The importance of ultrasound preceding cell-free DNA screening for fetal chromosomal abnormalities

Imogen Brown¹, Shavi Fernando¹, Melody Menezes^{2,3}, Fabricio da Silva Costa^{1,4},
Jayshree Ramkrishna², Simon Meagher², Daniel L. Rolnik^{1,2}

¹ Department of Obstetrics and Gynecology, Monash University, Melbourne, Australia

² Monash Ultrasound for Women, Melbourne, Australia

³ Department of Paediatrics, University of Melbourne, Melbourne, Australia

⁴ Department of Gynecology and Obstetrics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Corresponding Author:

Imogen Brown

Monash University, Clayton, Victoria, Australia

E-mail: imogenkatebrown@gmail.com

Phone: +61 429 539 913

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CONTRIBUTION

What is already known about this topic?

- Cell-free DNA (cfDNA) testing for fetal chromosomal abnormalities (also known as non-invasive prenatal testing – NIPT) is not recommended in the context of a non-viable pregnancy, demised twin, fetal structural abnormality or when gestational age is below ten weeks. It is also important to know the number of fetuses as this affects test interpretation.
- The detection of any of these conditions is possible on obstetric ultrasound and therefore an ultrasound performed before blood collection for cfDNA has the potential to change patient management.
- An ultrasound before non-invasive prenatal testing has previously been shown to have the potential to change patient management in a population of advanced maternal age patients.

What does this study add?

- This is the largest study to date to examine the importance of an ultrasound before cell-free DNA screening for fetal chromosomal abnormality.
- An ultrasound before cfDNA can detect these clinical situations and thus has the potential to change patient management in approximately one in ten cases.
- This proportion increases with advancing maternal age and is lower in IVF conceptions.

Data Availability Statement: The data supporting the findings of this study are not publicly available due to patient privacy. For enquiries regarding data, please contact the corresponding author, IB.

Keywords

Ultrasound, non-invasive prenatal testing, NIPT, cell-free DNA, early pregnancy, aneuploidy screening, chromosomal abnormalities

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ABSTRACT

Objective: This study aims to determine the incidence of ultrasound findings that may change clinical management on the day of blood-sampling for cell-free DNA (cfDNA) testing.

Methods: A retrospective study was conducted at a tertiary provider of obstetric and gynecological ultrasound in Melbourne, Australia. Individual patient files were reviewed, and results were collated for maternal characteristics, pre-cfDNA ultrasound reports, results and test characteristics of both cfDNA and diagnostic testing, and genetic counselling notes. The primary outcome was a potential change in patient management due to findings detected on the pre-cfDNA ultrasound.

Results: Of 6,250 pre-cfDNA ultrasounds, 6,207 were included in analysis. Of these, 598 (9.6%) pregnancies had a finding on pre-cfDNA ultrasound that had the potential to change management. The reasons for this potential change in management were detection of gestational age below ten weeks (245, 3.9%), miscarriage (175, 2.8%), demised twin (43, 0.7%), fetal edema (115, 1.9%) and major structural abnormalities (20, 0.3%). These findings were more common in patients of advanced maternal age and in spontaneous conceptions.

Conclusions: An ultrasound prior to cfDNA testing has the potential to change clinical management in almost one in ten women. The proportion is higher in older age groups and lower in IVF-conceived pregnancies.

INTRODUCTION

The use of cell-free DNA (cfDNA) analysis in screening for fetal chromosomal abnormalities (also known as non-invasive prenatal testing – NIPT) has resulted in sensitivities over 95% and specificities over 99% for the most common trisomies.^{1, 2}

Placental cfDNA is sequenced from the maternal circulation, which, due to its shared embryonic origins with the fetus, is likely to closely reflect the fetus.³ The accuracy of the test is dependent on the proportion of fetal cfDNA in the maternal circulation (fetal fraction) which is generally sufficient for testing after ten weeks of gestational age.⁴

This examination of chromosomal material is highly effective; however, limitations to the implementation of cfDNA screening persist. A false-positive result may occur in the setting of a demised twin, as it continues to release DNA into the maternal circulation and, if the demise was due to a chromosomal abnormality, this might lead to a false-positive result for the surviving twin.⁵ A false-positive result is also possible in the case of confined placental mosaicism, when a chromosomal abnormality is present in the placenta, but not the fetus.⁵ An inconclusive or false-negative result may occur in cases of low fetal fraction, which is more likely to occur before ten gestational weeks (when cfDNA screening is not validated and thus not recommended). Most cfDNA screening providers do not report a result in the case of insufficient fetal DNA or when the fetal gestational age is below ten weeks.⁵ It is also essential that the number of fetuses is known, as this affects the interpretation of the test result.⁶ In addition, cfDNA screening is not recommended in the setting of fetal abnormality due

to the potential for false reassurance,⁷ and instead definitive genetic testing should be considered in the first instance.

