

Clinical Feasibility of Early First-Trimester Non-Invasive Prenatal Testing: Associations Between Gestational Age, Fetal Fraction, and No-Call Rates

Trinh The Son¹, Sang Trieu Tien², Tran Van Khoa², Hung Sy Ho³, Hien Le Thi Thu⁴, Nhat Ngoc Nguyen¹, Dang Thai Son⁵, Phong Nguyen Van², Minh Duc Pham¹, Hang Thi Doan¹

¹Department of Embryology and Histology, Military Institute of Clinical Embryology and Histology, Vietnam Military Medical University, Hanoi, 12108, Vietnam; ²Department of Biology and Genetics, Vietnam Military Medical University, Hanoi, 12108, Vietnam; ³Department of Obstetrics and Gynecology, Hanoi Medical University, Hanoi, 100000, Vietnam; ⁴Department of Andrology, Andrology and Fertility Hospital of Hanoi, Hanoi, 12108, Vietnam; ⁵Department of Biological and Food Technology, Hanoi Open University, Hanoi, 12108, Vietnam

Correspondence: Hang Thi Doan, Department of Embryology and Histology, Military Institute of Clinical Embryology and Histology, Vietnam Military Medical University, Hanoi, 12108, Vietnam, Email DoanthiHang@vmmu.edu.vn

Background: Non-invasive prenatal testing (NIPT) based on cell-free fetal DNA (cffDNA) is widely used for screening common fetal aneuploidies. Although fetal fraction (FF) increases with gestational age, the feasibility and performance of NIPT in the early first-trimester, particularly before 10 weeks of gestation, remain incompletely defined.

Methods: This retrospective cohort study included 9,708 singleton pregnancies undergoing first-trimester NIPT between 9 weeks 0 days and 13 weeks 6 days of gestation, which was determined by ultrasound using crown-rump length (CRL) measurement. Participants were stratified into two groups based on gestational age at testing (< 10 weeks and ≥ 10 weeks). Multivariable linear regression was used to assess factors associated with fetal fraction, and multivariable logistic regression was applied to evaluate predictors of no-call results (non-reportable NIPT outcome primarily attributed to fetal fraction <4%), adjusting for maternal age and body mass index (BMI).

Results: Fetal fraction increased significantly with advancing gestational age. After adjustment for maternal age and BMI, testing performed at ≥ 10 weeks was associated with a higher fetal fraction compared with testing at < 10 weeks ($\beta = 1.58$; 95% CI, 1.31 to 1.85; $p < 0.001$). However, gestational age was not independently associated with the risk of a no-call result (adjusted OR 0.88; 95% CI, 0.68 to 1.16; $p = 0.40$), and no significant difference in no-call rates was observed between the two gestational age groups (3.78% vs. 3.76%).

Conclusion: Although fetal fraction increases after 10 weeks of gestation, gestational age is not an independent determinant of no-call results once fetal fraction adequacy is achieved. NIPT performed in the early first-trimester (< 10 weeks of gestation) demonstrates acceptable analytical feasibility and reliable performance, supporting its use for early prenatal screening under appropriate clinical conditions.

Keywords: non-invasive prenatal testing, cell-free fetal DNA, fetal fraction, no-call rate

Introduction

Non-invasive prenatal testing (NIPT), based on the analysis of cell-free fetal DNA (cffDNA) circulating in maternal plasma, has become an established component of prenatal screening for common fetal chromosomal aneuploidies, particularly trisomy 21, trisomy 18, and trisomy 13.¹⁻³ Cell-free fetal DNA, consisting of short DNA fragments, was first identified in maternal plasma in 1997 by Lo et al.⁴ Subsequent studies demonstrated that cffDNA is predominantly derived from apoptotic trophoblastic cells of placental origin and can be detected as early as 5 weeks of gestation.^{5,6} The fetal fraction, defined as the proportion of cffDNA relative to total circulating cell-free DNA, increases with advancing

gestational age, rising from approximately 3% to 13% during the first-trimester.⁷ Most NIPT platforms require a minimum fetal cfDNA fraction of approximately 4% to ensure reliable analysis, regardless of the specific sequencing or analytical technologies employed.^{8,9} Insufficient fetal fraction has been consistently associated with higher no-call rates and reduced analytical reliability, particularly when testing is performed in early pregnancy.^{10,11}

