base model. Through various statistical tests it was determined if the model with fetal fraction included performed better in predicting risks of pregnancy complications compared to the base model without fetal fraction. We found that that for some pregnancy complications including high blood pressure, diabetes, a baby born too small, and a baby born to early (between 32 and 37 weeks of pregnancy), the model with fetal fraction performed better than the base model without fetal fraction. Although the results of this study indicate that fetal fraction seems useful in the prediction of risks of pregnancy complications, this model is not yet ready to be used in practice. Future studies could aim at establishing a model with fetal fraction included that can used in clinical practice, for instance by adding fetal fraction to already in use prediction models for pregnancy complications.

Abstract

Background The proportion of fetal cell-free DNA (cfDNA) in the maternal circulation (i.e., the fetal fraction) is universally measured as a quality parameter in non-invasive prenatal testing (NIPT) for fetal aneuploidies. As fetal cfDNA originates from the placenta, the fetal fraction might reflect placental health. We assessed the added prognostic value of the fetal fraction in the prediction of adverse pregnancy outcomes.

Methods We performed a retrospective cohort study of women with singleton pregnancies that opted for NIPT over a 1-year period between June 2018 and June 2019 within the Dutch national implementation study on NIPT for fetal aneuploidies (TRIDENT-2 study). The TRIDENT-2 study data were linked to the Dutch registry of prenatal screening results (Peridos) and the Dutch registry of pregnancy outcomes (Perined). Outcomes included hypertensive disorders of pregnancy (HDP), birthweight <p10 and <p2.3, spontaneous preterm birth (sPTB), diabetes, congenital anomalies, and a combined poor neonatal outcome. The prognostic value of the fetal fraction was assessed by comparing logistic regression models based on clinical predictors without the fetal fraction (base model) and with the fetal fraction included (extended model) in terms of goodness of fit (likelihood ratio test (LRT)). In case of a statistically significant LRT ($p<0.05$), the amount of prognostic value was quantified by predictive measures including the area under the curve (AUC), integrated discrimination improvement (IDI), and the percentage of new predictive information based on difference in variance of predicted probabilities.

Results The analysis included 56 110 pregnant women. Based on the LRT, fetal fraction showed significant ($p<0.05$) added prognostic value for HDP, birthweight <p10, birthweight <p2.3, total sPTB, moderate to late sPTB, and diabetes, but not ($p>0.05$) for extremely sPTB, very sPTB, congenital anomalies, and combined poor neonatal outcome. For outcomes with a statistically significant LRT, the AUC showed marginal, but statistically significant ($p<0.05$) improvements for birthweight <p10, birthweight <p2.3, and all sPTB. The IDI was statistically significant ($p<0.05$) for HDP, birthweight <p10, birthweight <p2.3, all sPTB, moderate to late sPTB, and diabetes. The percentage of new predictive information based on variance was 5.4%, 7.5%, 10.1%, 2.3%, 2.9%, and -0.5% for HDP, birthweight <p10, birthweight <p2.3, all sPTB, moderate to late sPTB, and diabetes respectively.

Conclusion This study shows that the fetal fraction has added prognostic value in the prediction of HDP, birthweight<p10, birthweight<p2.3, all sPTB, moderate to late sPTB, and diabetes. The utility of the fetal fraction for the prediction of adverse pregnancy outcomes in clinical practice needs to be established in future research.

1. Introduction

The presence of fetal cell-free DNA (cfDNA) in the maternal blood circulation allows for non-invasive prenatal testing (NIPT) for Down, Edwards, and Patau syndrome.[1,2] Since the introduction of NIPT in clinical practice in 2011[3], NIPT has rapidly become available to pregnant women worldwide.[4] The accuracy of NIPT depends on a sufficient amount of fetal relative to total (both fetal and maternal) cfDNA in the maternal plasma. This is known as the fetal fraction. The fetal fraction is estimated in most cfDNA tests as a quality control parameter and is known to vary by biological factors such as maternal BMI and gestational age, but also depends on the bioinformatical method and molecular platform used for its estimation.[5–8] As fetal cfDNA originates from apoptotic syncytiotrophoblastic placental cells, the fetal fraction could reflect placental health and maternal pregnancy adaptation.[9,10] It has been hypothesized that impaired placentation with a relatively poor placenta-maternal interface in early pregnancy leads to a decreased release of fetal cfDNA in the maternal circulation, resulting in a lower fetal fraction. Some studies of etiologic nature have already shown an association between a low fetal fraction in NIPT and pregnancy complications such as hypertensive disorders of pregnancy (HDP), small for gestational age (SGA) neonates, spontaneous preterm birth (sPTB), and gestational diabetes mellitus (GDM).[11–16] This suggests that the fetal fraction has the potential to be an important biomarker in the prediction of these outcomes. Identifying pregnant women at risk for adverse pregnancy outcomes at an early gestational age allows for timely interventions and tailored pregnancy management in order to prevent complications. The prognostic value of the fetal fraction in the prediction of adverse pregnancy outcomes, in addition to other important predictors, has not yet been established. Here, we studied the added prognostic value of the fetal fraction in NIPT in the prediction of adverse pregnancy outcomes in a nationwide cohort of pregnant women who participated in the Dutch national implementation study on NIPT for fetal aneuploidies (the TRIDENT-2 study[17]).

2. Methods

2.1 Study design and linking of national registries

In the Netherlands, NIPT was introduced in April 2017 as a first-tier screening test for Down, Edwards, and Patau syndrome and offered to all pregnant women within a national implementation study: Trial by Dutch Laboratories for Evaluation of Non-Invasive Prenatal Testing (TRIDENT-2 prospective cohort study).[17] In the current retrospective cohort study, we used the data of all women who participated in the TRIDENT-2 study during a 1-year period between June 1st 2018 and June 1st 2019. Within the TRIDENT-2 study, NIPT results and fetal fraction estimates are collected in the Dutch national prenatal screening registry (Peridos) that includes data regarding maternal characteristics, prenatal ultrasound findings, and prenatal testing results.[18] For the current study, the Peridos registry was linked to the Dutch national perinatal registry (Perined) that includes maternal characteristics and pregnancy outcomes of all pregnant women in the Netherlands.[19] 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. This link was facilitated by a trusted third party (ZorgTTP) in order to comply with the European General Data Protection Regulation. The structure and coherence of the registries is graphically presented in supplemental Figure S1 and the method and process of linking the

Registries is explained in supplemental Document S1.

2.2 Inclusion and exclusion criteria

Women with singleton pregnancies who opted for NIPT within the TRIDENT-2 study were eligible for inclusion between June 1st 2018 and June 1st 2019. Women were excluded when they had not given consent for use of their data in follow-up research beyond the TRIDENT-2 study.[17]

2.3 Laboratory analysis

During the study period, the fetal fraction was uniformly measured in all three assigned NIPT laboratories in the Netherlands (i.e., the laboratory of Amsterdam University Medical Centers [location VU University Medical Center (VUMC)], Maastricht University Medical Center, and Erasmus University Medical Center). Blood sample collection and cfDNA isolation were performed as previously described.[17] Genome-wide shallow sequencing was performed with either the Illumina HiSeq4000 or the NextSeq500 sequencer (Illumina) and WISECONDOR software (v2.0.1) was used for bioinformatics analysis.[20] A whole-genome sequencing based methodology was used for fetal fraction estimation (Illumina VeriSeq, v1).

2.4 Definition of outcomes

Outcome variables were measured at the time of diagnosis of the outcome. Outcomes included hypertensive disorders of pregnancy (HDP), birthweight <p10 and <p2.3, spontaneous preterm birth (sPTB), diabetes, congenital anomalies, and a combined poor neonatal outcome. HDP comprised pregnancy-induced hypertension (PIH), preeclampsia (PE), and/or HELLP syndrome. PIH, PE, and HELLP syndrome were defined according to the ISSHP classification.[21] Birthweight <p10 and <p2.3 were determined by the Hoftiezer birthweight curve.[22] sPTB was defined as a spontaneous birth between 24-37 weeks of gestation (GA) and subdivided into moderate to late (32-37 weeks GA), very preterm (28-32 weeks GA), and extremely preterm (24-28 weeks GA).[23] Due to the nature of the Perined registry, diabetes included both gestational diabetes mellitus (GDM; determined by a 75 g 2-h oral glucose tolerance test between 24 and 28 weeks GA[24]) and pre-existing diabetes mellitus. Congenital anomalies were classified as major anomalies according to the guidelines of European Surveillance of Congenital anomalies (EUROCAT).[25] A combined poor neonatal outcome was also analysed, including neonatal death occurring within the first 4 weeks after birth, APGAR score <5 (5 minutes after birth), or NICU admission ≥32 weeks of GA.