In Australia, cfDNA screening is not publicly funded. Therefore, the patient is responsible for the entire cost of the test. Most women who utilise the test do so as a first-tier test between 10-11 weeks gestational age.^{2, 8}

An ultrasound performed just prior to cfDNA screening (pre-cfDNA ultrasound) determines the number of fetuses, fetal viability, gestational age, identifies a demised twin and sometimes could identify a major fetal structural abnormality.⁹ Performance of an ultrasound before cfDNA screening is recommended by many governing bodies based on expert opinion;^{10, 11} however, there is a lack of evidence to support this practice in the general obstetric population. One study of women of advanced maternal age (AMA) found that a pre-cfDNA screening ultrasound changed management in 16.1% of cases.¹²

The aim of this study was to determine the proportion of patients in an unselected population who had a finding detected on pre-cfDNA ultrasound that had the potential to change clinical management, in consecutive pregnancies attending for cfDNA screening.

METHODS

Ethical Approval

Ethical approval was obtained from the Monash Health Human Research Ethics Committee in January 2019 (Reference Number: HREC/46571/MonH-2018-152982(v1), Monash Health Ref: RES-18-0000-567L). Patient consent was not required as this was a retrospective analysis of data that was originally collected as part of routine clinical care.

Study Design and Population

The “Ultrasound PREceding Non-Invasive Prenatal Testing (PRENIPT)” was a single-center, retrospective cross-sectional study including all consecutive patients attending for a pre-cfDNA screening ultrasound at Monash Ultrasound for Women, (a tertiary provider of obstetric and gynecological ultrasound, prenatal screening and diagnosis in Melbourne, Australia) from January 2017 to December 2018. Patients are referred to this service either at their own request, or the recommendation of the practitioner. Provision of a pre-cfDNA screening transvaginal ultrasound performed on the day of venipuncture is standard care at the center where the study was conducted. If, at the pre-cfDNA screening ultrasound, it was found that the gestational age was below ten weeks, the patient was rebooked for venipuncture at a later date. If any contraindications to cfDNA screening were identified (such as a non-viable pregnancy or demised twin), cfDNA screening was not performed, and other screening or diagnostic methods were pursued (first trimester combined screening was recommended in the case of a demised twin). If, at the pre-cfDNA screening ultrasound, a fetal abnormality was detected, patients were seen by a genetic

counsellor and the option of invasive testing (in the form of chorionic villous sampling (CVS) or amniocentesis) was discussed, due to the increased risk of underlying chromosomal or genetic abnormalities. If no contraindications to cfDNA screening were found, the patient proceeded with blood sampling for cell-free DNA testing.

All tests were done by Next Generation Sequencing at a local provider (nestTM, Repromed, Adelaide, South Australia), using the Illumina platform (Illumina Inc., San Diego, CA, USA).

Figure 1 demonstrates the protocol for cfDNA screening provision at this service provider.

Data collection

A manual review of individual patient files was conducted. The following maternal demographics were collected: age, height, weight, body mass index (BMI) and parity. Maternal obstetric history was gathered; including method of conception of the current pregnancy and history of a chromosomal abnormality in a previous pregnancy.

Pre-cfDNA screening ultrasound reports were reviewed individually for viability, the number of fetuses, gestational age (defined according to previous ultrasound, crown-rump length on pre-cfDNA ultrasound examination or fetal biometry (when after 14 weeks of gestational age, except in cases of *in vitro* fertilization, in which the transfer date was used) fetal heart rate (FHR), presence of fetal edema, demised twin and presence of fetal structural abnormality. Fetal edema was considered present if the ultrasound specialist reported increased nuchal thickness of 2.2 mm or more (the 95th

percentile at ten weeks of gestational age) or generalized subcutaneous edema, with or without fluid present in one or more body cavities (*hydrops fetalis*).¹³ Those patients who had edema or increased nuchal thickness, with or without additional fetal abnormalities, were offered genetic counselling on the same day. The options to reconsider cfDNA screening until after the follow-up ultrasound at 11-14 weeks or to have cfDNA performed on the same day were offered, and ultimately the decision to proceed with cfDNA screening was made by the patient.

