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# OPTIMIZATION OF NIPT TIMING FOR MALE FETUSES AND ABNORMALITY DETECTION IN FEMALE FETUSES BASED ON QUALITY-CORRECTED MODELS

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**Abstract:** This study aims to enhance the accuracy of non-invasive prenatal testing (NIPT) by optimizing detection timing for male fetuses and improving abnormality diagnosis for female fetuses. For male fetuses, a multivariate nonlinear regression model quantified the associations of Y-chromosome fraction with gestational weeks and maternal BMI, revealing quadratic and decreasing trends, respectively. Ordered clustering and a risk-based optimization identified four BMI groups with distinct optimal testing windows, where higher BMI delayed the best timing. Monte Carlo simulations further confirmed sequencing quality as the main error source. For female fetuses, a combined logistic regression–random forest model was developed using standardized Z-scores and quality indicators, with an optimal diagnostic threshold ($\tau = 0.35$). The resulting workflow integrates quality screening, Z-score assessment, probability evaluation, and BMI-specific correction. These models provide clinically interpretable guidance to improve NIPT reliability and prenatal decision-making.
**Keywords:** NIPT; Nonlinear regression; Ordered clustering; Multi-objective optimization; Female fetal abnormality detection

## 1 INTRODUCTION

Non-invasive prenatal testing (NIPT) has become a widely adopted screening approach for common fetal chromosomal abnormalities, such as trisomy 21, trisomy 18, and trisomy 13 [1]. By analyzing cell-free fetal DNA (cffDNA) fragments circulating in maternal plasma, NIPT provides an accurate, safe, and non-invasive alternative to traditional prenatal diagnostic techniques [2-3]. Compared with invasive methods, NIPT substantially reduces the risks of miscarriage and maternal complications, while offering high sensitivity and specificity within the clinical detection window of 10–25 weeks of gestation [4-5].

However, the accuracy of NIPT results depends strongly on the proportion of fetal chromosomal fractions in maternal blood [6]. In male fetuses, Y-chromosome concentration serves as the primary marker [7], whereas in female fetuses, Z-scores of autosomes and the X-chromosome are generally adopted [8]. Clinical evidence indicates that fetal chromosomal fraction is significantly influenced by maternal gestational weeks and body mass index (BMI) [9]. In particular, higher BMI is associated with lower fetal fraction due to increased background DNA from adipose tissue, which may lead to test failure, false negatives, or delayed detection. Conversely, earlier gestational testing (before 12 weeks) may yield insufficient fetal fraction, but postponing testing increases clinical intervention risks [10]. Thus, selecting the optimal testing time point is a critical factor for improving NIPT reliability and ensuring effective clinical decision-making.

Previous studies have attempted to address this challenge by grouping pregnant women according to BMI and adopting uniform testing schedules [11]. Yet, such approaches fail to fully account for individual differences in maternal characteristics, sequencing quality, and biological variability. Consequently, there is a pressing need for quantitative methods to model the relationship between fetal fraction and maternal factors, so as to identify optimal NIPT timing that balances early detection with diagnostic accuracy.

Another challenge lies in abnormality detection for female fetuses. Since female fetuses lack Y-chromosome markers, alternative approaches rely on autosomal and sex-chromosomal Z-scores, adjusted by sequencing quality indicators. Nevertheless, standard Z-score thresholds may be unreliable under conditions of low sequencing depth or high maternal BMI, leading to missed or ambiguous diagnoses. Developing quality-corrected models that integrate statistical learning with clinical interpretability can therefore provide more robust detection pipelines.

In this study, a comprehensive quality-corrected modeling framework for NIPT is proposed. For male fetuses, multivariate nonlinear regression models are constructed to quantify the effects of gestational weeks and BMI on Y-chromosome fraction, followed by ordered clustering and risk-based optimization to determine optimal detection windows across BMI subgroups. Monte Carlo simulations are further employed to assess robustness under measurement errors and sequencing variability. For female fetuses, a dual-model diagnostic strategy combining logistic regression with random forest classification, integrating Z-score normalization, sequencing quality correction, and BMI-specific adjustments, is introduced [12].

