

**RESEARCH ARTICLE****Identification of Gender in Early Pregnancy Using Cell-Free Fetal DNA**Budi Junio Hermawan^{1,*}, Bobby Indra Utama², Andani Eka Putra³¹ *Obstetric and Gynecology Department, Dr. M. Djamil Central General Hospital/Faculty of Medicine Andalas University, Padang, West Sumatra, Indonesia*² *Urogynecologic Division, Obstetric and Gynecology Department, Dr. M. Djamil Central General Hospital/Faculty of Medicine, Andalas University, Padang, West Sumatra, Indonesia*³ *Center for Diagnostic and Research on Infectious Diseases (PDRPI), Faculty of Medicine, Andalas University, Padang, West Sumatra, Indonesia***Corresponding Author:** Budi Junio Hermawan, budijunio97@gmail.com**Submitted:** September 16, 2025;**Accepted:** January 20, 2026;**Published:** February 02, 2026**DOI:** <https://doi.org/10.25077/aoj.10.1.135-144.2026>**Abstract**

Introduction: Traditionally, chorionic villus sampling and amniocentesis can be used to help identify fetal gender around 11 weeks of pregnancy. However, the risk of miscarriage with these procedures is approximately 1 to 3%. The discovery of cell-free fetal DNA (cffDNA) fragments in the plasma and serum of pregnant women carrying male fetuses has made reliable and accurate diagnosis using non-invasive procedures an option for determining fetal gender.

To determine the success rate of fetal gender identification in early pregnancy using Cell Free Fetal DNA.

Methods: An analytical observational research method to identify fetal gender in early pregnancy through Cell Free Fetal DNA examination in maternal blood. A total of 70 samples were collected using quota sampling technique. Data analysis was performed using sensitivity and specificity tests, as well as cross-sectional analysis to determine the relationship between PCR test results and gender at birth.

Results: The PCR test has low sensitivity (14.29%), meaning the test is less effective in correctly detecting male gender, resulting in many false negatives. However, it has high specificity (98.11%), indicating its excellent ability to detect female gender with very few false positives. Additionally, the Pearson Chi-Square statistical test resulted in a p-value of 0.033 ($p < 0.05$), indicating a statistically significant relationship between PCR test results and gender at birth.

Conclusion: Statistically, this test is better at identifying females than males, with a lower error rate for females.

Keywords: Pregnancy, cell-free fetal DNA, gender, early pregnancy

INTRODUCTION

Contemporary methods for determining fetal sex largely rely on ultrasonographic imaging in the second trimester to identify the presence of the penis and scrotum in male fetuses or labial folds in female fetuses. However, ultrasonographic determination of fetal sex has been reported to be inaccurate in up to 40% of cases. Traditionally, chorionic villus sampling (CVS) and amniocentesis have been used to identify fetal sex as early as 11 weeks of gestation; nevertheless, these procedures are associated with a miscarriage risk of approximately 1–3%.^{1,2}

Prenatal diagnostic techniques are broadly classified into invasive and non-invasive procedures. At present, invasive methods such as amniocentesis and CVS are routinely employed, although both carry a small but significant risk to both the fetus and the mother. Conversely, non-invasive procedures such as ultrasonography have relatively low sensitivity and are typically performed during the second trimester of pregnancy, resulting in delayed diagnosis particularly problematic in pregnancies at high risk of genetic disorders.³

In 1997, the discovery of cell-free fetal DNA (cffDNA) fragments in the plasma and serum of pregnant women carrying male fetuses marked a major breakthrough, enabling reliable and accurate prenatal diagnosis using non-invasive techniques. Subsequent studies using quantitative real-time PCR demonstrated that cffDNA concentrations in maternal plasma originating primarily from apoptotic placental trophoblasts reach an average of 25.4 genome equivalents (GEq)/mL in early pregnancy and 292.2 GEq/mL in late pregnancy. Notably, the proportion of cffDNA increases with gestational age, from approximately 3.4% to 6.2% of total circulating DNA in maternal plasma. Furthermore, cffDNA becomes undetectable within 4–30 minutes after delivery due to its rapid clearance from maternal circulation, with a mean half-life of 16.3 minutes, making it an ideal source for prenatal investigation without residual confounding effects.^{4,5}

