



SneakPeek Early Gender Test: The Earliest and Most Accurate Method for Fetal Sex Determination at 6-Weeks Gestation

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ABSTRACT

Background: The SneakPeek[®] Early Gender Test was previously shown to be 99.9% accurate in determining fetal sex as early as seven weeks of gestation. To our knowledge, no prior study has shown fetal sex identification from maternal plasma earlier than seven weeks of gestation with high accuracy. The SneakPeek assay was further optimized to enable fetal sex identification at earlier gestations and its performance characteristics were determined at 6-weeks' gestation.

Method: Between May and September 2021, 156 pregnant women were recruited from 12 clinics in the United States. After excluding participants that reported miscarriage during the study, reported twin/triplet pregnancy, or miscalculated gestational age at the time of the initial sample collection, 115 individuals completed the trial in its entirety. Three to four milliliters (mL) of maternal blood were drawn from each participant *via* venipuncture at three gestational ages (i.e., 6, 7, and 8-10 weeks' gestation). Maternal plasma was separated from whole blood *via* centrifugation. Using a commercial DNA isolation kit, circulating cell-free DNA (ccfDNA) was extracted from the maternal plasma samples. Real-time quantitative PCR was utilized to detect male fetal DNA using a multi-copy sequence on the Y chromosome and total cell-free DNA using an autosomal control target gene. Fetal sex was determined from the qPCR results using an algorithm that includes the Quantification Cycle (Cq) values of the Y-target sequence and autosomal control gene. Since the SneakPeek[®] Early Gender Test was found to be 99.9% accurate at 7-weeks' and 8-weeks' gestation or later, the fetal sex test results for six-week gestation samples were confirmed with the 7-week and 8-week to 10-week sample results. Sonography was also used to confirm SneakPeek test results for 103 pregnancies in the study.

Results: SneakPeek accurately determined fetal sex at six weeks for 113 subjects in this study, results for two subjects were inconclusive. Test results at later gestational ages (7 and 8+ weeks) matched the six-week test results. All sonogram results matched the SneakPeek test results in this study. SneakPeek accuracy, sensitivity, and specificity were 100%, 100%, and 100% for fetal sex identification, respectively. No false positive or false negative results were observed in this study.

Conclusion: SneakPeek[®] Early Gender Test was shown to be 100% accurate for fetal sex determination at 6 weeks gestation. SneakPeek gender test is the earliest and most accurate method for fetal sex determination. The SneakPeek Early Gender DNA Test can provide prenatal genetic information a month earlier than traditional noninvasive prenatal tests (e.g., NIPS).

Keywords: Sneak peek; NIPT; Early gender test; Pregnancy; Fetal sex; Maternal blood; Maternal plasma; Cell-free fetal DNA

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INTRODUCTION

Since the discovery of circulating cell-free fetal DNA (ccffDNA) in maternal plasma [1], noninvasive prenatal testing has been used for multiple diagnostic applications, including fetal RhD genotyping, aneuploidy detection, monogenic disorder screening, fetal sex identification, and X-linked abnormality determination [2]. It is generally acknowledged that knowing fetal sex as early as possible for pregnant women is essential to long term planning and further screening for sex-related genetic disorders. For instance, the mother may be a carrier of an X-linked condition like Duchenne muscular dystrophy, hemophilia, or other heritable disorders [3,4].

Non-medical reasons for learning fetal sex during pregnancy also exist. Multiple studies have shown that for over 50% of the participating parents [5], it was crucial to seek fetal sex determination for non-medical (social) reasons [6]. The option to determine fetal sex before delivery is a key element of the initial introduction of the unborn child to its parents. According to a study conducted in the United Kingdom, planning, preparation, and satisfying someone's curiosity are the most prevalent reasons parents want to learn fetal sex. Participants in another survey also highlighted the desire for a sense of control, peace of mind, and the belief that additional information would be advantageous [7]. Additional motivations for learning fetal sex include the willingness to establish a closer emotional connection with the fetus and the feeling of parenthood [8].