The American College of Obstetricians and Gynecologists (ACOG) currently recommends offering NIPT to all pregnant women, regardless of maternal age, as an effective screening tool for the early detection of fetal chromosomal aneuploidies. Early NIPT screening provides clinically meaningful information that supports timely risk assessment and informed decision-making in prenatal care. Although several professional guidelines recommend initiating NIPT from 10 weeks of gestation to ensure an adequate fetal fraction and minimize no-call,¹² the ACOG has stated that NIPT based on cfDNA can be performed as early as 9 weeks of gestation. Nevertheless, ACOG also acknowledges that higher fetal fractions achieved at 10 weeks of gestation and beyond are associated with lower no-call rates.⁷ Despite these recommendations, evidence directly comparing NIPT performance between the early first-trimester (9–10 weeks) and later first-trimester remains limited.¹³ In addition to gestational age, several maternal characteristics have been reported to influence fetal fraction and NIPT performance, most notably maternal age and body mass index (BMI).⁷ Advanced maternal age and increased BMI have been associated with lower fetal fraction, potentially due to increased maternal cfDNA contribution and altered placental biology, thereby increasing the likelihood of no-call results.¹⁴ However, the relative contribution of gestational age compared with maternal factors in early pregnancy remains incompletely understood. In addition, population-specific factors may influence fetal fraction distribution. In Asian populations, maternal body mass index (BMI) tends to be lower compared to Western populations, which may contribute to relatively higher fetal fraction values and potentially improved test performance in early gestation.^{15,16} Despite this, data evaluating NIPT performance before 10 weeks in Asian populations remain limited.

The performance of NIPT is commonly evaluated using a combination of analytical quality metrics and clinical performance indicators. Among these, fetal fraction and no-call rate represent fundamental feasibility parameters.^{3,17} Therefore, the present study aimed to evaluate the association between gestational age at the time of NIPT and cfDNA levels, with particular emphasis on comparisons between pregnancies tested before and after 10 weeks of gestation. Multivariable logistic regression analyses were performed to assess the independent effect of gestational age on fetal fraction and no-call result while adjusting for potential confounders, including maternal age and BMI.

Materials and Methods

Study Design and Setting

This retrospective observational cohort study was conducted at the Medical Genetics Laboratory, Department of Biology Medical Genetics, Vietnam Military Medical University. The study included Vietnamese pregnant women who underwent first-trimester non-invasive prenatal testing (NIPT) between April 2023 and November 2024. The primary objective was to evaluate gestational age specific performance metrics of NIPT, including fetal fraction (FF), no-call rate, with particular emphasis on early first-trimester testing.

Participants

Eligible participants included women with singleton pregnancies, with gestational age determined by ultrasound (CRL measurement) and ranging from 9 weeks 0 days to 13 weeks 6 days at the time of blood sampling. Exclusion criteria were defined as follows: (1) multiple gestations; (2) pregnancies achieved via in vitro fertilization (IVF); (3) maternal history of malignant disease or organ transplantation; and (4) cases with incomplete clinical records or missing fetal fraction results.

Following the selection process, a total of 9,708 pregnant women met the inclusion criteria and were included in the final analysis. To facilitate comparative evaluation, the cohort was stratified into two groups based on the timing of NIPT: < 10 weeks and $\geq$ 10 weeks of gestation.