2.5 Definition of predictors

Relevant predictors were selected based clinical expertise and previous literature on prediction models for the selected outcomes. Predictor variables considered were measured at timing of NIPT or at the start of pregnancy. The main predictor of interest was the fetal fraction (%). Other predictors differed by outcome and included Body Mass Index (BMI, kg/m^2) at time of NIPT, maternal age at time of NIPT (in years), ethnicity (white/other), gravidity (number of previous conceptions), parity (number of previous pregnancies beyond 16 weeks of gestation), smoking (yes/no), method of conception (spontaneous/assisted), socio economic status (SES) score based on postal code area, and conditions of obstetric history. Conditions of obstetric history included previous PE, previous PTB, previous SGA, and previous miscarriage. An overview of the predictors used by outcome is provided in supplemental Table S1.

2.6 Statistical analysis

To assess if missing data in the database was missing completely at random (MCAR), an MCAR table was created in which characteristics of pregnant women without missing data were compared to pregnant women with missing data in at least one variable (supplemental Table S2). Data was assumed not to be MCAR, because statistically significant differences were found between pregnant women with at least one missing variable versus no missing variables, as can be concluded from supplemental Table S2. Since a complete case analysis can thus result in imprecision and bias in estimates in the presence of missing data, missing data in the dataset were imputed using multiple imputation.[26] If the amount of missing data of a variable exceeded a 50% threshold, the variable was not considered in further analyses. All outcomes and predictor variables were used in the imputation model and 20 imputations were performed. Descriptive analyses were performed to describe the study cohort. Associations between all continuous predictors and all outcomes were assessed for potential non-linearity by spline plots using restricted cubic splines and multiple fractional polynomials and if needed continuous predictors were transformed. To determine the association between the fetal fraction and adverse pregnancy outcomes univariable and multivariable logistic regression analysis were first performed.

The added prognostic value of the fetal fraction was assessed by comparing multivariable logistic regression models with and without the fetal fraction as a predictor in terms of measures of goodness of fit and predictive performance.[27] A base model was fitted with known clinical predictors from previous literature and based on clinical expertise. An overview of the chosen predictors by outcome is provided in supplemental Table S1. Next, an extended model was fitted by adding the fetal fraction as a continuous variable to the base model. Multivariable logistic regression analysis was used for all models. A hierarchical based step-wise method was applied to assess the added prognostic value of the fetal fraction. First, a likelihood ratio chi-square test (LRT) was performed to compare log likelihoods of the base model versus the extended model. The LRT was used as a golden standard test, meaning that if no statistically significant result was found ($p>0.05$), it was assumed that the fetal fraction did not add prognostic value for that outcome.[28] Second, to quantify to what extent the fetal fraction adds prognostic value in case of a statistically significant LRT($p<0.05$), several predictive performance measures were calculated. The discriminative performance, i.e. the ability of the model to discriminate between pregnant women that did and did not develop the outcome, was described by the Area Under the Curve (AUC)[29] and the difference in AUC of the base model versus the extended model was calculated. To formally test if this difference was statistically significant ($p<0.05$) the Hanley McNeil method was used.[30] Risk classification was assessed by the Integrated Discrimination Improvement (IDI) index, in which a larger IDI indicates increased estimated risks for women with the outcome and decreased estimated risks for women without the outcome because of the addition of the fetal fraction to the model.[31] Additionally, the distribution in predicted risks of the outcomes was visualised by plotting these risks for the base and extended model, and quantified by calculating the variance. Higher variance (a greater variety in predicted risks) indicates increased discriminative power of the model. The fraction of new predictive information based on variance, i.e. the proportion of total predictive information that was

added to the model by the fetal fraction based on variance, was calculated by one minus the ratio of the variance in the base model to the variance in the extended model.[32] Additionally, to visualise the change in predicted risks by adding the fetal fraction to the model, the predicted risks for each participant before and after adding the fetal fraction to the model were plotted. All analyses were performed in each imputed dataset separately and results were pooled using Rubin's rules.[33] All statistical analyses were performed in R version 4.0.3 (The R Foundation for Statistical Computing).[34]

2.7 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 Amsterdam UMC, location VUMC (No.2017.165). The Medical Ethical Committee of the VU University Medical Center (VUMC) declared that the Medical Research Involving Human Subjects Act (WMO) did not apply to this present study (No.2020.10).

3. Results

Baseline characteristics and pregnancy outcomes of the study cohort based on imputed data and before imputation are presented in Table 1. 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 between June 1st 2018 to June 1st 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.