Sex determination for humans derives from analysis of the sex chromosomes. At the time of conception, the pairing of XX or XY typically results in the well-recognized biological sex. During embryonic development, the internal and external sexual structures begin to appear ten weeks postconception. Sonography, which identifies fetal sex through visual measures of sexual structure, can only be used for fetal sex determination after ten weeks of gestation. Furthermore, it is important to note that sonography only shows high accuracy after 13 weeks of gestation [9]. Several studies have shown that the accuracy of this method varies considerably, even when applying more advanced 3D ultrasound techniques. With further embryonic development, sonography shows greater reliability during the second trimester of pregnancy [10,11].

The first report of the existence of cell-free Y chromosome DNA sequences in the plasma of pregnant women was in 1997. Since that report, numerous groups worldwide have confirmed that Y chromosome sequences can be amplified and detected in maternal blood and used to differentiate between male and female fetuses. This research has since expanded by utilizing various techniques, sex-specific markers, and sample types throughout all gestational ages [12].

Over a decade ago, the findings from several studies suggested that the earliest gestational age of a successful noninvasive sex

detection is 4-5 weeks [13,14]. However, some of these earlier studies that revealed sex marker detection sooner than seven weeks of gestation could not be considered reliable for the following reasons: lack of controls, insufficient sample numbers, or incomplete sensitivity data (e.g., only male samples were tested) [15,16]. This presumption was also concluded by a systematic review of 57 selected studies regarding the overall test performance of noninvasive fetal sex determination using cell-free fetal DNA which found that the accuracy of these assays is more reliable after 7-weeks of pregnancy [12]. These findings are in agreement with current limitations of commercially available products.

To our knowledge, no prior study has shown fetal sex determination from maternal plasma earlier than seven weeks of gestation with high accuracy.

The present article shows the results from our most recent multicenter study for SneakPeek Early Gender Test. SneakPeek is an ultrasensitive qPCR-based assay for the detection of Y-chromosome DNA in maternal plasma. This study sought to determine the performance characteristics of SneakPeek at six weeks gestation. As a provider of an early gender test service, Gateway Genomics' goal is to provide expectant parents with knowledge of their baby's sex early in pregnancy. Previous studies demonstrated SneakPeek's ability to accurately determine fetal sex as early as seven weeks gestation with 99.9% accuracy [17-19]. The SneakPeek assay was further optimized to enable fetal sex identification at six weeks' gestation. In order to confirm the validity of the results, fetal sex was determined at three consecutive time points of the pregnancy (6, 7, and 8-10 weeks). Additionally, SneakPeek test results were compared with anatomy scan results completed around 18-20 weeks gestation.

METHODOLOGY

This study recruited 156 participants from 12 ultrasound clinics in the United States between May and September 2021. All participants provided informed consent following the Declaration of Helsinki's principles for human beings. The first blood sample was collected from each patient at six weeks gestation (calculated based on the first day of the last menstrual period, LMP). A second sample was collected at seven weeks gestation, followed by a third sample collected between 8 and 10 weeks (8+ weeks) of pregnancy. The study inclusion criteria were the following: woman, six weeks of gestational age with a willingness to provide maternal blood samples for three consecutive weeks. The exclusion criteria were: reported miscarriage at any gestational age during the study, reported twin/triplet pregnancy or miscalculated gestational age at the time of the first sample collection. In this study, twenty-seven subjects were excluded due to miscarriage, four subjects were excluded due to inaccurate gestational age at the time of enrollment, and ten individuals were excluded due to

insufficient blood volume collection. The final number of subjects that completed the study was 115.

At each time point, three to four milliliters (mL) of maternal blood was collected by venipuncture. Blood samples were sent to the SneakPeek laboratory at ambient temperature. Plasma was separated from whole blood by centrifugation at 1,600 g for 15 minutes. cfDNA was extracted from 100 uL plasma utilizing a proprietary DNA extraction method or stored at -80°C until further utilization.

Using real-time quantitative Polymerase Chain Reaction (RT-qPCR), male cell-free DNA and total cfDNA (maternal and fetal DNA) levels were analyzed in each plasma sample. A target sequence on the Y-chromosome was used to detect male cell-free DNA. Simultaneously, an autosomal control gene was used to demonstrate that an adequate quantity of cfDNA was extracted from each plasma sample. qPCR analysis was performed on male and female control samples (known concentration male/female plasma and male/female gDNA).