Blood Sample Collection

Maternal peripheral blood (10 mL) was collected from each participant into Cell-Free DNA BCT vacuum tubes (Streck, USA). To ensure proper stabilization of nucleated cells, tubes were gently inverted 5 times immediately after collection. Samples were transported and stored at temperatures ranging from 6°C to 37°C in accordance with the manufacturer's instructions.

cfDNA Extraction

Plasma separation was performed using a dual-centrifugation protocol. Initially, whole blood samples were centrifuged at $1,600 \times g$ for 10 minutes at 4°C. The resulting plasma supernatant was carefully aliquoted into 2.0 mL microcentrifuge tubes and subjected to a second centrifugation step at $16,000 \times g$ for 10 minutes at 4°C to remove residual cellular debris. The clarified plasma was recovered, and cell-free DNA (cfDNA) was extracted using a magnetic bead-based nucleic acid extraction and purification kit (S10020), following the established laboratory protocol.

Library Preparation and Sequencing

DNA libraries were constructed using the Fetal Aneuploidies Detection Kit (S30030). Following quantification, libraries were normalized and pooled by barcode. Template preparation and emulsion PCR (emPCR) were conducted on the Ion OneTouch™ 2 system (Thermo Fisher Scientific, USA). Enrichment of template-positive ion sphere particles was performed using the automated Ion OneTouch™ ES system. The enriched libraries were then loaded onto Ion PI™ Chip v3 and subjected to semiconductor sequencing on the BioelectronSeq 4000 platform. The detailed procedures for library preparation and semiconductor sequencing have been previously validated and described.^{18,19}

Bioinformatics Analysis

Fetal fraction (FF) was estimated using a fragment length based method that exploits the distinct size distributions of fetal and maternal cell-free DNA (cfDNA) in maternal plasma. Fetal DNA concentration was calculated using a linear regression model of Y-chromosome proportion as well as a linear regression model based on the length distribution of cell-free DNA. No explicit FF enrichment procedure was performed by the investigators. However, the commercial kit used in this study is designed for cfDNA analysis and may introduce preferential representation of shorter cfDNA fragments during library preparation as an inherent feature of the workflow.

A minimum threshold of 3.5 million single-end reads per sample was required for analysis; otherwise, resequencing was performed. The detection of trisomies 21, 18, and 13 was based on a Z-score approach, which evaluates the proportional representation of sequencing reads for each target chromosome. A result was considered negative when $Z \leq 1.96$; a gray zone was defined as $1.96 < Z < 3$ (repeat sampling is recommended if the fetal fraction is low, $FF < 4\%$); and positive when $Z \geq 3$.

Statistical Analysis

Data were collected and managed using Microsoft Excel 2019. Statistical analyses were primarily performed using R software (R Foundation for Statistical Computing, Austria). Continuous variables, including fetal fraction (FF), maternal age, and body mass index (BMI), were summarized using the mean $\pm$ standard deviation (SD) and median, as appropriate. Categorical variables were presented as counts and percentages. To identify factors associated with fetal fraction, multivariable linear regression analysis was conducted, with adjustment for maternal age and BMI. Multivariable logistic regression analysis was used to assess the independent association between gestational age at testing and the risk no-call results.

Results

A total of 9,708 pregnant women undergoing first-trimester NIPT were included in the analysis. The median gestational age at the time of testing was 11.0 weeks (range: 9.0–13.6 weeks). The mean maternal age was 29.9 ± 5.1 years, and the

Table 1 Summary of Population's Characteristics and the Fetal Fraction

Characteristics	Median	Mean $\pm$ SD	Min	Max
BMI (kg/m ²)	21.24	21.25 $\pm$ 2.04	16.60	27.51
Maternal age (years)	29.5	29.9 $\pm$ 5.1	18.0	50.0
Gestational age (weeks)	11.0	10.9 $\pm$ 1.2	9.0	13.6
Fetal fraction (%)	16.05	16.29 $\pm$ 5.09	4.20	42.50

Abbreviation: SD, standard deviation.

mean maternal body mass index (BMI) was 21.25 ± 2.04 kg/m². The median fetal fraction (FF) was 16.05%, with values ranging from 4.20% to 42.50% (Table 1).