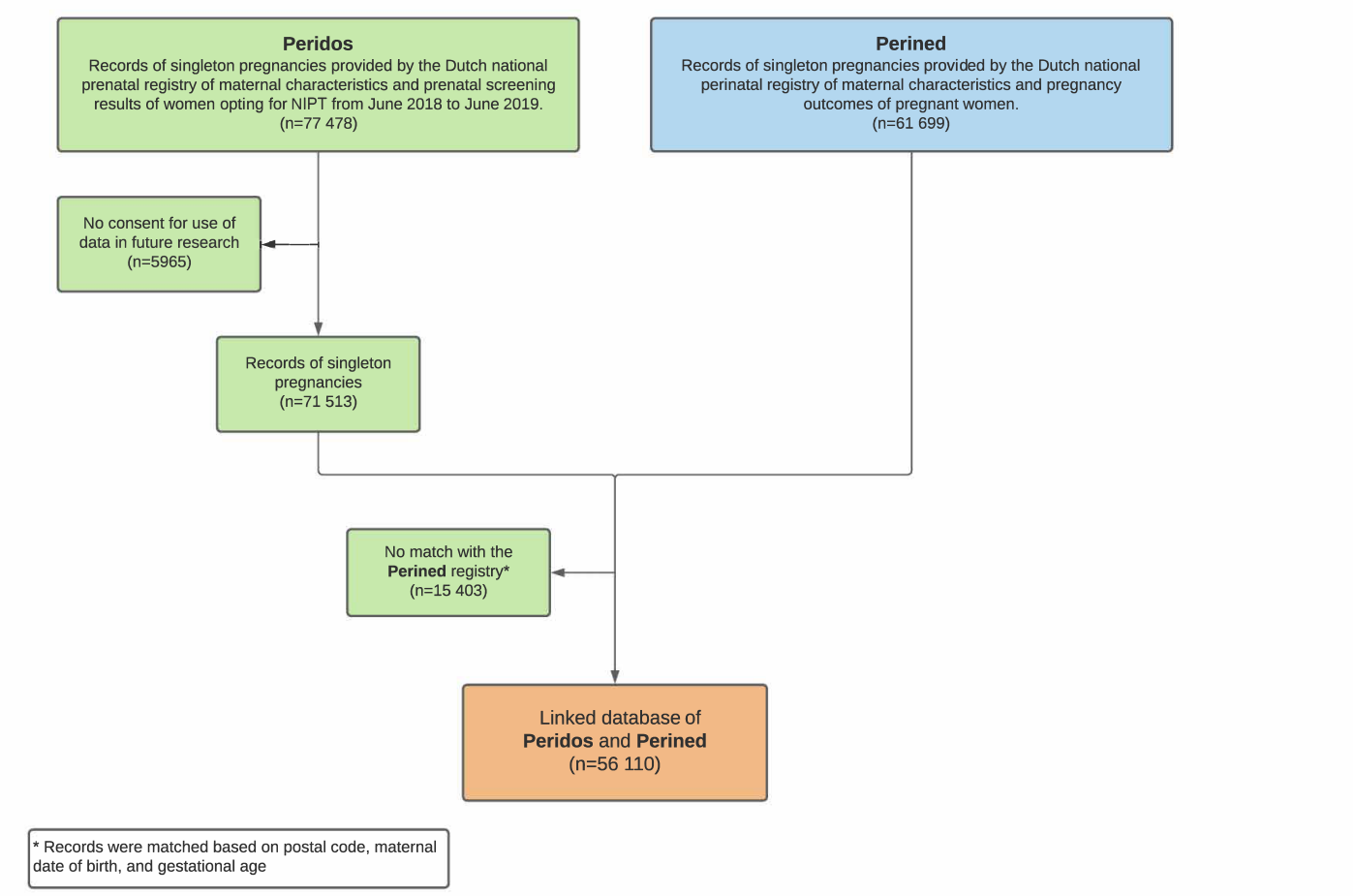

Figure 1. Flowchart of the study population

Table 1. Characteristics of the study cohort

	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 (years)	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 NIPS blood draw (weeks$^{+\text{ days}}$)	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%)	
Obstetric history			0 (0%)
Previous preeclampsia*	160/27 066 (0.6%)	160/27 066 (0.6%)	
Previous preterm birth*	785/27 066 (2.9%)	785/27 066 (2.9%)	
Previous small for gestational age*	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 (weeks)	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 caesarean section	4148 (7.4%)	4036 (7.6%)	
Emergency caesarean section	4750 (8.5%)	4602 (8.6%)	
Birthweight (gram)	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 weeks)	1891 (3.4%)	1747 (3.2%)	1069 (1.9%)
Extremely sPTB (24 - 28 weeks)	76 (0.1%)	61 (0.1%)	
Very sPTB (28 - 32 weeks)	140 (0.3%)	104 (0.2%)	
Moderate to late sPTB (32 - 37 weeks)	1675 (3.0%)	1582 (2.8%)	
Diabetes†	1902 (3.4%)	1902 (3.4%)	0 (0%)
Congenital anomalies‡	741 (1.3%)	741 (1.3%)	0 (0%)
Combined poor neonatal outcome¶	1471 (2.6%)	1468 (2.7%)	993 (1.8%)

IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; sPTB, spontaneous preterm birth.

* Data of multiparous women only (n=27 066).

† Including both pre-existing diabetes mellitus and gestational diabetes mellitus.

¶ Including neonatal death occurring within the first 4 completed weeks of life, Neonatal Intensive Care Unit (NICU) admission >32 weeks of GA

‡ Excluding pregnancies with confirmed Down, Edwards, or Patau syndrome

For 15 403 pregnancies, no match with the Perined registry could be accomplished based on postal code, maternal date of birth, and gestational age. 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 in NIPT between June 1st 2018 and June 1st 2019.

Table S3 shows the comparison of baseline characteristics of the study cohort of 56 110 women compared to the total population of women opting for NIPT and the total Dutch obstetric population. Median fetal fraction and BMI in the study cohort was 8% (IQR 6%-11%) and 23.2 (IQR 21.2-26.1) respectively, which was similar to the median fetal fraction and BMI in the total Dutch NIPT population during the study period of 8% (IQR 6%-11%) and 23.1 (IQR 20.6-25.6) respectively. Similarly, mean maternal age of the study cohort was 31.6 (SD 4), which was comparable to the mean maternal age in the total Dutch NIPT population of 31.6 (SD 4.2) and the total Dutch obstetric population of 31.3 (source Statistics Netherlands). Women in the study cohort were more often of white ethnicity compared to the total Dutch obstetric population (93% versus 85%), and were more often nulliparous (51.8% versus 43.6%).

3.1 Univariable and multivariable logistic regression analysis

Results of the univariable and multivariable logistic regression analysis are displayed in Table 2.

Table 2. Association of the fetal fraction with adverse pregnancy outcomes

Pregnancy complication (n= 54 711)	Outcome/total (%)	Univariable OR (95 % CI)	p-value	Multivariable OR	p-value
Hypertensive disorders of pregnancy	3186/54 711 (5.8)	0.22 (0.19 - 0.26)	<0.0001	0.44 (0.36 - 0.53)	<0.0001
Birthweight < p10	4784/54 711 (8.7)	0.80 (0.75 - 0.85)	<0.0001	0.73 (0.69 - 0.78)	<0.0001
Birthweight < p2.3	1104/54 711 (2.0)	0.39 (0.29 - 0.52)	<0.0001	0.38 (0.28 - 0.51)	<0.0001
All sPTB (24 - 37 weeks)	1891/54 711 (3.5)	0.98 (0.96 - 0.99)	0.00013	0.98 (0.97 - 0.99)	0.0014
Extremely sPTB (24 - 28 weeks)*	76/52 139 (0.1)	1.01 (0.95 - 1.07)	0.78	1.02 (0.95 - 1.08)	0.63
Very sPTB (28 - 32 weeks)*	140/52 275 (0.3)	0.98 (0.94 - 1.03)	0.39	0.98 (0.94 - 1.03)	0.47
Moderate to late sPTB (32 - 37 weeks)*	1675/54 345 (3.1)	0.97 (0.96 - 0.99)	<0.0001	0.98 (0.96 - 0.99)	0.00087
Diabetes†	1890/54 711 (3.5)	0.92 (0.91 - 0.93)	<0.0001	0.97 (0.96 - 0.98)	<0.0001
Congenital anomalies‡	741/55 956 (1.3)	0.97 (0.95 - 0.99)	0.0059	0.98 (0.96 - 1.00)	0.13
Combined poor neonatal outcome¶	1369/54 711 (2.5%)	0.98 (0.97-0.99)	0.0034	0.99 (0.97 - 1.00)	0.06

OR, odds ratio; sPTB, spontaneous preterm birth.
Shown is the univariable OR and the multivariable OR. 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 transformations used for fetal fraction per outcome is provided in supplemental Table S1. Only pregnancies with a gestational age at delivery ≥24 weeks were analysed, except if mentioned otherwise.
* Pregnancies with a sPTB within this gestational age range were compared to term pregnancies (gestational age ≥37 weeks); pregnancies outside of this range were excluded for this analysis.
† Including both pre-existing diabetes mellitus and gestational diabetes mellitus.
‡ Excluding all pregnancies with confirmed Down, Edwards, or Patau syndrome.
¶ The outcome included neonatal death occurring within the first 4 completed weeks of life, APGAR<5 (5 minutes after birth), Neonatal Intensive Care Unit (NICU) admission>32 weeks of GA.

In multivariable analysis, fetal fraction was associated with HDP (OR 0.44, 95% CI [0.36 to 0.53]), birthweight <p10 (OR 0.73, [95% CI; 0.69 to 0.78]), birthweight <p2.3 (OR 0.38 [95% CI; 0.28 to 0.51]), all sPTB (OR 0.98 [95% CI; 0.97 to 0.99]), moderate to late sPTB (OR 0.98 [95% CI; 0.96 to 0.99]), and diabetes (OR 0.97 [95% CI; 0.96 to 0.98]). For these outcomes, a higher fetal fraction corresponded to a lower odds of the outcome, and vice versa, a lower fetal fraction corresponded to a higher odds of the outcome assessed. No evidence of an association was found between fetal fraction and extremely sPTB (OR 1.02 [95% CI; 0.95 to 1.08]) or very sPTB (OR 0.98 [95% CI; 0.94 to 1.03]), congenital anomalies (OR 0.98 [95% CI; 0.96 to 1.00]), and combined poor neonatal outcome (OR 0.99 [95% CI; 0.97 to 1.00]). Results of the full multivariable logistic regression analyses including all model parameters with possible transformations of the extended model by outcome are displayed in Supplemental Document S2.

3.2 Likelihood ratio test

The added prognostic value of the fetal fraction is displayed in Table 3. Based on the LRT, fetal fraction showed significant ($p<0.05$) added prognostic value for HDP, birthweight <p10, birthweight <p2.3, total sPTB , moderate to late sPTB, and diabetes. No significant ($p>0.05$) added value was found for extremely sPTB, very sPTB, congenital anomalies, and combined poor neonatal outcome based on the LRT, indicating that there was no added prognostic value of the fetal fraction in the prediction of these outcomes.

3.3 Measures of predictive performance by outcome

The amount to which the fetal fraction added prognostic information varied by type of outcome assessed. The measures of predictive performance by outcome are displayed in Table 3.

The distribution in predicted risks of the outcome by the base model and the extended model including the variance are graphically presented in Figure 2. The left side of the histograms (blue) represent the distribution of predicted risks of the outcome by the base model. The right side of the histograms (green) represent the distribution of predicted risks of the outcome by the extended model with the fetal fraction included. A wider distribution of predicted risks and increased variance are an indication of better discrimination of a model. The change in predicted risks of the outcome by the base model versus the extended model is displayed graphically in Supplemental Figure S2. The X-axis shows the probability of the outcome for each participant by the base model, and the Y-axis shows the probability of the outcome when fetal fraction was added to that model (extended model). The red lines show the 0.1 and 0.9 quantile of the estimated risk by the extended model as a function of the estimated risk by the base model. Changes in predicted risks from the base model to the extended model indicate that low and high risk individuals can be more easily distinguished by adding fetal fraction to the model.