Recorded cfDNA screening test characteristics included indication, reason the test was not done (if not performed), “no-call” results (inconclusive on the first attempt), fetal fraction, results for trisomy 21, 18, 13 and sex chromosome abnormalities (SCA) when available, and test failures (no result on resampling after a no-call). cfDNA screening indication was considered a first tier screening test when the main indication was maternal age or choice. The test was deemed to be second tier when preceded by a high-risk result from another screening method or when a previous history of pregnancy affected by chromosomal abnormality was present.

All consecutive pregnancies attending for cfDNA screening were included. Women were excluded from the analysis if they were under the age of 18 at the time of the pre-cfDNA screening ultrasound. Women who were incorrectly booked for a pre-cfDNA screening ultrasound (i.e. appointment errors) and never intended to have cfDNA screening, but instead wanted to book for a viability ultrasound were also excluded.

Statistical Analysis and Outcome Measures

The primary outcome was a composite of the number of times a finding that had the potential to change pregnancy management was detected at the pre-cfDNA screening ultrasound. A potential change in management was determined if there was a missed miscarriage, fetal edema or structural abnormality, a demised “vanishing” twin, or the patient was “too early” (gestational age below ten weeks). The findings were recorded regardless of the actual management of the patient.

Data on maternal weight, height and BMI were missing for 240 (3.9%) and on parity for 231 (3.7%) women, and multiple imputation using regression techniques was applied with ten iterations.

Descriptive statistics were obtained, and data were checked for normality by inspection of histograms. Categorical variables were expressed as absolute number and proportion, and compared between groups with the chi-squared test. Continuous variables were expressed as the mean and standard deviation (SD) and compared with Student t test when normally distributed and as the median and interquartile range (IQR) when the distribution was not symmetric. The frequency of the primary outcome is reported within the total and among subgroups according to maternal age and mode of conception. Categorical variables between age and mode of conception subgroups were compared with chi-squares, and odds ratios were calculated with their respective 95% confidence intervals (CI). Continuous variables were compared using independent-samples t-test or Wilcoxon ranks sum test, depending on the distribution of the data.

Lastly, to investigate the independent effect of maternal age as a continuous variable and in vitro fertilization (IVF) on the primary outcome, multiple logistic regression analysis was performed including age, maternal weight and height, parity and mode of conception to obtain the regression coefficients and the adjusted odds ratios controlling for the other variables in the model. Variables that are part of the outcome definition or are highly correlated with the primary outcome, such as CRL and twin pregnancy, were not included in the model.

Statistical analysis was performed in SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for Macintosh, Version 26.0. Armonk, NY: IBM Corp.), and two-tailed P values of less than 0.05 were considered statistically significant.

RESULTS

Overall, 6,250 pre-cfDNA screening ultrasound examinations were booked at the between 1st January 2017 and 31st December 2018. Of these, one patient (<0.1%) was excluded as she was underage at the time of the pre-cfDNA screening ultrasound and another 42 (0.7%) were excluded as they were mistakenly booked for a pre-cfDNA screening ultrasound. Therefore, 6,207 ultrasound examinations were included in the analysis.

The baseline characteristics of the study population are summarized in Table 1. The mean maternal age at the pre-cfDNA screening ultrasound was 33.4 years (SD 4.6

years). Pregnancies were spontaneously conceived in 85.9% of cases and by IVF in 14.1% of cases. Of the IVF conceptions, 88.0% were autologous eggs, and 12.0% were donor eggs. The median CRL at the time of pre-cfDNA screening ultrasound was 40.8 mm, which corresponds to a gestational age of ten weeks and six days. cfDNA screening test characteristics are shown in Table 2. cfDNA screening was performed by maternal choice or age alone in 94% of pregnancies.

An ultrasound finding that may lead to a change in management was detected in 598 (9.6%) cases. The most common reason for a potential change in management due to the pre-cfDNA screening ultrasound was gestational age below ten weeks, which was detected in 245 (3.9%) cases. A missed miscarriage was detected in 175 (2.8%) cases, and a demised twin was detected in 43 (0.7%) cases. Fetal edema and fetal structural abnormalities were detected in 115 (1.9%) and 20 (0.3%) cases, respectively. Table 3 summarizes the potential changes in management due to the pre-cfDNA screening ultrasound, and Figure 2 demonstrates a breakdown of the reasons for a potential change in management due to findings on the pre-cfDNA screening ultrasound by age subgroup.

In IVF pregnancies, there was a significantly higher incidence of a demised twin (demised twin present in 1.5% of IVF conceptions compared with 0.6% of spontaneous conceptions, $P = 0.006$). Additionally, the finding of gestational age less than ten weeks leading to delay of cfDNA screening was significantly less common, (4.3% in spontaneous conceptions compared with 2.1% of IVF conceptions, $P = 0.001$, Table 4).