Fetal fraction increased progressively with advancing gestational age during the first-trimester. Scatter plot analysis demonstrated a positive correlation between gestational age and fetal fraction (Figure 1).

Multivariable linear regression analysis was performed to identify independent factors associated with fetal fraction (FF), adjusting for gestational age at testing, maternal body mass index (BMI), and maternal age (Table 2). After adjustment for potential confounders, gestational age remained significantly associated with FF. Specifically, pregnancies tested after 10 weeks of gestation had a significantly higher mean FF compared with those tested at <10 weeks (16.64 ± 5.14 vs. 15.1 ± 4.74 ; $\beta = 1.58$; 95% CI, 1.31–1.85; $p < 0.001$).

Maternal BMI was not significantly associated with FF in the adjusted model ($\beta = 0.01$; 95% CI, -0.04 to 0.06 ; $p = 0.70$). Maternal age showed a weak inverse association with FF; however, this relationship did not reach statistical significance ($\beta = -0.02$; 95% CI, -0.04 to 0.00 ; $p = 0.053$).

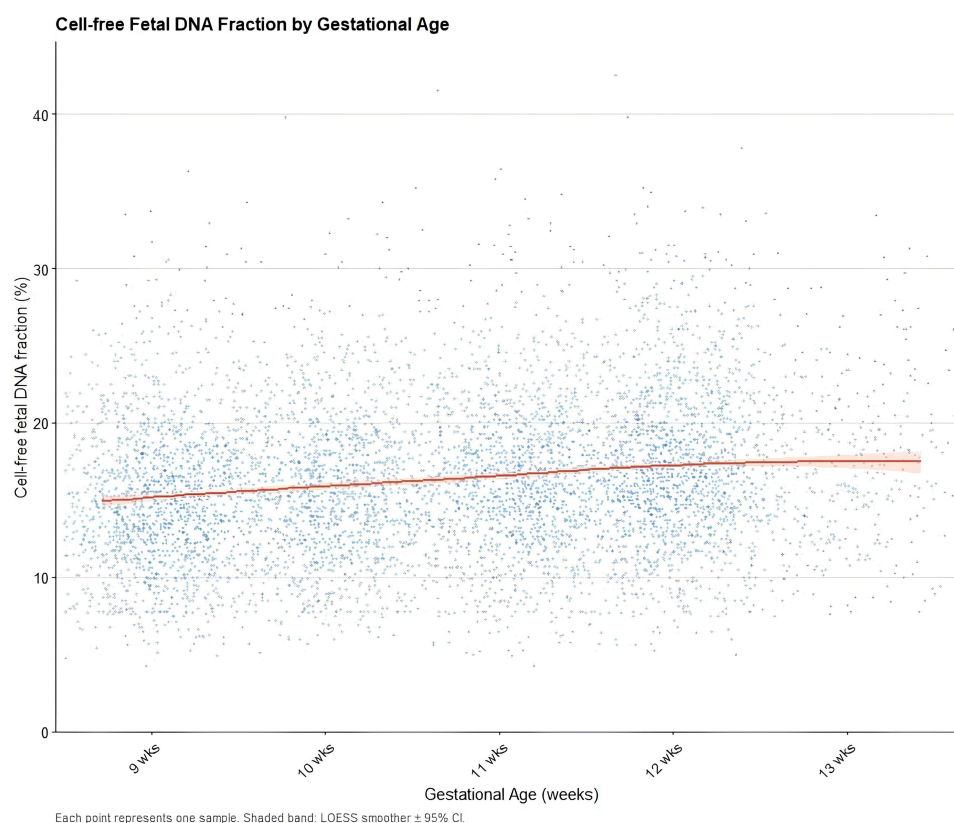
**Figure 1** Correlation between Gestational Age and Fetal Fraction.