The contributions of this study are threefold:

(1) Model-based optimization of NIPT timing: A nonlinear regression and clustering approach identifies BMI-specific optimal testing windows for male fetuses.
(2) Robust risk assessment under uncertainty: Monte Carlo simulations highlight sequencing quality as the dominant error factor, particularly in high-BMI groups.
(3) Integrated abnormality detection pipeline for female fetuses: A logistic–random forest model with quality correction improves detection accuracy and clinical applicability [13].
Overall, this work provides both methodological and clinical contributions by offering interpretable, data-driven solutions for NIPT timing optimization and abnormality detection, which can enhance prenatal screening outcomes and reduce maternal-fetal health risks.

## 2 METHODS

### 2.1 Data Preprocessing

The raw sequencing data and supporting clinical information (including maternal height, weight, gestational week records, and fetal Y-chromosome concentration detection data, etc.) required for this study were all obtained from the Maternal and Child Health Special Dataset officially provided by the Chinese Society for Industrial and Applied Mathematics (CSIAM). To ensure the reliability of subsequent analysis results, strict multi-dimensional quality control was conducted on the acquired raw data, with the specific process as follows:
Samples with abnormal GC content (<40% or >60%) or poor sequence alignment quality (<70% unique mapping ratio) were excluded [14]. For gestational week records, those in the mixed "weeks + days" format (e.g., "38 weeks + 5 days") were converted into continuous numerical values (e.g., 38.71 weeks) to unify the measurement standard. Maternal BMI was recalculated using the formula BMI = weight/height² from the recorded maternal height (unit: m) and weight (unit: kg) to correct for potential input errors in the original data. For the fetal Y-chromosome concentration index, outliers (including negative values and extreme values greater than 0.20) were identified and removed using the interquartile range (IQR) method, and were secondarily validated against clinically recognized thresholds (the normal reference range for fetal Y-chromosome concentration is 0.02-0.18) to ensure the data are clinically meaningful.
After data preprocessing, descriptive statistics (including mean, standard deviation, median, quartiles, etc.) were generated for the three core indicators of gestational weeks, maternal BMI, and fetal Y-chromosome concentration to establish a database of baseline characteristics of the study subjects and lay the foundation for subsequent analyses.

### 2.2 Modeling Fetal Y-Chromosome Concentration (Male Fetuses)

To quantify the relationship between fetal Y-chromosome concentration and maternal characteristics, both linear and nonlinear models were explored. Scatterplots suggested a quadratic association with gestational weeks and an exponential decline with BMI. Consequently, a multivariate nonlinear regression model was constructed, incorporating quadratic terms for gestational weeks, interaction terms between BMI and X-chromosome fraction, and logarithmic transformations for GC content. The model was expressed as:

$$Y=\beta_0+\beta_1(\text{Gestational Weeks})^2+\beta_2\cdot BMI+\beta_3(BMI\times X)+\beta_4\ln(GC)+\varepsilon \quad (1)$$

where YYY denotes Y-chromosome fraction. Model fit was evaluated using coefficient of determination ($R^2$), root mean square error (RMSE), and residual analysis. The final model achieved $R^2 = 0.6888$, confirming strong explanatory power.