Although the presence of circulating cell-free nucleic acids (cfNA) in human blood was first described in 1948 by Mandel and Métais, cell-free DNA has only recently attracted significant research interest. The identification of cffDNA in maternal plasma has expanded the boundaries of non-invasive prenatal diagnosis, enabling fetal sex determination at an early stage of pregnancy. This advancement is particularly important for the management of X-linked genetic disorders, such as haemophilia, fragile X-linked intellectual disability, and Duchenne muscular dystrophy, in which early diagnosis is crucial.^{1,2}

Non-invasive prenatal diagnosis, unlike invasive techniques such as CVS or amniocentesis, does not carry a risk of miscarriage which is highest during the first trimester and can be performed earlier in pregnancy. Circulating cffDNA originates from placental trophoblasts and accounts for approximately 3–13% of the total maternal DNA isolated from venous blood. The amount of cffDNA increases as pregnancy progresses and can be detected as early as 4–5 weeks of gestation. The average fragment size of cffDNA is approximately 150 base pairs.^{6,7}

One of the primary applications of prenatal diagnosis is fetal sex determination, mainly to guide the management of X-linked genetic disorders. Fetal sex determination using cffDNA in maternal plasma is largely limited to paternally inherited sequences absent from the maternal

genome, such as the SRY, DYS14, and DAZ sequences located on the Y chromosome. Consequently, detection of these sequences is only possible in pregnancies carrying male fetuses.^{4,6}

Several studies have reported early fetal sex determination; however, most utilised cffDNA extracted from maternal venous blood, excluding the possibility of capillary blood sampling for individuals who cannot tolerate venepuncture. Capillary blood sampling is preferred by many patients, as it is simpler and less painful. When contamination with exogenous male DNA at the sampling site is avoided, this method may serve as a reliable alternative to conventional venous sampling. To date, the only reference to analysis performed using capillary blood appears in the abstract eBook of the 69th Annual Scientific Meeting of the American Association for Clinical Chemistry (AACC), without methodological details. Additionally, a study by Xiong *et al.* described fetal sex determination using dried maternal capillary blood spots (DYS14 marker; specificity = 91.6%) in a sample of 19 cases.⁸

This study aims to evaluate the success rate of early fetal sex identification through detection of cell-free fetal DNA in maternal blood, thereby avoiding the risks associated with invasive procedures such as CVS and amniocentesis. This non-invasive approach has demonstrated high accuracy and holds promise for future large-scale implementation, enabling early determination of fetal sex during pregnancy.

METHODS

This study employed an analytical observational design to evaluate early fetal sex determination in early pregnancy through detection of cell-free fetal DNA (cffDNA) in maternal blood. The study was conducted at Puskesmas Seberang Padang, Indonesia, between January and February 2024.

The study population consisted of 70 pregnant women in the first trimester who attended routine antenatal care during the study period. All eligible participants with a gestational age of 6–14 weeks were included using a quota sampling approach. Gestational age was determined based on the first day of the last menstrual period and confirmed using antenatal records. Inclusion criteria were confirmed pregnancy, gestational age between 6 and 14 weeks, and provision of written informed consent. Exclusion criteria included suspected abortion, molar pregnancy, blighted ovum, a history of coagulation disorders, or refusal to participate.

Venous blood samples were collected from all participants using standard phlebotomy techniques under aseptic conditions. Maternal plasma was separated for DNA extraction. Detection of cffDNA was performed using polymerase chain reaction (PCR). Fetal sex determination was based on the detection of Y-chromosome-specific sequences in maternal plasma, indicating a male fetus, while absence of these sequences was interpreted as female.

