

Accurate fetal sex determination from maternal blood at 7 weeks gestation

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Abstract

Background: Multiple studies have demonstrated SneakPeek® Early Gender Test's ability to accurately determine fetal sex as early as 8 weeks gestation with greater than 99% accuracy. The qPCR-based assay was further optimized to determine fetal sex at 7 weeks gestation. The objective of this study was to evaluate SneakPeek's clinical performance for noninvasive prenatal testing (NIPT) to determine fetal sex as early as 7 weeks gestation.

Method: A multicenter non-randomized, controlled study was carried out at twelve healthcare clinics in the United States to assess the performance of the SneakPeek Early Gender Test for fetal sex identification at 7 weeks gestational age. In this study, 134 pregnant women consented to two blood draws: one draw at 7 weeks gestation and a second draw between 8- and 10-weeks' gestation. Plasma was separated from whole blood samples by centrifugation. Circulating cell-free DNA was isolated from maternal plasma using a DNA isolation kit for the 7- and 8 to 10-week gestation samples. After extracting the cell-free DNA from these samples, real-time quantitative PCR was used to detect fetal DNA using a multi-copy sequence on the Y chromosome. Maternal and fetal circulating free DNA (total cell-free DNA) was measured using an autosomal control gene. Test results from the 8 to 10-week gestation blood samples were used to confirm the 7-week test results.

Results: Male Y-chromosome was detected in all samples from participants carrying a male fetus. Inconclusive results were obtained for 3 pregnancies. SneakPeek correctly identified fetal sex in the remaining 131 pregnancies at 7 weeks gestation. Fetal sex status for all samples was unknown prior to genetic testing. Since SneakPeek® Early Gender Test was previously shown to be 99.9% accurate at 8 weeks or later gestation, the results for 7 week gestation samples were confirmed with the 8 to 10-week sample results. In this study, SneakPeek accuracy was 100% for fetal sex identification at 7 weeks gestation.

Conclusion: This study demonstrated that SneakPeek® Early Gender Test is highly accurate for fetal sex determination in pregnancy as early as 7 weeks gestation.

Keywords: 7 weeks gender blood test; SneakPeek accuracy; Gender blood test; SneakPeek; Gender test

Introduction

Noninvasive prenatal testing (NIPT) through the analysis of cell-free fetal DNA (cffDNA) has been prevalent in the biotech and health industry to provide useful information to expectant parents. Previous studies have shown that the SneakPeek® Early Gender DNA Test (SneakPeek) utilizes NIPT to confidently tell patients the sex of their baby as early as 8 weeks gestation [1-3].

Early fetal sex determination has evolved from invasive testing (ex: chorionic villus sampling, amniocentesis, etc) to less invasive means such as sonography and NIPT [4]. Since the first study of cffDNA in maternal plasma and serum was described by Dr. Y. M. Dennis Lo, the development of NIPT has expanded and studies have shown detectable cffDNA in maternal plasma as early as 5-weeks' gestation, although most tests are not highly accurate until after 7 weeks gestation [5,6].

SneakPeek surveyed 1,113 expectant mothers in 2019 behind the motivations of wanting to know fetal sex early [7]. The majority of general motivation for wanting to know early was straight curiosity (77.5%). For planning purposes, most parents (78.6%) wanted to know so they can start shopping for a baby. Another reason for wanting to know was so they can connect with family and friends with 54.2% of parents wanting to let grandparents or other relatives know. Another survey was conducted to evaluate the benefits of early fetal sex determination. Most expectant mothers (92.7%) indicated it was simply to satisfy their curiosity, while 51.3% of mothers also stated that it gave them a sense of control and a peace of mind [8]. Being able to

report fetal sex at 7 weeks will let mothers satisfy their curiosity, plan, and share the good news even earlier in the pregnancy.

A large number of benefits have been reported for non-invasive fetal sex identification [9,11-12]. For example, knowledge of fetal sex early in pregnancy can indicate if sex-linked genetic disorders are present. Psychological advantages of knowing fetal sex early include satisfying curiosity as well as normalization of pregnancy [9,12]. Furthermore, parents want to plan for the arrival of their new baby and knowing the baby's sex early in pregnancy helps with this planning [8].

Although sonography is still the most common method used for determining fetal sex, anatomy scans typically are not completed until 18-20 weeks gestation and not accurate until 12-13 weeks gestation. NIPT tests are most commonly provided at 9-10 weeks gestation [7]. Most pregnant women will not be seen by an OBGYN prior to 8 weeks gestation and will not have an option to determine fetal sex when they go in for their first prenatal checkup. Previous studies demonstrated SneakPeek's ability to accurately determine fetal sex as early as 8 weeks

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gestation with 99.9% accuracy [3]. The SneakPeek assay was further optimized to enable fetal sex identification at 7 weeks gestation. In this study, we aimed to validate the SneakPeek® Early Gender Test for fetal sex determination at 7 weeks gestation.

Methods

One hundred thirty-four participants from twelve ultrasound clinics were recruited for this study. Informed consent was obtained from all participants in accordance with the Declaration of Helsinki guidelines for human subjects. All participants provided a first blood sample at 7 weeks gestation and a second blood sample between 8- and 10-weeks' (8+-weeks) gestation.

During each sample collection, 3-4 mL of maternal blood was drawn from participants by standard venipuncture. Blood samples were sent to the SneakPeek lab and were tested 1-3 days after collection. The blood tubes were centrifuged at 1,600 g for 15 minutes to separate plasma from whole blood. cfDNA was isolated from 100uL plasma using proprietary DNA extraction technology.