### 2.3 BMI Grouping and Optimal NIPT Timing

To determine optimal NIPT windows, maternal BMI values were grouped using ordered clustering guided by the elbow method. Candidate cut-off points (28.0, 32.0, 36.0, and 40.0) were evaluated under the criterion of minimizing within-group variance in time-to-threshold attainment ($Y \geq 0.04$). Four groups were retained, each containing ≥30 samples.
An expected risk function was defined to balance diagnostic accuracy and clinical intervention risk:

$$E(R,t)=P(t)\cdot R(t)+\big(1-P(t)\big)\cdot R(t+2) \quad (2)$$

where $P(t)$ is the proportion of samples reaching the threshold at time $t$, and $R(t)$ represents gestational risk (0.3 for early, 0.5 for mid, 0.7 for late window). The optimal time point was selected as the earliest $t$ satisfying $P(t)\geq 90\%$ and minimizing $E(R,t)$. Results indicated that higher BMI groups required later detection windows, with optimal timings ranging from 16.5 weeks (BMI < 28) to 23.5 weeks (BMI ≥ 40).

### 2.4 Robustness Analysis under Measurement Errors

To account for sequencing variability, error modeling was performed using residual distributions from repeated samples (≥3 tests). Measurement error was assumed to follow a normal distribution:

$$Y_{obs}=Y_{true}+\epsilon, \quad \epsilon \sim N(0,\sigma^2) \quad (3)$$

Monte Carlo simulations (n=1000 per group) were conducted under low, medium, and high error levels. The analysis showed that optimal timing shifts positively with error magnitude (0.5–1.5 weeks delay), with high-BMI groups more sensitive to errors. Sequencing depth was identified as the dominant factor; increasing reads to >5 million significantly reduced variability.

### 2.5 Abnormality Detection in Female Fetuses

Unlike male fetuses, female fetuses lack Y-chromosome markers. Abnormality detection was therefore based on standardized Z-scores of chromosomes 13, 18, 21, and X [15-16]. Quality correction was implemented by down-weighting unreliable samples (e.g., low sequencing quality score, abnormal GC content).
A dual-model strategy was adopted:
(1) Logistic Regression – to provide interpretability by modeling abnormality probability from selected features (Z-scores, BMI category, sequencing quality index).
(2) Random Forest – to enhance classification accuracy by capturing nonlinear interactions among variables.
The Youden index was applied to determine the optimal decision threshold ($\tau = 0.35$). The final clinical workflow included four steps: (i) quality screening, (ii) rapid Z-score evaluation, (iii) probability-based classification, and (iv) BMI-specific correction. This approach enabled robust classification into "normal," "suspected abnormal," and "abnormal" categories.

## 3 METHODS

### 3.1 Descriptive Statistics

After preprocessing, 445 valid male-fetus samples were retained. The mean gestational age was 16.8 weeks (SD = 4.1), with BMI averaging 32.3 (SD = 3.0). The mean fetal Y-chromosome fraction was 0.077, ranging from 0.01 to 0.23. As shown in Table 1, Y-chromosome concentration exhibited moderate variability (SD = 0.0335), with skewness (0.71) and kurtosis (1.01) indicating slight right-tailed distribution.

**Table 1** Descriptive Statistics of Maternal and Fetal Indicators

| Indicator | Mean | Median | SD | Min | Max |
|---|---|---|---|---|---|
| Gestational weeks | 16.85 | 16 | 4.08 | 11 | 29 |
| Maternal BMI | 32.29 | 31.81 | 2.97 | 20.7 | 46.9 |
| Y-chromosome (%) | 0.077 | 0.075 | 0.0335 | 0.01 | 0.23 |

Scatterplot visualization confirmed nonlinear associations: Y-chromosome fraction increased quadratically with gestational age ($R^2 = 0.58$ for quadratic fit vs. 0.32 linear), while declining exponentially with BMI ($R^2 = 0.49$ for exponential fit).

### 3.2 Model Fitting for Y-Chromosome Concentration

The multivariate nonlinear regression model incorporating quadratic, logarithmic, and interaction terms achieved $R^2 = 0.6888$ and RMSE = 0.0284, outperforming linear models ($R^2 = 0.52$). Residual analysis showed no systematic deviations, validating model adequacy. These results indicate that both gestational weeks and BMI are significant predictors of fetal fraction, with BMI exerting stronger negative influence.