Data were recorded using standardised research forms and analysed using SPSS version 26 (IBM Corp., 2019). Univariate analysis was performed to describe the distribution of the study variables.

Ethical approval for the study was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Andalas/RSUP Dr. M. Djamil Padang (Approval Number: DP.04.03/D.XVI.XI/158/2024). Written informed consent was obtained from all participants prior to enrolment, and the study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Participant Characteristics

A total of 70 pregnant women in early pregnancy were included in the analysis. The majority of participants were aged 26–35 years. Most participants were housewives, and the predominant gravidity was gravida 2. Gestational age at the time of blood sampling ranged from 3 to 12 weeks, with most samples obtained between 6 and 9 weeks of gestation. Detailed demographic and obstetric characteristics are presented in Table 1.

Table 1. Maternal Characteristics of the Study Participants

Characteristic	Category	Number (n)	Percentage (%)
Age (years)	18–25	23	32.86
	26–35	31	44.29
	36–41	16	22.86
Occupation	Housewife	50	71.43
	Labourer	1	1.43
	Physician	1	1.43
	Teacher	1	1.43
	Employee	6	8.57
	Civil servant	4	5.71
	Self-employed	6	8.57
	Sales promotion staff	1	1.43
Gravidity	1	21	30.88
	2	26	38.24
	3	13	19.12
	4	4	5.88
	5	2	2.94
	6	2	2.94
Gestational age (weeks)	3	1	1.43
	5	9	12.86
	6	12	17.14
	7	13	18.57
	8	7	10.00
	9	14	20.00
	10	7	10.00
	11	4	5.71
	12	3	4.29

PCR-Based Fetal Sex Detection According to Gestational Age

The performance of PCR-based fetal sex determination varied according to gestational age (Table 2). No correct identification was observed at 3 and 5 weeks of gestation. Initial detection of male fetuses was observed at 6 and 7 weeks, with one correctly identified male fetus at each gestational age. The highest number of correctly identified male fetuses was observed at 9 weeks of gestation, although misclassification still occurred.

Across gestational ages below 9 weeks, the majority of male fetuses were not detected by PCR, while female fetuses were predominantly classified correctly. Beyond 9 weeks, no consistent improvement in detection rate was observed.

Table 2. Comparison of PCR Test Results with Sex at Birth According to Gestational Age

Gestational Age (weeks)	Sex at Birth	PCR Result: Female	PCR Result: Male
3	Female	0	0
	Male	1	0
	Total births	1	0
5	Female	6	0
	Male	2	0
	Total births	8	0
6	Female	9	0
	Male	2	1
	Total births	11	1
7	Female	10	0
	Male	2	1
	Total births	12	1
8	Female	5	0
	Male	2	0
	Total births	7	0
9	Female	8	1
	Male	4	1
	Total births	12	2
10	Female	5	0
	Male	2	0
	Total births	7	0
11	Female	1	0
	Male	3	0
	Total births	4	0
12	Female	3	0
	Male	0	0
	Total births	3	0

Diagnostic Performance of PCR-Based Fetal Sex Determination

Comparison between PCR results and fetal sex at birth is shown in Table 3. Of 20 male neonates, only 3 cases were correctly identified prenatally as male, whereas 17 cases were incorrectly classified as female. Among 50 female neonates, 49 cases were correctly identified, with only one false-positive male result.

The association between PCR results and fetal sex at birth was statistically significant ($p = 0.034$). However, the confidence interval was wide (95% CI: 0.842–88.825), indicating substantial variability.

Table 3. Association between PCR Test Results and Sex at Birth

PCR Test Result	Sex at Birth: Male	Sex at Birth: Female	95% CI	p-value
Male	3	1	0.842 – 88.825	0.034*
Female	17	49	-	-

*Note: CI = confidence interval; p-value indicates statistical significance. $p < 0.05$ indicates a statistically significant association.