3.3.1 Hypertensive disorders of pregnancy

For HDP, the AUC of the base model versus the extended model with fetal fraction was 0.67 [95% CI; 0.66 to 0.68] versus 0.68 [95% CI; 0.67 to 0.69], with a p-value for the difference in AUC of 0.14. The IDI was 0.0018 [95% CI; 0.0013 to 0.0024] with a p-value <0.0001. The variance of the base model increased from 0.00135 to 0.00143 when adding fetal fraction to the model. The fraction of new predictive information added to the base model by the fetal fraction based on variance was 5.4%.

Table 3. Added prognostic value of the fetal fraction in NIPT.

Pregnancy complication (n= 54 711)	Likelihood ratio test (LRT)			Area Under the Curve (AUC)			Integrated Discrimination Improvement (IDI) index		Fraction of new predictive information based on variance		
	Residual deviance base model	Residual deviance extended model	LRT p-value	Base model (95% CI)	Extended model (95% CI)	p-value	IDI (95% CI)	P-value	Variance base model	Variance extended model	Fraction of new information
Hypertensive disorders of pregnancy	23245	23165	<0.0001	0.67 (0.66-0.68)	0.68 (0.67-0.69)	0.14	0.0018 (0.0013-0.0024)	<0.0001	0.00135	0.00143	5.4%
Birthweight < p10	31134	31037	<0.0001	0.67 (0.65-0.66)	0.69 (0.67-0.70)	<0.0001	0.0023 (0.0017-0.0028)	<0.0001	0.00211	0.00229	7.5%
Birthweight < p2.3	10341	10300	<0.0001	0.67 (0.66-0.69)	0.69 (0.67-0.71)	<0.0001	0.0011 (0.00065-0.0016)	<0.0001	0.000120	0.000222	10.1%
All sPTB (24 - 37 weeks)	16011	16000	0.0010	0.63 (0.61-0.64)	0.63 (0.62-0.64)	0.021	0.00028 (0.00010-0.00046)	0.0023	0.000332	0.000340	2.3%
Extremely sPTB (24 - 28 weeks)*	1111	1111	0.56	0.68 (0.62-0.74)	0.69 (0.63-0.75)	0.11	-0.0000077 (-0.000050-0.000035)	0.72	0.00000134	0.00000136	1.2%
Very sPTB (28 - 32 weeks)*	1909	1908	0.42	0.63 (0.58-0.68)	0.63 (0.58-0.68)	0.68	0.000037 (-0.000027-0.00010)	0.26	0.00000250	0.00000254	1.6%
Moderate to late (sPTB 32 - 37 weeks)*	14566	14554	0.00055	0.63 (0.61-0.64)	0.63 (0.62-0.64)	0.061	0.00030 (0.00011-0.00050)	0.0023	0.000270	0.000278	2.9%
Diabetes†	15463	15441	<0.001	0.72 (0.70-0.73)	0.72 (0.70-0.73)	0.35	0.00055 (0.00026-0.00083)	0.00015	0.00111	0.00110	-0.5%
Congenital anomalies‡	7864	7862	0.11	0.54 (0.52-0.56)	0.54 (0.52-0.56)	0.47	0.000040 (-0.000022-0.000010)	0.20	0.00000417	0.00000474	12.0%
Combined poor neonatal outcome¶	12779	12776	0.06	0.54 (0.52-0.55)	0.54 (0.53-0.56)	0.25	0.000073 (-0.0000020-0.00015)	0.060	0.0000116	0.0000132	12.0%

sPTB, spontaneous preterm birth.

An overview of the variables used in the multivariable analysis and the transformation used for fetal fraction per outcome is provided in supplemental Table S1. Results of all model parameters of the multivariable logistic regression analyses are available in Appendix 2. Only pregnancies with a gestational age at delivery ≥24 weeks were analysed, except if mentioned otherwise.

* Pregnancies with a sPTB within this gestational age range were compared to term pregnancies (gestational age ≥37 weeks); pregnancies outside of this range were excluded for this analysis.

† Including both pre-existing diabetes mellitus and gestational diabetes mellitus.

‡ Excluding all pregnancies with confirmed fetal trisomy 21, trisomy 13, or trisomy 18.

§ Low APGAR was defined as an APGAR score <5, 5 minutes after birth.

¶ Including neonatal death occurring within the first 4 completed weeks of life, APGAR<5 (5 minutes after birth), Neonatal Intensive Care Unit (NICU) admission within the first week after birth >32 weeks of GA

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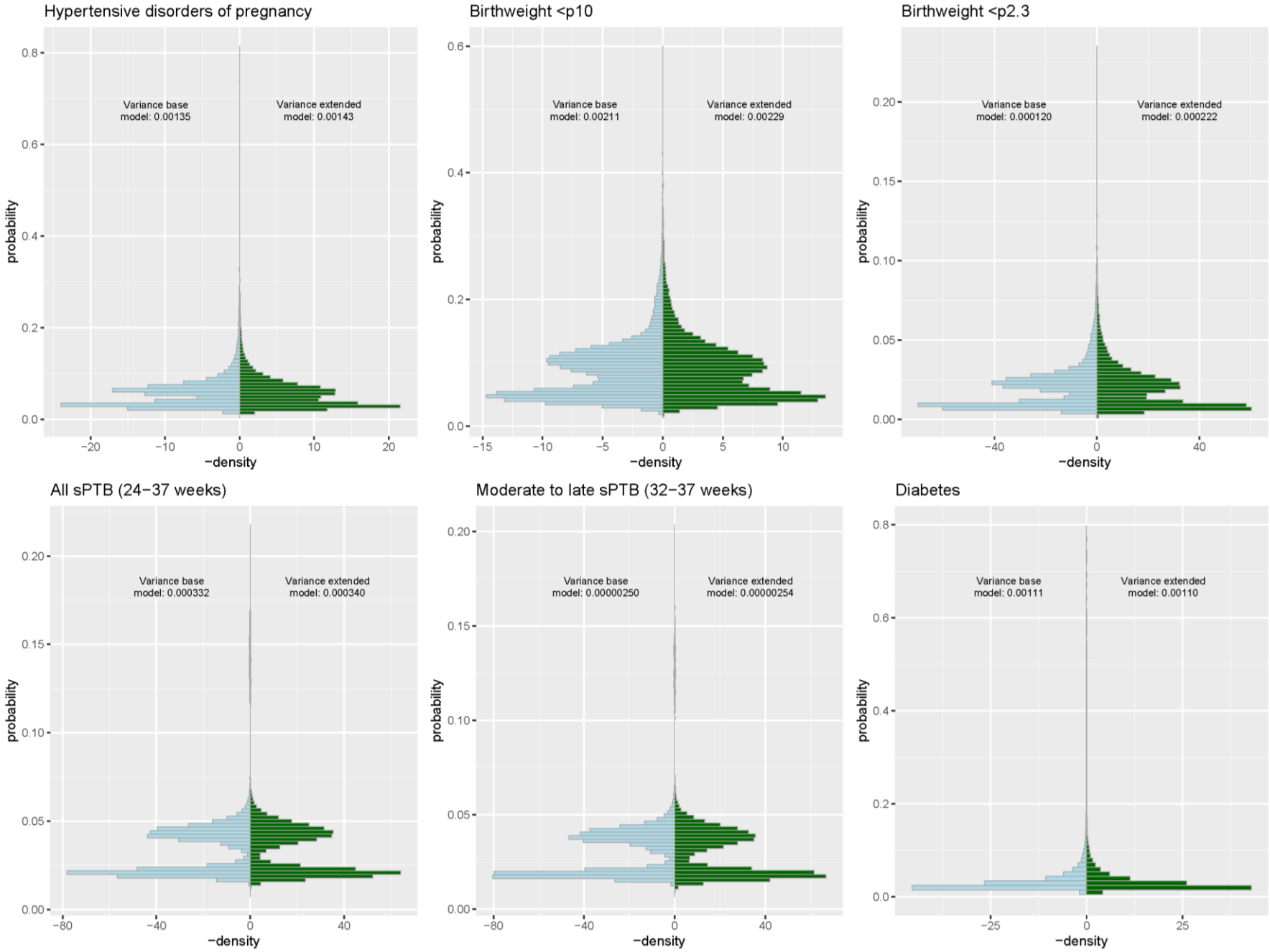

Figure 2. Distribution of predicted risks and variance of the base model and extended model by outcome

3.3.2 Birthweight <p10 and <p2.3

The AUC of the base model versus the extended model for birthweight<p10 was 0.67 [95% CI; 0.65 to 0.66] versus 0.69 [95% CI; 0.67 to 0.70] (p-value of the difference in AUC<0.0001). The IDI was 0.0023 [95% CI; 0.0017 to 0.0028] (p-value<0.0001). The variance of the base model (0.00211) increased when adding fetal fraction to the model (0.00229). The fraction of new predictive information by adding fetal fraction to the base model was 7.5% For birthweight <p2.3, the AUC of the base model versus the extended model increased from 0.67 [95% CI; 0.66 to 0.69] to 0.69 [95% CI; 0.67 to 0.71] (p-value of the difference <0.0001) with an IDI of 0.0011 [95% CI; 0.00010 to 0.0016] (p-value<0.0001). The variance increased from 0.000120 to 0.000222 by adding fetal fraction to the base model, resulting in 10.1% of new predictive information added by the fetal fraction.