### 3.3 BMI Grouping and Optimal Testing Time

Using ordered clustering and the elbow method, BMI thresholds of 28, 32, and 36 were identified, producing four groups with ≥30 cases each. Within-group variance in time-to-threshold attainment was minimized, confirming robust subgrouping.
The optimal NIPT detection time was defined as the earliest gestational week satisfying $P(t) \geq 90\%P(t) \geq 90\backslash\%P(t) \geq 90\%$ and minimizing expected risk. As shown in Figure 1, higher BMI shifted the optimal detection time later. Final results were:
(1) Group 1 (BMI < 28): 16.5 weeks
(2) Group 2 (28 ≤ BMI < 32): 18.0 weeks
(3) Group 3 (32 ≤ BMI < 36): 21.5 weeks
(4) Group 4 (BMI ≥ 36): 23.5 weeks

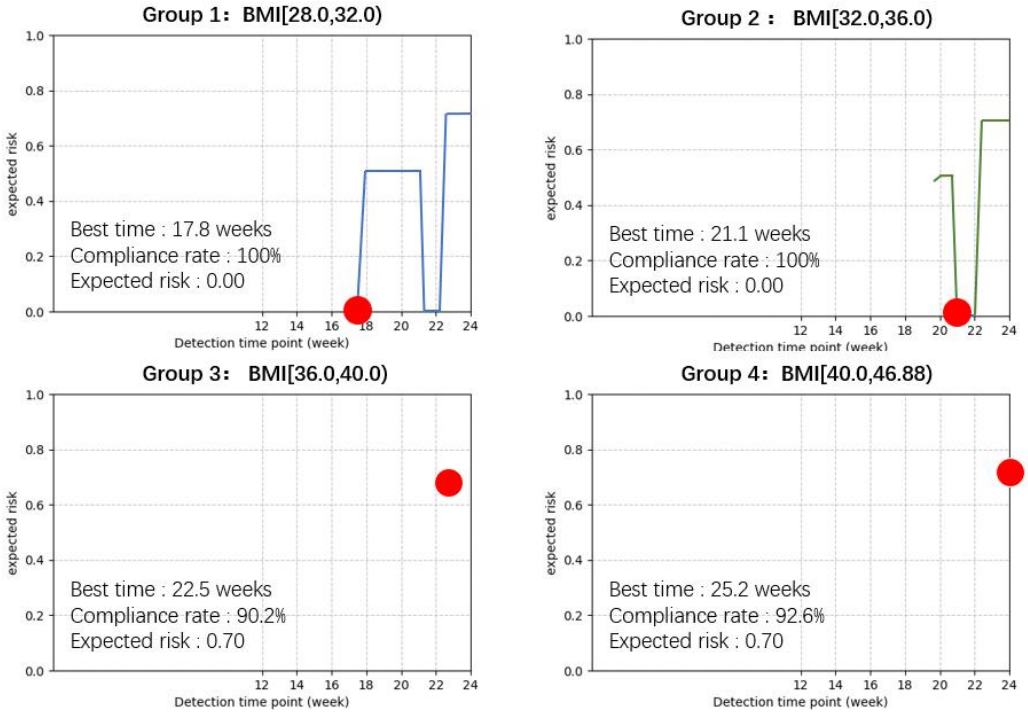


**Figure 1** Expected Risk Across Gestational Weeks in Different BMI Groups

This finding highlights that universal testing schedules may be suboptimal, and BMI-specific adjustment is required for accurate and timely detection.

### 3.4 Robustness to Measurement Errors

Monte Carlo simulations demonstrated that measurement error level was positively correlated with optimal testing delay. Under high-error scenarios ($\sigma > 0.015$), detection was delayed by 1–1.5 weeks compared to low-error settings. Group 4 (BMI $\geq$ 40) was most sensitive, with optimal timing shifting from 23.5 to 24.5 weeks. Sensitivity analysis identified sequencing depth as the dominant error source; increasing reads to >5 million stabilized detection windows across BMI groups.