Sensitivity and Specificity

The diagnostic performance of PCR-based fetal sex determination is summarised in Table 4. The sensitivity for detection of male fetuses was 15%, whereas the specificity was 98%. This indicates a marked discrepancy between the ability of the test to correctly identify male fetuses and its ability to correctly exclude male sex in female fetuses.

Table 4. Sensitivity and Specificity of PCR Test Results

Measure	Value
True Positive (TP)	3
False Negative (FN)	17
Sensitivity	15%
True Negative (TN)	49
False Positive (FP)	1
Specificity	98%

DISCUSSION

This study evaluated the performance of cell-free fetal DNA (cffDNA) analysis using PCR for early fetal sex determination during the first trimester of pregnancy. The main finding is the very low sensitivity for detecting male fetuses, contrasted with a very high specificity for identifying female fetuses. Although a statistically significant association was observed between PCR results and sex at birth, the diagnostic performance was clearly asymmetric, indicating that the test was more reliable for excluding male sex than for confirming it.

cffDNA is a non-invasive prenatal testing method that detects fragmented fetal DNA released into the maternal circulation from placental trophoblasts. Since its initial description by Lo et al. in 1997, cffDNA has gained widespread clinical interest due to its safety and earlier applicability compared with invasive procedures such as amniocentesis and chorionic villus sampling.⁹⁻¹¹ Detection of Y-chromosome-specific sequences forms the molecular basis for identifying male fetal sex, whereas the absence of these markers is interpreted as female sex determination.

Despite these advantages, the present findings demonstrate limited sensitivity of cffDNA-based PCR for male fetal sex detection in early gestation. In this study, only a small proportion of male fetuses were correctly identified, resulting in a high false-negative rate. This observation is consistent with previous reports indicating that early gestational testing is constrained by low fetal DNA concentration in maternal plasma, particularly before 7 weeks of gestation.^{4,12}

Gestational age plays a critical role in test performance. Fetal DNA becomes detectable in maternal plasma from approximately 5–7 weeks of gestation, with the fetal fraction increasing progressively thereafter.³ At very early gestational ages, insufficient fetal DNA may lead to failure in amplifying Y-chromosome markers, explaining the low sensitivity observed in this study. Similar gestational age-dependent variations in diagnostic accuracy have been reported in previous studies evaluating early non-invasive prenatal testing.^{12,13}

Technical factors may further contribute to reduced sensitivity. PCR-based assays are highly dependent on sample integrity, DNA extraction efficiency, and amplification accuracy. Suboptimal sample handling, delayed processing, and DNA degradation may disproportionately affect the detection of low-abundance fetal DNA fragments, particularly in early pregnancy.^{14,15} In addition, the predominance of maternal DNA in circulation can obscure fetal genetic signals, increasing the likelihood of false-negative results.

Biological variability among pregnancies also influences cffDNA test performance. Differences in placental DNA shedding, maternal physiological conditions, and fetal genetic characteristics may affect the fetal fraction available for analysis.¹⁶⁻¹⁹ These biological factors may explain why cffDNA testing performs well in some pregnancies but remains unreliable in others, particularly during early gestation.

From a clinical perspective, these findings indicate that cffDNA-based PCR testing in early pregnancy should be interpreted cautiously. While a positive result indicating male fetal sex is highly reliable due to the high specificity observed, a negative result does not reliably exclude male sex in early gestation. Previous studies have similarly emphasised the need for confirmatory testing at later gestational ages when fetal DNA concentration is higher and test accuracy improves.^{11,13}

CONCLUSION

In conclusion, although cffDNA analysis offers clear advantages as a non-invasive prenatal testing method, its diagnostic accuracy for fetal sex determination in early pregnancy is limited by low sensitivity for male fetuses. These results highlight the importance of gestational age selection, technical optimisation, and cautious clinical interpretation when applying cffDNA testing in early

pregnancy. Further refinement of analytical methods and gestational age–stratified testing strategies may enhance its clinical utility in the future.

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