Real-time quantitative polymerase chain reaction (RT-qPCR) was used to detect male cell-free DNA and total cfDNA (maternal and fetal DNA). A multi-copy target sequence on the Y-chromosome was used to detect male cell-free DNA while an autosomal control gene was detected to confirm that a sufficient amount of cfDNA was extracted from the sample.

An algorithm that included the cycle threshold (CT) value of the Y-target sequence and autosomal control gene for the PCR was applied to determined fetal sex. The fetal sex results of the SneakPeek assay at 7-weeks were confirmed by the fetal sex result obtained from the 8+-week samples. The SneakPeek assay was previously shown to be 99.9% accurate at 8+ week's gestation [3].

Results

One hundred thirty-four participants from twelve ultrasound clinics participated in this study and provided a blood sample at 7 weeks gestation and a second blood sample at 8+ weeks gestation. At the time of the first draw, the gestational age of all pregnant women

ranged from 7.00 to 7.86 weeks. At the second draw, the gestational age of all pregnant women ranged from 8.00 to 10.00 weeks (Table 1).

Fetal sex at 8+ week's gestation was determined for all 134 participants that completed the study. In this study, there were 59 male bearing pregnancies and 72 female bearing pregnancies. SneakPeek correctly identified 59 of 59 male bearing pregnancies, demonstrating 100% sensitivity for Y-chromosome DNA detection. There were no false negatives resulting in a positive predictive value of 100%. SneakPeek correctly identified 72 of 72 female bearing pregnancies. There were no false positives resulting in a negative predictive value of 100%. There were 3 inconclusive results from the 7-week samples (Table 2).

Discussion

The SneakPeek® Early Gender Test was previously shown to be 99.9% accurate for determining fetal sex as early as 8 weeks gestation [2,3]. In this study, the SneakPeek® Early Gender Test was shown to be 100% accurate at 7 weeks gestation, demonstrating that SneakPeek can accurately determine fetal sex using a microvolume amount (100 uL) of maternal plasma as early as 7 weeks gestation.

SneakPeek utilizes a multi-copy Y-chromosome target sequence alongside an autosomal control gene to measure male fetal cfDNA and total cfDNA, respectively, using a real-time quantitative polymerase reaction (RT-qPCR). An algorithm was used that incorporated the cycle threshold (CT) of both the Y-target sequence and the autosomal control gene to accurately identify fetal sex in all participants for this study. Fetal sex results at 7 weeks gestation were verified by a test result obtained from blood samples collected between 8 and 10 weeks gestation. Since all fetal sex results matched at both timepoints, this study showed that SneakPeek Early Gender Test provides accurate fetal sex determination as early as 7 weeks gestation. This study represents the earliest gestational age for >99% accurate fetal sex determination to date.

The gold standard against which non-invasive prenatal diagnosis of fetal sex is measured is the baby's sex on examination at birth [13]. A limitation of this study is that fetal sex was confirmed based off the SneakPeek test results from a blood sample drawn between 8 and 10

Table 1: Gestational age of the 134 participants during initial and final draw.

First Draw (7 weeks)		Second Draw (8 weeks)	
Gestational Age (week)		Gestational Age (week)	
Range	7.0-7.86	Range	8.00-10.14
Median	7.14	Median	8.14
Mean	7.25	Mean	8.30

Table 2: Statistical parameters of the study 7 weeks gestation.

Number of Participants	134
7 Week Samples Analyzed	134
Female Fetuses	72
Male Fetuses	59
Inconclusive Results	3
False Positives	0
False Negative	0
Sensitivity	100%
Specificity	100%
Accuracy	100%
Positive Predictive Value	100%
Negative Predictive Value	100%

weeks gestation, which does not meet the gold standard. However, SneakPeek was previously shown to be greater than 99% accurate at identifying fetal sex from maternal blood samples collected at 8 weeks gestation based on ultrasound confirmation by licensed sonographers [14]. In 2020, SneakPeek was shown to be 99.9% accurate for identifying fetal sex from maternal blood samples collected between 8 and 37 weeks of gestation based on live birth confirmation [3]. Taken together, the results from previously published studies demonstrate that SneakPeek test results from the 8+ week blood samples in this study may be used to confirm the results of the SneakPeek test at 7 weeks gestation.

Between May through August 2021, blood samples were collected from 134 pregnant women at 7 weeks gestation. A second blood sample was collected at 8+ week's gestation. 134 of 156 participants completed the study. Three participants received inconclusive results at the 7-week time period. The remaining 131 of 131 7 week results matched the 8+ week result. SneakPeek was shown to be 100% accurate at identifying fetal sex based off of 8+ week results. To our knowledge, SneakPeek is the most accurate blood test available for determining fetal sex as early as 7 weeks into pregnancy. A majority of expectant parents want to learn the sex of their baby as early as possible in pregnancy. This study demonstrates that SneakPeek can provide this information as early as 7 weeks into pregnancy. This study further opens the door to testing at even earlier timepoints in pregnancy, such as 6 or even 5-weeks' gestation. With further optimization and enhancement of cfDNA extraction for SneakPeek, we believe fetal sex identification at early timepoints in pregnancy will be available to expectant parents in the future.

Conclusion

In conclusion, this multicenter study demonstrated that women can learn the sex of their baby as early as 7 weeks into pregnancy with SneakPeek Early Gender DNA Test. Further optimization of the SneakPeek assay may allow for fetal sex identification at even earlier timepoints in pregnancy.

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