### 3.5 Abnormality Detection in Female Fetuses

For female fetuses, standardized Z-scores of chromosomes 13, 18, 21, and X were analyzed. Logistic regression provided baseline interpretability, while random forest improved classification accuracy. The Youden index identified 0.35 as the optimal probability threshold.

The ROC curves of the two classifiers in the determination of chromosomal abnormalities in female fetuses ( including the dynamic correspondence between sensitivity and 1-specificity ) are shown in Figure 2 ( the ROC curve for the determination of chromosomal abnormalities in female fetuses ). It can be visually observed that the ROC curve of the random forest model is located above the logistic regression model, reflecting its advantages in the ability to distinguish abnormal classification. However, there is a common problem of decreased sensitivity in the high-specificity interval ( 1-specificity < 0.2 ), which also provides an optimization basis for the subsequent integration model and BMI specificity correction.

Cross-validation showed that logistic regression achieved 67.0% accuracy (AUC = 0.55), while random forest yielded higher overall accuracy (75.7%) but lower sensitivity (~12%). Integrating both models with sequencing quality screening and BMI-specific correction produced a four-step workflow (quality check → Z-score evaluation → probability assessment → BMI correction). This pipeline enhanced robustness and reduced false negatives in high-BMI subgroups.

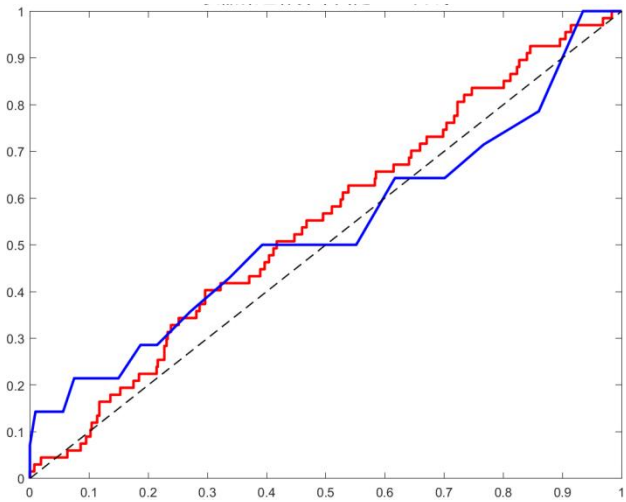


**Figure 2** ROC Curves of Logistic Regression and Random Forest Classifiers

### 3.6 Summary of Findings

(1) Fetal Y-chromosome fraction follows a quadratic growth with gestational weeks and decreases exponentially with BMI.
(2) BMI-specific testing windows significantly improve NIPT accuracy, with high-BMI women requiring later detection times.
(3) Sequencing quality is the primary source of error; improving read depth effectively stabilizes outcomes.
(4) Female fetal abnormality detection benefits from a dual-model strategy with quality correction, enhancing clinical applicability.

## 4 DISCUSSION

### 4.1 Model Advantages

The proposed quality-corrected framework demonstrates several advantages over conventional NIPT practices. First, it effectively addresses variability in raw sequencing data by integrating multi-dimensional quality control measures. For example, GC content filtering and alignment ratio thresholds ensured data reliability, while recalculated BMI values corrected potential entry errors. This preprocessing strategy eliminated noise and provided a robust foundation for downstream modeling.
Second, the framework offers strong interpretability and logical consistency. The nonlinear regression model for male fetuses was grounded in clinical and biological principles, with coefficients corresponding to gestational growth dynamics, BMI-related dilution effects, and sequencing quality factors. Likewise, the ordered clustering of BMI groups produced results consistent with clinical experience, revealing that higher BMI systematically delays the optimal NIPT timing. For female fetuses, the dual-model strategy balanced interpretability (logistic regression) with predictive performance (random forest), aligning with the dual clinical requirements of "accuracy" and "explainability."
Third, the clinical practicality of the models is significant. The expected risk function explicitly incorporated routine re-testing intervals, thereby enhancing real-world applicability. BMI-specific detection windows provided a feasible guideline for obstetricians, replacing the "one-size-fits-all" approach. The female abnormality detection pipeline, incorporating Z-score thresholds and BMI-specific corrections, ensured flexible yet reliable decision-making in diverse clinical contexts.