3.3.3 All and moderate to late spontaneous preterm birth

For the base model of all sPTB the AUC was 0.63 [95% CI; 0.61 to 0.64] versus 0.63 [95% CI; 0.62 to 0.64] for the extended model (p-value of the difference in AUC=0.021). The IDI was 0.00028 [95% CI; 0.00010 to 0.00046] (p=0.0023). The variance of the base model (0.000332) increased when adding fetal fraction to the model (0.000340) and 2.3% new predictive information based on variance was found. The AUC for moderate to late sPTB without the fetal fraction was 0.63 [95% CI; 0.61 to 0.64] versus 0.63 [95% CI 0.62 to 0.64] with the fetal fraction included in the model (p=0.061). IDI was 0.00030 [95% CI; 0.00011 to 0.00050] (p=0.0023). Variance increased from 0.000270 to 0.000278 in the base model to the extended model, and the fraction of new information based on variance was 2.9%.

3.3.4 Diabetes

For diabetes, the AUC of the base model was 0.72 [95% CI; 0.70 to 0.73) versus 0.72 [95% CI; 0.70-0.73] in the extended model (p-value of the difference in AUC=0.35). IDI was 0.000555 [95% CI; 0.00026 to 0.00083] (p=0.00015). The variance of the base model decreased from 0.00111 to 0.00110 in the extended model, with -0.5% of predictive information retracted by the fetal fraction based on variance.

3.3.5 Other outcomes

No significant added prognostic value of the fetal fraction was found in the prediction of extremely sPTB, very sPTB, congenital anomalies and combined poor neonatal outcome based on the LRT. For reasons of completeness of information, results of the other predictive measures for these outcomes were calculated and are displayed in Table 3.

4. Discussion

This aim of this nationwide retrospective cohort study of 56 110 pregnant women opting for NIPT in the Netherlands was to assess the prognostic value of the fetal fraction additional to other known clinical predictors of adverse pregnancy outcomes. Based on the LRT, Significant added prognostic value was found for HDP, birthweight <p10, birthweight <p2.3, all sPTB, moderate to late sPTB, and diabetes. The fetal fraction did not add prognostic value for very sPTB, extremely sPTB, congenital anomalies, and a combined poor neonatal outcome.

The extent to which the fetal fraction added prognostic value varied by outcome and predictive measure assessed. Discriminative performance of the models was moderate, and addition of the fetal fraction to the base model resulted in a marginal but statistically

significant increase in the AUC for birthweight <p10, birthweight <p2.3, and all sPTB. These increases can be considered clinically irrelevant as the maximum increase only reached 0.02. A possible explanation for reaching statistical significance with these marginal differences in AUC may be the large sample size of the study cohort. Most improvement in risk classification was found by adding fetal fraction to the base models of HDP, birthweight<p10, and birthweight<p2.3, as the IDI was highest for these outcomes. To a lesser extent, risk classification improved by adding the fetal fraction to the base model for all sPTB, moderate to late sPTB, and diabetes. The fraction of new predictive information by the fetal fraction was also reasonably high for HDP (5.4%), birthweight <p10 (7.5%) and <p2.3 (10.1%), but was lower for all sPTB (2.3%), moderate to late sPTB (2.9%) and even slightly negative for diabetes (-0.5%).

The added prognostic value of the fetal fraction for adverse pregnancy outcomes found in this study is in line with the hypothesis that the level of fetal fraction level is closely related to placental health. As fetal cell-free DNA is believed to originate from trophoblastic placental cells undergoing apoptosis, the release of fetal cell-free DNA in the maternal circulation is thought to be connected to placental function.[5,9] We hypothesize that in the case of abnormal placental development, which is characterised by impaired trophoblast invasion and failed spiral artery transformation in the first trimester and consequently an increased risk of adverse pregnancy outcomes, less fetal cell-free DNA is released in the maternal circulation, resulting in a lower fetal fraction. This is supported by the ORs of the univariable and multivariable analysis our study, that all indicate that a higher fetal fraction relates to a lower odds of the adverse pregnancy outcome, and vice versa, a lower fetal fraction relates to a higher odds of the outcome assessed. Some previous retrospective cohort studies of etiologic nature found an association between low fetal fraction in NIPT and adverse pregnancy outcomes including preeclampsia, fetal growth restriction, sPTB and gestational diabetes[35], but little evidence exists that aims at establishing the prognostic value of the fetal fraction in the prediction of these outcomes. To our knowledge, only one study specifically evaluated the performance of the fetal fraction in the prediction of HDP, and found an AUC of 0.61 [95% CI; 0.55-0.66] in a model with fetal fraction, maternal age, and maternal weight.[36] This is lower than the AUC we found for HDP 0.68 [95% CI; 0.67 to 0.69], which may be explained by the lower amount of predictors included in their model.

The Netherlands is one of the few countries worldwide in which NIPT is performed within a government-supported national screening programme for fetal aneuploidies (the TRIDENT-2 study). A strength of this study is that we were able to use data of this large and nationwide cohort of pregnant women that opted for NIPT. This enabled us to use data of pregnant women opting for NIPT within a one-year time period and to link these data to the Dutch national registry of prenatal screening outcomes (Peridos) and the Dutch perinatal registry of pregnancy outcomes (Perined). Data originating from the TRIDENT-2 study, including information on the fetal fraction and BMI, were accurately collected in a standardized manner, and fetal fraction was consistently measured by the same WGS-based methodology in all NIPT laboratories during the study period. Also, to reduce bias in the presence of missing data, multiple imputation was performed.

A limitation of our study was that routinely collected data in the perinatal registry (Perined) are known to be subject to under registration or misclassification of certain maternal characteristics and pregnancy outcomes, which may have underestimated their true incidence.[37] Due to the nature of the perinatal registry we were also not able to fully distinguish pre-existing diabetes from gestational diabetes mellitus. Similarly, we cannot exclude that some cases of pre-existing hypertension may have been included in the outcome HDP. This means that in some cases the outcome may have already been present at the time of measurement of the fetal fraction. Although 56 110 pregnancies were included in the analysis, this was 72·4% (56 110/77 478) of the total cohort of pregnant women with singleton pregnancies that opted for NIPT within the study period which may limit generalizability of our results. However, based on the information presented in supplemental Table S3, maternal characteristics of the study cohort were similar to the total NIPT population. Our results may not be fully generalizable to the total Dutch obstetric population, as certain characteristics including parity and ethnicity were different in the study cohort compared to the total Dutch obstetric population (supplemental Table S3).

Fetal fraction is currently being universally assessed as a quality parameter in NIPT for fetal aneuploidies, but could potentially also be used as a biomarker for placental health and maternal pregnancy adaptation. 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 allows for timely preventive measures or intensified monitoring. For instance, administration of aspirin starting at ≤16 weeks of pregnancy is thought to improve placental function and is available at low cost and low complication rate.[38] A systematic review and meta-analysis showed that aspirin initiated ≤16 weeks of pregnancy reduced the incidence of PE (RR 0.57 [95% CI; 0.43-0.75]) and fetal growth restriction (RR 0.56 [95% CI; 0.44-0.70]) in high risk women.[39] Similarly, women with a previous preterm birth that received low-dose aspirin in their second pregnancy had a reduced risk of preterm birth (RR 0.87 [95% CI; 0.77-0.99]).[40] Our findings show that the fetal fraction has some added prognostic value on top of known predictors for specific adverse pregnancy outcomes, but the utility of the fetal fraction in clinical practice still needs to be established in future studies. Internal and external validation of models with fetal fraction included still needs to be performed. Future research could also aim at adding the fetal fraction as a predictor to internationally used prediction models for adverse pregnancy outcomes such as the National Institute for Health and Care Excellence (NICE) model or the Fetal Medicine Foundation (FMF) model for PE or fetal growth restriction.[41,42]

Conclusion

Our findings indicate that the fetal fraction has prognostic value additional to known clinical predictors of adverse pregnancy outcomes including HDP, birthweight <p10, birthweight <p2.3, all sPTB, moderate to late sPTB, and diabetes. The extent to which the fetal fraction added prognostic value differed by outcome and predictive measure assessed. The utility of the fetal fraction in the prediction of adverse pregnancy outcomes in clinical practice still needs to be established in future research.