Twenty patients (0.3%) had a significant structural abnormality detected at the pre-cfDNA screening ultrasound. The median CRL of the fetuses with a structural abnormality was 41.3 mm (corresponding to eleven weeks and one day of gestation), which ranged from 30.7 mm (corresponding to ten weeks and one day of gestation) to 54.3 mm (corresponding to eleven weeks and six days of gestation). The fetal abnormalities that were detected encompassed the cardiac, neurological, abdominal and musculoskeletal systems, and are summarized in Supplementary Table 1.

The fetuses with edema detected at pre-cfDNA screening ultrasound had varied outcomes. Of the 115 cases with edema, 18 patients (15.7%) did not proceed and 97 (84.3%) proceeded with cfDNA screening. Of the 96 patients who proceeded with the test despite detection of edema on pre-cfDNA ultrasound, six (6.3%) women had a high risk cfDNA screening result. On follow-up, 252 patients (4% of the cohort) had an abnormality at the first-trimester ultrasound, 42 (16.7%) of whom had had edema detected at pre-cfDNA screening ultrasound. There were 4128 patients who returned for a first-trimester anatomy ultrasound and, of these, only four patients who had had edema detected at pre-cfDNA ultrasound and had had a first trimester ultrasound performed at the center had no abnormality. Of the patients who had edema at pre-cfDNA screening ultrasound, 25 had fetal chromosomal analysis performed (CVS, amniocentesis or postnatal). Eleven (44%) of these patients had an abnormal result and another three (12%) had variation of unknown significance (VOUS). The pregnancy outcomes among women with fetal edema who underwent fetal chromosomal analysis included six cases of T21, three of T18, one of mosaic triploidy

(demised in utero), two of Turner syndrome. There were three patients who had no fetal chromosomal analysis performed, two patients had a miscarriage and one had pregnancy termination at a later stage.

On multiple logistic regression analysis, pre-cfDNA screening ultrasound findings were positively associated with maternal age ($\beta = 0.49$, adjusted odds ratio 1.05, 95% CI 1.03–1.07, $P < 0.001$) and negatively associated with IVF conception ($\beta = -0.35$, adjusted odds ratio 0.69, 95%CI 0.53–0.93, $P = 0.013$, Nagelkerke R^2 0.01), after controlling for all other variables in the model. No other maternal characteristics (weight, height, or parity) were independently associated with unexpected ultrasound findings. The regression coefficients and adjusted odds ratios are given in Supplementary Table 2.

DISCUSSION

Main findings and clinical implications

The use of a pre-cfDNA screening ultrasound is currently recommended based on expert opinion alone.^{10, 11} The PRENIPT study is the first to examine the use of an ultrasound before cfDNA screening in an unselected obstetric population. We found that, for every ten women who have a pre-cfDNA screening ultrasound, one inappropriate episode of cfDNA screening can be avoided. Ultrasound is more likely to detect a finding that has the potential to change management in older women. This

can prevent the costly uptake of clinically inappropriate cfDNA screening, particularly in advanced maternal age groups. In Australia, not all cfDNA providers perform an ultrasound before venipuncture. Therefore, in many instances, the gestational age of the fetus will not be confirmed when cfDNA screening is performed.

Women aged 35 years and over were significantly more likely to have a miscarriage or demised twin. These findings are consistent with previous studies, which have shown an increased risk of pregnancy loss even in chromosomally normal fetuses, due to poorer oocyte quality in this population.^{14, 15} These women are also more likely to have a fetal abnormality due to age-related non-disjunction meiotic errors.¹⁵

The detection of a structural abnormality or fetal edema on ultrasound was an indication for consideration of invasive testing, due to the potential of an associated chromosomal abnormality. The detection of early fetal edema has not been widely studied; however, recent data suggest that this finding is less benign than previously thought. A study by Grande *et al.* demonstrated the utility of nuchal translucency in fetuses in the first trimester (with CRL below 45 mm).¹⁶ The authors found an increased nuchal translucency in two thirds of trisomy 21 fetuses, nearly three quarters of fetuses with trisomies 18 and 13, and all fetuses with monosomy X.¹⁶ In contrast to this, a study by Scholl *et al.* that found that fetuses with a cystic hygroma detected at an early gestation (when the CRL was 45 mm and below) often had a normal karyotype (26 fetuses out of 28 with a cystic hygroma at CRL 45 mm or below).¹⁷

The rate of IVF conceptions was considerably higher in our cohort than in the general obstetric population, since 14.1% of pregnancies were conceived via IVF, compared

with 4.7% of births in Australia that were conceived using some type of assisted reproductive technology.¹⁸ This is likely due to the practice's affiliation with an IVF provider, who refer a significant number of their patients to this clinic.