Table 2 Multivariable Linear Regression Analysis for Fetal Fraction

Variable	Fetal Fraction (Mean $\pm$ SD)	Beta Coefficient	95% CI	p value
Gestational age				
< 10 weeks	15.1 $\pm$ 4.74	1.00 (Ref)	–	–
$\geq$ 10 weeks	16.64 $\pm$ 5.14	1.58	1.31, 1.85	<0.001
BMI (kg/m ²)	–	0.01	–0.04, 0.06	0.70
Maternal age (years)	–	–0.02	–0.04, 0.00	0.053

Note: The model was adjusted for all variables listed.

Abbreviations: CI, confidence interval; BMI, body mass index; Ref, reference group.

Table 3 Logistic Regression Analysis for No-Call Results

Variable	No-Call Rate (%)	Adjusted OR	95% CI	p value
Gestational age				
< 10 weeks	3.78 (83/2194)	1.00 (Ref)	–	–
$\geq$ 10 weeks	3.76 (283/7514)	0.88	0.68, 1.16	0.40
BMI (kg/m ²)	–	0.92	0.87, 0.97	0.001
Maternal age (years)	–	0.98	0.96, 1.00	0.06

Note: Adjusted for all variables listed in the table.

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; Ref, reference group.

In multivariable logistic regression analysis, gestational age at the time of NIPT was not significantly associated with the risk of a no-call result (Table 3). Compared with testing at < 10 weeks' gestation, testing at $\geq$ 10 weeks showed a lower but non-significant odds of a no-call result (adjusted OR 0.88; 95% CI, 0.68 to 1.16; $p = 0.40$).

Maternal BMI was independently associated with the no-call rate, with higher BMI associated with a reduced likelihood of a no-call result (adjusted OR 0.92 per 1 kg/m² increase; 95% CI, 0.87 to 0.97; $p = 0.001$). Maternal age showed a non-significant inverse association with the risk of a no-call result (adjusted OR 0.98 per year; 95% CI, 0.96 to 1.00; $p = 0.06$).

Discussion

In this large cohort study, we assessed key performance metrics of non-invasive prenatal testing (NIPT), including fetal fraction (FF), no-call rate, to evaluate the feasibility and clinical effectiveness of NIPT performed as early as 9–10 weeks of gestation.^{3,17} Our findings indicate that, although fetal fractions are lower in early first-trimester pregnancies, NIPT before 10 weeks remains feasible and demonstrates reliable analytical feasibility in a large real world population.

Fetal fraction emerged as the central analytical parameter influencing NIPT performance in early pregnancy. The relatively high median fetal fraction (~16%) observed in this cohort likely reflects population-specific characteristics, particularly the predominantly Asian population with lower maternal BMI, rather than differences in analytical performance. We observed a clear increasing trend in FF across the first-trimester with advancing gestational age, reflecting the dynamic nature of placental development and fetal DNA contribution to the maternal circulation. After adjustment for maternal age and body mass index (BMI), pregnancies tested after 10 weeks of gestation exhibited significantly higher FF compared with those tested at or before 10 weeks, underscoring the critical role of gestational timing in determining circulating cfDNA levels. The observed increase in FF with advancing gestational age is consistent with previous studies

and is biologically plausible, as placental derived cfDNA primarily originates from trophoblast and syncytiotrophoblast turnover, which increases as placental mass and cellular activity expand during the first-trimester.^{20,21} Furthermore, placental remodeling and the transition from a hypoxic to a more oxygenated environment around 10–12 weeks of gestation may further enhance trophoblast differentiation and shedding, thereby contributing to rising FF levels.²² In the adjusted model, maternal age showed a weak inverse association with FF that did not reach statistical significance, consistent with prior studies reporting small and inconsistent effects.^{23–25} Maternal BMI was also not significantly associated with FF, likely reflecting the relatively narrow and predominantly normal BMI distribution in this study population. This finding contrasts with reports from Western cohorts, where higher BMI has been linked to lower FF, potentially due to increased maternal cfDNA contribution and plasma volume dilution.²⁶ In our study, the mean BMI was relatively low (21.25 kg/m²), consistent with the leaner characteristics of Asian populations. Therefore, the observed pattern should not be interpreted as a protective effect of higher BMI, but rather as a population-specific phenomenon driven by limited BMI variability and a low prevalence of obesity. Within this context, modest variations in BMI may not exert the same negative influence on fetal fraction or no-call rates as observed in populations with higher rates of overweight and obesity. Overall, these findings suggest that gestational age is the primary determinant of fetal fraction in the first trimester, whereas the effects of maternal BMI and age appear modest and context-dependent.