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Supplementals

Legends:

Figure S1. Structure and coherence of national registries and linked database

Figure S2. Change in predicted probabilities of the base model versus the extended model by outcome

Table S1. Overview of outcomes, exclusions, transformations used for fetal fraction, and predictors by outcome

Table S2. Characteristics of pregnant women without missing data and pregnant women with missing data in ≥1 variable

Table S3. Maternal characteristics of the study cohort, the total cohort of women opting for NIPT and the entire Dutch obstetric population.

Document S1. Method and process of linking national registries.

Document S2. Results of the multivariable logistic regression analysis.

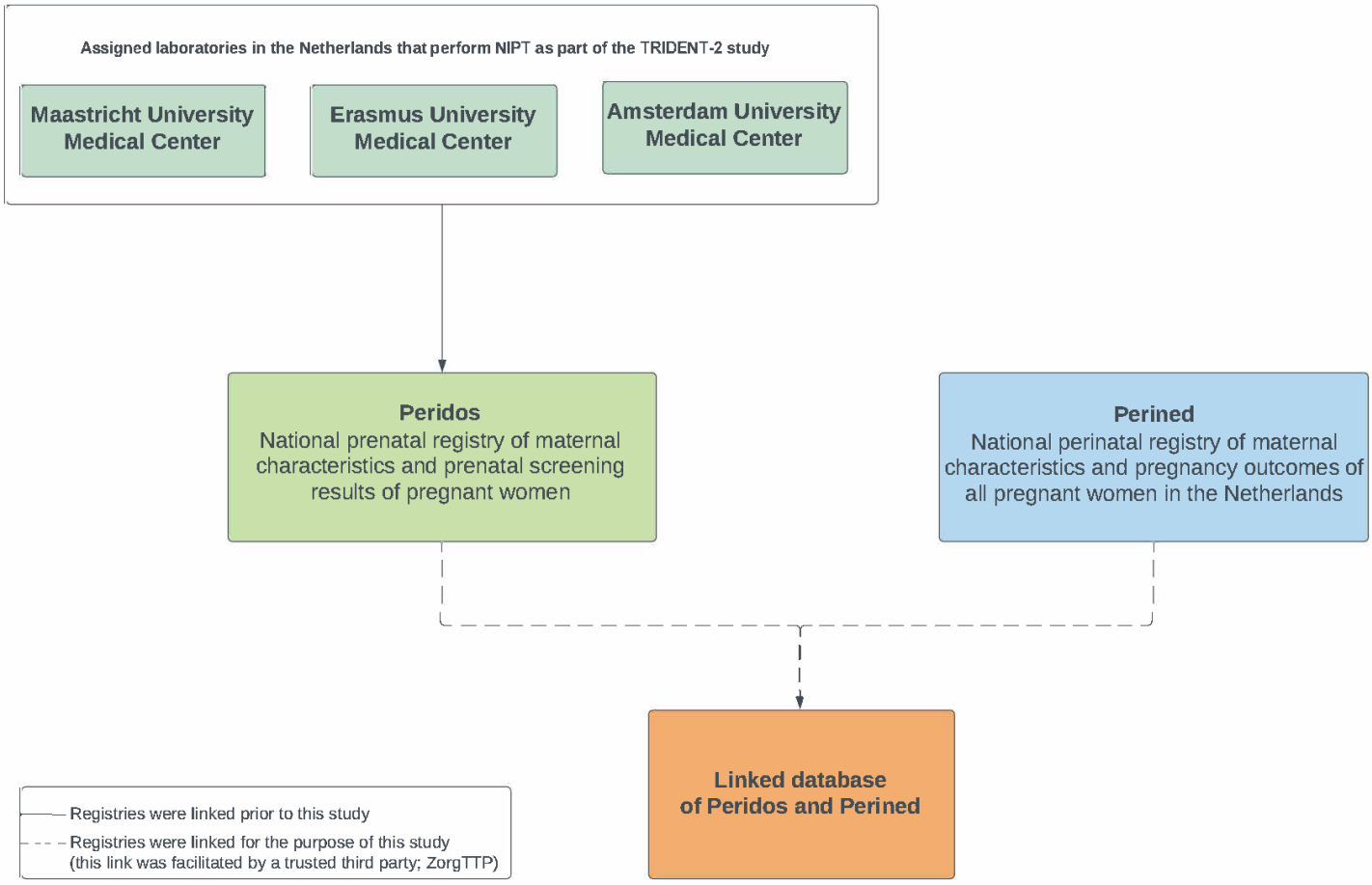

Figure S1. Structure and coherence of national registries and linked database

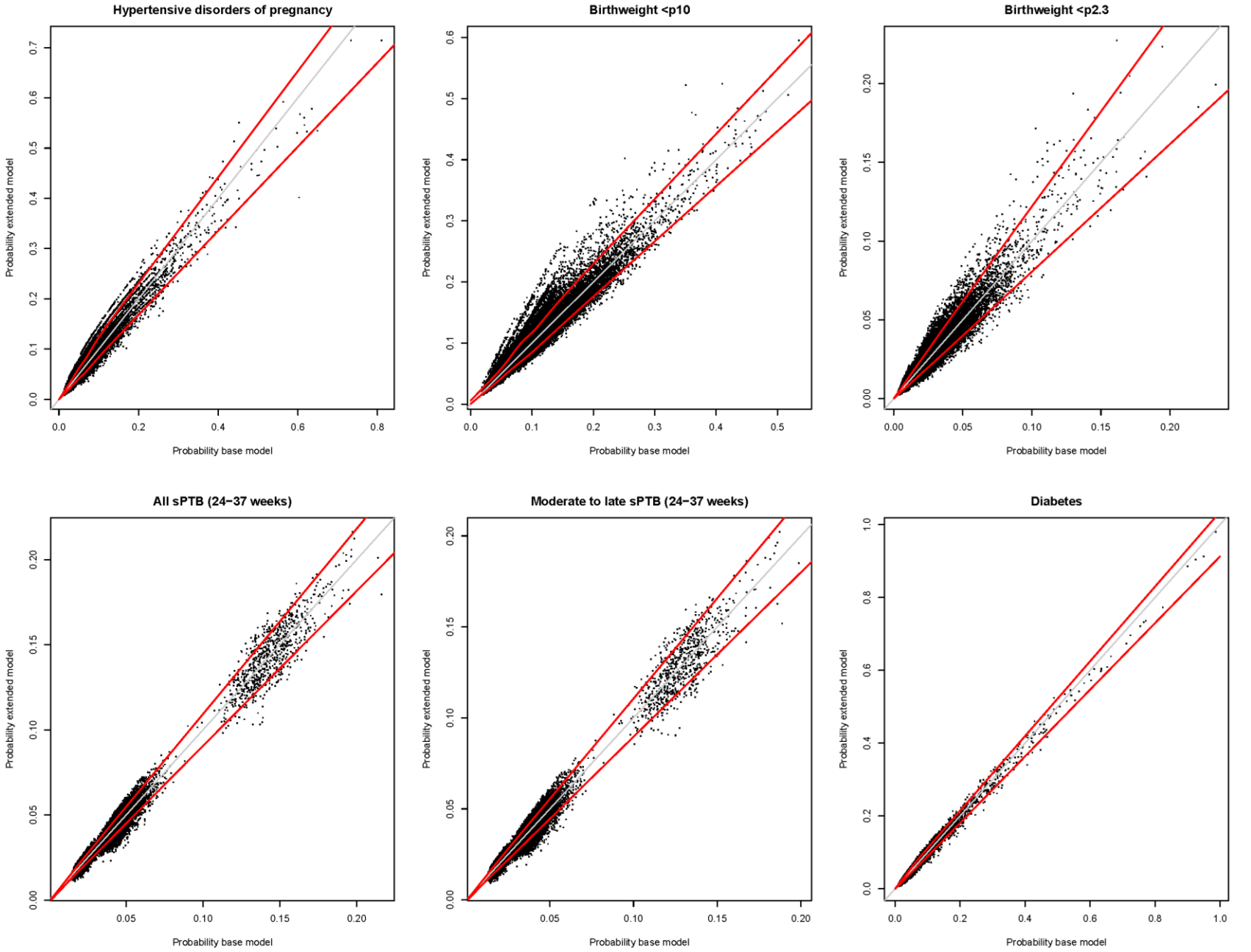

Figure S2. Change in predicted probabilities of the base model versus the extended model by outcome

Table S1. Overview of outcomes, exclusions, transformations used for fetal fraction, and pedictors by outcome.