### 4.2 Clinical Implications

The study findings have direct implications for prenatal care. For male fetuses, BMI-adjusted testing schedules can reduce false negatives and avoid unnecessary repeat testing. By demonstrating that optimal detection shifts later with increasing BMI, clinicians can personalize testing recommendations, improving accuracy without compromising timeliness. The robustness analysis also highlighted sequencing depth as the primary determinant of stability, suggesting that increasing read coverage is a cost-effective intervention for reducing risk in high-BMI populations.
For female fetuses, the proposed dual-model workflow provides a structured decision pipeline that aligns with clinical practice. The integration of sequencing quality screening prevents unreliable data from driving false diagnoses, while BMI-specific corrections mitigate under-detection in high-BMI subgroups. Importantly, the inclusion of SHAP-based interpretability enhances clinician confidence, facilitating transparent communication with patients and improving the uptake of clinical recommendations.

### 4.3 Limitations

Despite these strengths, the study has several limitations. The models were developed under a set of assumptions that may not fully generalize to complex clinical settings. For instance, comorbidities such as diabetes, hypertension, or placental dysfunction were not explicitly included, though these factors could affect fetal fraction dynamics. Similarly, the abnormality detection framework focused on trisomies 13, 18, 21, and the X chromosome, while rare aneuploidies (e.g., chromosomes 16 or 22) were not considered. Extreme technical errors, such as sequencing platform failures or contamination, were also outside the model scope.

Moreover, the relatively low sensitivity of the random forest classifier in female-fetus detection suggests that further refinement is necessary, particularly for balancing specificity with recall. Additionally, the dataset was drawn primarily from high-BMI populations, which may limit generalizability to broader maternal demographics.

### 4.4 Future Directions

Future research should expand the scope of maternal covariates by incorporating clinical comorbidities, lifestyle factors, and genetic history into predictive models. Multi-center validation across diverse populations will be essential to ensure robustness and external applicability. On the methodological side, hybrid machine learning approaches—such as ensemble stacking or deep learning models—may improve female abnormality detection without sacrificing interpretability. Finally, integrating cost-effectiveness analysis into model design could support clinical guideline development, ensuring both scientific rigor and healthcare efficiency.

## 5 CONCLUSION

This study proposed a quality-corrected modeling framework to optimize the timing of non-invasive prenatal testing (NIPT) for male fetuses and to improve abnormality detection strategies for female fetuses. By integrating nonlinear regression, ordered clustering, and risk-based optimization, we demonstrated that Y-chromosome fraction is strongly influenced by both gestational weeks and maternal BMI, and that higher BMI systematically delays the optimal detection window. The derived BMI-specific testing schedules, ranging from 16.5 to 23.5 weeks, provide actionable guidance for clinicians to improve reliability and reduce unnecessary re-testing.

Robustness analyses confirmed that sequencing quality is the dominant error source, especially in high-BMI groups, highlighting the importance of maintaining sufficient read depth in clinical practice. For female fetuses, a dual-model diagnostic approach combining logistic regression and random forest achieved reliable performance when integrated with quality control and BMI-specific correction. The structured workflow supports clinical decision-making by balancing accuracy, interpretability, and practicality.

Overall, the proposed framework enhances the scientific basis of NIPT, offering interpretable and clinically implementable solutions that can improve prenatal screening outcomes, reduce maternal-fetal health risks, and inform the development of personalized testing guidelines. Future extensions incorporating comorbidities, larger datasets, and advanced learning methods will further strengthen the generalizability and impact of this work.

## COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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