A no-call result, defined as a non-reportable NIPT outcome, is primarily attributed to an insufficient fetal fraction (<4%). Such results have been reported to occur in 0.1% to 6.1% of cases, depending on clinical protocols and the specific sequencing platform used.^{27,28} In this study, the overall no-call rate was approximately 3.76–3.78%, indicating favorable testing conditions and adequate fetal fraction in most cases.^{29,30} Although FF is known to increase with gestational age, gestational age itself was not an independent predictor of no-call risk after multivariable adjustment. This finding is consistent with previous studies suggesting that, once a minimum FF threshold is achieved, other factors such as maternal characteristics and placental biology (including PAPP-A and free β -hCG) may have a more relevant influence on test performance.³¹ When comparing testing before and after 10 weeks of gestation, no significant difference in no-call rates was observed, and adjusted analyses confirmed that testing at ≥ 10 weeks did not significantly reduce no-call risk. These results suggest that the commonly used 10-week threshold may not represent a strict cutoff for no-call outcomes. Accordingly, NIPT performed in the early first-trimester may achieve reportable result rates comparable to those obtained later in the first-trimester under appropriate clinical and laboratory conditions.

Several limitations of this study should be acknowledged. First, the retrospective and single genetic center design may introduce selection bias and limits the ability to infer causal relationships. Additionally, the treatment of no-call results as a binary outcome represents a technical limitation. According to the manufacturer's recommendations, any sample with an initial FF below 4% requires re-sampling and re-testing to ensure diagnostic accuracy. Consequently, the specific numerical FF values from these initial sub-threshold runs were not archived for primary analysis, limiting our ability to perform a more granular continuous variable analysis for the no-call group. Furthermore, the relationship between maternal BMI and no-call results in our cohort appears to differ from findings reported in Western populations. Accordingly, this finding should be interpreted with caution and may not be generalizable to other populations. Further studies in more diverse cohorts are warranted to better clarify the relationship between maternal BMI and NIPT performance. Notably, the lower no-call rates observed here and in other Asian studies compared to Western cohorts may be attributed to a lower prevalence of obesity.³² Despite these limitations, the large cohort size and real-world clinical setting provide valuable evidence supporting the analytical feasibility of NIPT performed in the early first-trimester.

Conclusion

In this study, gestational age at the time of NIPT (confirmed by ultrasound CRL measurement) was positively associated with fetal fraction; however, it was not an independent predictor of no-call results after adjustment for maternal factors. Although higher fetal fractions were observed after 10 weeks of gestation, no significant difference in no-call rates was identified between pregnancies tested before and after this threshold. No-call results in our study were primarily related to insufficient fetal fraction (<4%). Taken together, these findings suggest that NIPT performed in the early first trimester

(from 9–10 weeks) may be feasible under appropriate clinical and laboratory conditions, provided that minimum fetal fraction requirements are achieved and gestational age is accurately determined.

Data Sharing Statement

All data supporting the findings of this study are available from the corresponding author on request.

Ethics Approval

The study was approved by the Ethical Review Committee of Vietnam Military Medical University (Approval No. 02 / 2026/CNChT-HDDD) and conducted in accordance with the Declaration of Helsinki. All participants received comprehensive information regarding the study objectives, procedures, and potential risks. Written informed consent was obtained from each participant prior to enrollment and data collection. Participant confidentiality was strictly maintained throughout the study.

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Author Contributions

The corresponding author provided the conceptual framework for this study. Additionally, All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors have no conflicts of interest in this study and declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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