Pregnancy complication	Exclusions	N (remaining/total)	Transformation fetal fraction	Covariables included in the base model
Hypertensive disorders of pregnancy	Gestational age <24 weeks	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
Birthweight < p10	Gestational age <24 weeks	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
Birthweight < p2.3	Gestational age <24 weeks	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*	Gestational age <24 weeks	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 weeks)	Gestational age <24 weeks	54 711/56 110	No transformation	BMI, maternal age, ethnicity, parity, gravidity, Method of conception, smoking, previous preterm birth, socio economic status
Extremely sPTB (24 - 28 weeks)	Gestational age <24 weeks 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
Very sPTB (28 - 32 weeks)	Gestational age <28 weeks and between 32 - 37 weeks	52 275/56 110	No transformation	BMI, maternal age, ethnicity, parity, gravidity, method of conception, smoking, previous preterm birth, socio economic status
Moderate to late sPTB (32 - 37 weeks)	Gestational age < 32 weeks	54 345/56 110	No transformation	BMI, maternal age, ethnicity, parity, gravidity, method of conception, smoking, previous preterm birth, socio economic status
Congenital anomalies†	Cases with confirmed trisomy 21, 13, or 18	55 956/56 110	No transformation	BMI, maternal age, socio economic status
Combined poor neonatal outcome¶	Gestational age <24 weeks	54 711/56 110	No transformation	BMI, maternal age, socio economic status

PTB, spontaneous preterm birth.

* Including both pre-existing diabetes mellitus and gestational diabetes mellitus.

† Excluding pregnancies with confirmed Down, Edwards, or Patau syndrome.

¶ Including neonatal death occurring within the first 4 completed weeks of life, APGAR<5 after 5 minutes, Neonatal Intensive Care Unit (NICU) admission within the first week after birth >32 weeks of GA.

Table S2. Characteristics of pregnant women without missing data and pregnant women with missing data in ≥1 variable.

	Pregnant women without missing data	Pregnant women with missing data	p-value
n	34873	21237	
Fetal fraction (mean (SD))	8.35 (3.86)	8.43 (3.90)	0.028
Gestational age (mean (SD))	277.11 (11.36)	272.68 (26.26)	<0.001
Gravidity (mean (SD))	2.03 (1.19)	2.04 (1.23)	0.334
Parity (mean (SD))	0.64 (0.78)	0.62 (0.78)	0.001
Maternal length (mean (SD))	169.48 (6.67)	169.49 (6.90)	0.881
Maternal weight (mean (SD))	69.30 (13.09)	69.85 (13.88)	<0.001
Maternal age (mean (SD))	31.53 (4.07)	31.79 (4.22)	<0.001
Socio economic status score (mean (SD))	0.08 (1.14)	0.05 (1.18)	<0.001
Previous abortion/miscarriage (%)	143 (0.4)	206 (1.0)	<0.001
Previous hypertensive disorder of pregnancy (%)	97 (0.3)	63 (0.3)	0.751
Previous preterm birth (%)	407 (1.2)	378 (1.8)	<0.001
Previous SGA (%)	253 (0.7)	157 (0.7)	0.893
Level of urbanisation (%)			<0.001
>2500 inhabitants/m^2	16578 (47.5)	10736 (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)	<0.001
Ethnicity = white (%)	32789 (94.0)	18693 (91.3)	<0.001
Smoking = no (%)	33370 (95.7)	4479 (94.8)	0.005
Deprived area of living = yes (%)	3266 (9.4)	2375 (11.3)	<0.001
Diabetes = yes (%)	1243 (3.6)	659 (3.1)	0.004
Hypertensive disorders of pregnancy (%)	2035 (5.8)	1049 (4.9)	<0.001
Preeclampsia/HELLP (%)	133 (0.4)	65 (0.3)	0.166
Hoftiezer percentile (mean (SD))	50.96 (28.61)	50.55 (29.05)	0.103
Start of birth (%)			<0.001
Spontaneous	24693 (70.8)	12604 (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 caesarean section	2602 (7.5)	1434 (7.6)	
Foley catheter	3512 (10.1)	1894 (10.1)	
Congenital anomaly = yes (%)	398 (1.1)	441 (2.1)	<0.001
Apgar score 5 minutes after birth (mean (SD))	9.65 (0.85)	9.45 (1.57)	<0.001
Neonatal mortality (%)			<0.001
Alive	34828 (99.9)	19700 (92.8)	
Death before birth	0 (0.0)	124 (0.6)	
Not viable	0 (0.0)	1262 (5.9)	
Death through birth	0 (0.0)	64 (0.3)	
Death <24h after birth	20 (0.1)	57 (0.3)	
Death 2^{nd}-7^{th} day after birth	12 (0.0)	18 (0.1)	
Death 8^{th}-28^{th} day after birth	7 (0.0)	8 (0.0)	

Death>28 days after birth	6 (0.0)	4 (0.0)	
NICU admission (%)	833 (2.4)	656 (3.1)	<0.001
Trisomy 21 in NIPT (%)	6 (0.0)	129 (0.6)	<0.001
Trisomy 13 in NIPT (%)	10 (0.0)	20 (0.1)	0.002
Trisomy 18 in NIPT (%)	5 (0.0)	29 (0.1)	<0.001
Additional findings in NIPT (%)	28 (0.1)	93 (0.4)	<0.001
missing = TRUE (%)	0 (0.0)	21237 (100.0)	<0.001

Table S3. Maternal characteristics of the study cohort, the total population of women opting for NIPT and the total Dutch obstetric population.

Characteristic	Study cohort (n = 56,110)	Total NIPT population (n = 77,478)	Total Dutch obstetric population
Maternal age (mean, SD)	31.6 (4)	31.6 (4.2)	31.3
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} weeks (1^{+1} weeks)	11^{+6} weeks (1^{+3} weeks)	Not applicable
Ethnicity	93% white 7% other	Not known	87% white 13% other
Parity	51.8% nulliparous	Not known	43.6% nulliparous

Document S1. 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 pseudonymisation process was carried out by a Trusted Third-Party specialised in secure transfers of personal data.

The Peridos registry contained 77 478 records of all women with singleton pregnancies who opted for NIPT from June 1st 2018 to June 1st 2019. After exclusion of pregnancies of women that 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 (i.e. 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.

Document S2. Results of the multivariable logistic regression analysis.

Hypertensive disorders of pregnancy	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-3.54	0.18	0.03	0.02	0.04	<0.0001
(BMI/10)^1) + (BMI/10)^2	0.10	0.01	1.10	1.09	1.11	<0.0001
(Age/10)^2 + (Age/10)^3	0.00	0.00	1.00	1.00	1.00	0.43
Ethnicity (other)	-0.50	0.09	0.60	0.51	0.72	<0.0001
(Parity+ 1)^-2	1.01	0.05	2.75	2.47	3.06	<0.0001
Method of conception (assisted)	0.22	0.11	1.25	1.01	1.54	0.04
Smoking (no)	0.02	0.11	1.02	0.83	1.26	0.83
Previous abortion/miscarriage	0.17	0.28	1.18	0.69	2.04	0.54
Previous preeclampsia	2.29	0.18	9.87	6.87	14.17	<0.0001
Previous SGA	0.17	0.24	1.18	0.73	1.90	0.50
(Socio economic status + 6.5)/10)^3	-0.11	0.13	0.89	0.69	1.16	0.40
(fetal fraction+1/10)^0.5	-0.83	0.09	0.44	0.36	0.53	<0.0001