Fetal sex was determined using an algorithm that includes both the quantification Cycle (Cq) value of the Y-target sequence and the autosomal control gene for the qPCR. The prenatal sex results of the SneakPeek assay at 6 weeks were verified by the fetal sex results of the 7 and the 8+ week samples as well as follow up sonogram results. The SneakPeek assay was previously shown to be 99.9% accurate at seven weeks of gestation.

RESULTS

At the time of the initial draw, the gestational ages of study subjects ranged between 5.87 and 6.86 weeks. At the second draw, the gestational ages of study subjects ranged between 7.00 and 7.86 weeks. At the third and final draw, study subjects gestational ages ranged from 8.0 to 10.0 weeks (Table 1).

First draw (6 weeks)		Second draw (7 weeks)		Third draw (8 weeks)	
Gestational age (weeks)		Gestational age (weeks)		Gestational age (weeks)	
Range	6-6.86	Range	7-7.86	Range	8-10
Median	6.14	Median	7.14	Median	8
Mean	6.32	Mean	7.28	Mean	8.26

Table 1: Gestational ages of the 115 participants during each draw in the study.

SneakPeek correctly determined fetal sex at six weeks for 113 subjects in this study; two samples were inconclusive at this early time point. Testing at later gestational ages (7 and 8+ weeks) matched the six-week test results for all 113 samples. There were 48 male-bearing pregnancies and 65 female-bearing pregnancies in this study. SneakPeek accurately detected 48 of 48 male-bearing pregnancies, exhibiting a sensitivity of 100% for Y-chromosome DNA detection. No false negatives were observed in this study resulting in a 100% positive predictive value. SneakPeek accurately detected 65 of 65 female bearing

pregnancies in women. There were no false positives, resulting in a 100% negative predictive value. There were two inconclusive results among the six-week blood samples. Test performance results are shown in Table 2.

Number of Participants	115
6 Week samples analyzed	115
Female fetuses male	65
Male fetuses	48
Inconclusive results	2
False positives	0
False negative sensitivity	0
Ultrasound confirmation	103/103
Sensitivity	100%
Specificity	100%
Accuracy	100%
Positive predictive value	100%
Negative predictive value	100%

Table 2: Statistical parameters of the study at 6 weeks gestation.

Sonographic scans are considered a gold standard for fetal sex determination. Ultrasound screening results were obtained for 103 study participants. Sonogram results for fetal sex matched the SneakPeek test results for all 103 participants.

DISCUSSION

In summary, we report an effective and noninvasive method for accurate fetal sex determination at 6-weeks of pregnancy. Our approach utilizes a real-time qPCR assay in combination with an algorithm that relies on the presence/absence of a sex chromosome marker while considering the level of the autosomal target. This method can reduce the need for invasive procedures in pregnant women carrying an X-linked chromosomal abnormality and clarify inconclusive readings by ultrasound while providing knowledge about the unborn child and helping families plan and prepare very early in the pregnancy.

The SneakPeek® Early Gender Test was previously shown to be 99.9% accurate in detecting fetal sex as early as seven weeks of gestation. In this study, the SneakPeek® Early Gender Test was confirmed to be 100% accurate at six weeks gestation. This study demonstrates that SneakPeek can accurately identify fetal sex utilizing a microvolume amount (100 uL) of maternal plasma as early as six-weeks into pregnancy. Sonographic findings were also gathered and compared to confirm the accuracy of SneakPeek, all sonogram results matched the SneakPeek test results at 6 weeks' gestation.

A limitation of this study is that demographical characteristics and other maternal conditions such as obesity, hypertonia, and preeclampsia that can have an effect on the level of cffDNA were not recorded and examined. Furthermore, the ethical aspects of early fetal sex determination are not the subject of this article since it was broadly discussed in other articles.

CONCLUSION

This multicenter study demonstrated that with the SneakPeek Early Gender DNA Test, parents can discover the sex of their baby as early as six weeks into their pregnancy. There is no other technology or method in the world that can predict fetal sex at 6 weeks with greater than 99% accuracy. Additional refinements of the SneakPeek assay may enable fetal sex detection at time points closer to the first awareness of a pregnancy (e.g., 4 weeks gestation).

Gateway Genomics develops leading-edge prenatal tests that allow expectant parents to learn more about their unborn child sooner than ever before. The SneakPeek Early Gender DNA Test can provide prenatal genetic information a month earlier than traditional noninvasive prenatal tests (e.g., NIPS).

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