Birthweight <p10	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-4.86	0.13	0.01	0.01	0.01	<0.0001
(BMI/10)^-2) + (BMI/10)^-2 * log(BMI/10)	3.10	0.22	22.22	14.51	34.03	<0.0001
(Age/10)^3) + (Age/10)^3 * log(Age/10)	0.00	0.00	1.00	1.00	1.00	<0.0001
Ethnicity (other)	0.60	0.05	1.82	1.65	2.01	<0.0001
(Parity + 1)^-1 + (Parity + 1)^-0.5	1.03	0.04	2.81	2.59	3.04	<0.0001
Method of conception (assisted)	0.03	0.09	1.03	0.86	1.24	0.76
Smoking (no)	-0.72	0.07	0.49	0.43	0.56	<0.0001
Previous preeclampsia	0.14	0.31	1.15	0.63	2.11	0.65
Previous SGA	1.83	0.12	6.24	4.96	7.85	<0.0001
Socio economic status	-0.02	0.01	0.98	0.95	1.00	0.07
log(fetal fraction+1/10)	-0.31	0.03	0.73	0.69	0.78	<0.0001

Birthweight <p2.3	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-5.80	0.28	0.00	0.00	0.01	<0.0001
(BMI/10)^-2 + (BMI/10)^-2 * log(BMI/10)	1.91	0.43	6.78	2.94	15.62	<0.0001
(Age/10)^2) + (Age/10)^2 * log(Age/10)	0.03	0.00	1.03	1.02	1.04	<0.0001
Ethnicity (other)	0.60	0.10	1.81	1.50	2.19	<0.0001
(Parity + 1)^-1 + (Parity + 1)^-0.5	1.34	0.09	3.83	3.23	4.53	<0.0001
Method of conception (assisted)	0.09	0.17	1.09	0.78	1.53	0.61
Smoking (no)	-0.92	0.11	0.40	0.32	0.50	<0.0001
Previous preeclampsia	0.36	0.59	1.44	0.45	4.61	0.54
Previous SGA	1.80	0.22	6.05	3.95	9.28	<0.0001
Socio economic status	-0.07	0.03	0.94	0.89	0.98	0.01
(fetal fraction+1/10)^0.5	-0.98	0.15	0.38	0.28	0.51	<0.0001

All sPTB (24-37 weeks GA)	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-1.36	0.64	0.26	0.07	0.90	0.03
rcs(BMI, 4)	-0.12	0.03	0.88	0.83	0.94	<0.0001
rcs(BMI, 4)	0.70	0.19	2.02	1.40	2.91	0.00017
rcs(BMI, 4)	-1.64	0.45	0.19	0.08	0.46	0.00022
(Age/10)^3) + (Age/10)^3 * log(Age/10)	0.00	0.00	1.00	1.00	1.00	0.027
Ethnicity (other)	0.10	0.09	1.10	0.93	1.32	0.27
(Parity+ 1)^-2	0.82	0.11	2.28	1.85	2.81	<0.0001
(Gravidity)^-2	0.22	0.10	1.24	1.03	1.50	0.02
Method of conception (assisted)	0.04	0.15	1.04	0.79	1.39	0.77
Smoking (no)	-0.16	0.12	0.85	0.67	1.07	0.17
Previous preterm birth	2.09	0.11	8.07	6.47	10.07	<0.0001
Socio economic status	-0.03	0.02	0.98	0.94	1.02	0.22
Fetal fraction	-0.02	0.01	0.98	0.97	0.99	0.0014

Extremely sPTB (24-28 weeks GA)	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-6.78	2.08	0.00	0.00	0.07	0.0011
rcs(Age, 3)	-0.06	0.06	0.94	0.84	1.06	0.30
rcs(Age, 3)	0.14	0.08	1.15	0.98	1.34	0.09
BMI	0.32	0.40	1.37	0.62	3.03	0.43
Ethnicity (other)	1.67	0.43	5.30	2.26	12.43	0.00012
Gravidity	0.14	0.12	1.15	0.91	1.44	0.23
(Parity+ 1)^-2	0.00	0.03	1.00	0.94	1.06	0.97
Method of conception (assisted)	0.48	0.55	1.61	0.55	4.74	0.39
Smoking (no)	0.20	0.68	1.22	0.32	4.59	0.77
Previous preterm birth	2.33	0.54	10.23	3.58	29.23	0.000014
Socio economic status	0.05	0.10	1.05	0.86	1.28	0.66
Fetal fraction	0.02	0.03	1.02	0.95	1.08	0.63

Very sPTB (28-32 weeks GA)	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-6.63	1.09	0.00	0.00	0.01	<0.0001
BMI	0.04	0.02	1.04	0.99	1.09	0.11
Age	0.47	0.29	1.60	0.92	2.80	0.10
Ethnicity (other)	-0.37	0.21	0.69	0.46	1.04	0.08
Parity	0.26	0.33	1.30	0.68	2.49	0.43
(Gravidity)^-2	-0.35	0.63	0.71	0.20	2.44	0.58
Method of conception (assisted)	-0.28	0.41	0.76	0.34	1.70	0.50
Smoking (no)	2.02	0.39	7.57	3.53	16.25	<0.0001
Previous preterm birth	-0.01	0.08	0.99	0.85	1.15	0.90
Socio economic status	0.00	0.02	1.00	0.96	1.04	0.89
Fetal fraction	-0.02	0.02	0.98	0.94	1.03	0.47

Moderate to late sPTB (32-37 weeks GA)	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-1.32	0.70	0.27	0.07	1.06	0.06
rcs(BMI, 4)	-0.13	0.03	0.87	0.82	0.93	<0.0001
rcs(BMI, 4)	0.75	0.20	2.11	1.44	3.11	0.00014
rcs(BMI, 4)	-1.74	0.47	0.18	0.07	0.44	0.00021
Age	0.01	0.01	1.01	0.99	1.02	0.25
Ethnicity (other)	0.05	0.10	1.05	0.87	1.28	0.59
(Parity+ 1)^-2	0.76	0.11	2.15	1.72	2.68	<0.0001
(Gravidity)^-2	0.26	0.10	1.30	1.06	1.58	0.01
Method of conception (assisted)	0.05	0.15	1.05	0.78	1.43	0.73
Smoking (no)	-0.17	0.12	0.85	0.67	1.08	0.17
Previous preterm birth	2.09	0.12	8.10	6.41	10.23	<0.0001
Socio economic status	-0.03	0.02	0.97	0.93	1.01	0.17
Fetal fraction	-0.02	0.01	0.98	0.96	0.99	0.00087

Diabetes	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-4.59	0.17	0.01	0.01	0.01	<0.0001
rcs(gravidity, 3)	0.15	0.07	1.17	1.02	1.33	0.02
rcs(gravidity, 3)	-0.22	0.08	0.80	0.68	0.94	0.01
(Age/10)^3	0.07	0.00	1.07	1.06	1.08	<0.0001
Ethnicity (other)	0.01	0.00	1.01	1.01	1.02	<0.0001
Parity	0.83	0.07	2.29	2.00	2.63	<0.0001
Method of conception (assisted)	-0.07	0.05	0.93	0.85	1.02	0.13
Smoking (no)	0.60	0.12	1.83	1.46	2.29	<0.0001
Previous preterm birth	-0.12	0.12	0.89	0.70	1.14	0.35
Socio economic status	-0.21	0.46	0.81	0.33	2.01	0.66
(BMI/10)^2 + (BMI/10)^2 * log(BMI/10)	-0.09	0.02	0.92	0.88	0.95	<0.0001
Fetal fraction	-0.03	0.01	0.97	0.96	0.98	<0.0001

Congenital anomalies	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-4.79	0.38	0.01	0.00	0.02	<0.0001
Age	0.00	0.01	1.00	0.98	1.02	0.83
BMI	-0.01	0.03	0.99	0.93	1.06	0.83
Socio economic status	0.03	0.01	1.03	1.01	1.04	0.00094
Fetal fraction	-0.02	0.01	0.98	0.96	1.00	0.13

Combined poor neonatal outcome	B-coefficient	bSE	OR	CI 95% LB	CI 95% UB	p-value
(Intercept)	-4.16	0.49	0.02	0.01	0.04	<0.0001
rcs(Age, 3)	0.00	0.02	1.00	0.97	1.03	0.92
rcs(Age, 3)	0.01	0.02	1.01	0.97	1.06	0.53
rcs(Socio economic status, 4)	-0.04	0.07	0.97	0.85	1.10	0.60
rcs(Socio economic status, 4)	0.29	0.15	1.34	0.99	1.80	0.06
rcs(Socio economic status, 4)	-2.63	1.07	0.07	0.01	0.59	0.01
BMI	0.02	0.01	1.02	1.01	1.03	0.0024
Fetal fraction	-0.01	0.01	0.99	0.97	1.00	0.06

If a transformation was used, this is indicated in the variable name. If restricted cubic splines were used, this is indicated by rcs(variable name, number of splines). Age represents the maternal age in years. Abbreviations: sPTB; spontaneous preterm birth, GA; gestational age.