Unsurprisingly, due to the higher incidence of multiple pregnancies in IVF conceptions, IVF patients were significantly more likely to have a vanishing twin detected at the pre-cfDNA screening ultrasound.¹⁹ Also, as one might expect, in IVF pregnancies, the finding on pre-cfDNA screening ultrasound of gestational age less than ten weeks was significantly less common, reflective of the higher accuracy of dating in IVF pregnancies using transfer date. Similarly, the slightly lower incidence of miscarriage may reflect earlier diagnosis in previous viability ultrasounds; a viability ultrasound at seven weeks is standard protocol for this IVF provider. Therefore, it is unlikely that this reflects a true lower miscarriage rate in IVF pregnancies, but instead represents earlier detection of non-viable pregnancies.

The abnormalities that were detected at the pre-cfDNA screening ultrasound were varied. It is well known that fetal abnormalities are more easily identified at a later gestation.^{20, 21} Nevertheless, recent evidence suggests that a significant number of abnormalities can be detected at the first trimester morphology ultrasound,²¹ some even before 11 weeks.⁹ However, the detection of structural abnormalities at this early gestation is highly dependent upon the technology available as well as the skill level of the clinician performing the ultrasound.

Comparison with previous studies

The results of our investigation are comparable with the findings presented in the study by Vora *et al.* which found that a pre-cfDNA screening ultrasound has the potential to alter patient management in 16.1% of cases in an AMA population.¹² Although we studied an unselected obstetric population, both studies had a similar number of women who were specifically of older age strata. In our AMA population, 11.6% of women over 35 years had a pre-cfDNA screening ultrasound finding which may have changed their management. We also found that AMA patients are more likely to have a missed miscarriage, vanishing twin or major structural abnormality than other women. Our study was larger, including all age groups, and mainly recorded findings that would lead to inappropriate implementation of cfDNA screening. Discrepancies in outcomes measured between our study and the aforementioned study (for example, the inclusion of unexpected twins) may account for the slight differences.¹²

Strengths and Limitations

Currently, this is the largest study exploring the importance of ultrasound before cfDNA screening in an unselected obstetric population having cfDNA screening mostly as a first-tier screening test.

The main limitation of our study is its retrospective study design. Nevertheless, the review of individual files with standardized reports minimizes this limitation. Due to records of any previous ultrasounds being unavailable and not usually collected as part of routine patient care, we are unable to describe the number of women who had previously had an early pregnancy ultrasound. This is a limitation of our study as we

are unable to comment on the proportion of women who had gestational age previously confirmed by another ultrasound examination or who were previously diagnosed with a missed miscarriage. We also relied on ultrasound reports to characterize fetal edema or increased nuchal thickness. These findings may have been under-reported, and the fact that nuchal thickness was not systematically measured before 11 weeks limits our conclusions and ability to comment on specific nuchal thickness cut-offs. It is likely, however, that the most severe cases of fetal edema were captured.

Our sample may not reflect the overall pregnant population in Australia. The proportion of IVF patients included in our study was three times that of the general population, and the BMI of the women in our study was also lower than that of the average Australian women of the relevant age group.¹⁸ Whilst these inconsistencies may bias our estimates, cfDNA screening is only available privately at a significant cost to patients, and therefore, there is only a selected group of patients available for analysis. We acknowledge that some structural abnormalities detected on pre-cfDNA screening ultrasound may not have been identified by all clinicians who perform obstetric ultrasound. As the clinic where this study was conducted is a tertiary specialist center, abnormalities are more likely to be identified when compared with non-tertiary centers or those who are less experienced in the detection of first-trimester anomalies. Therefore, the incidence of findings detected on pre-cfDNA screening ultrasound may not apply to all centers. However, the majority of significant findings were either a missed miscarriage or a gestational age less than ten weeks. It is reasonable to expect

that these outcomes should be detected by any clinician who performs obstetric ultrasound.

To expand on our findings from the PRE-cfDNA study, further investigation into the cost-effectiveness of pre-cfDNA screening ultrasound should be considered. Randomized controlled trials are needed to better estimate the clinical impact and cost implications of the pre-cfDNA screening ultrasound.

Conclusion

An ultrasound before blood sampling avoids unnecessary cfDNA screening in approximately one in ten women. The proportion is higher in older age groups and lower in IVF conceived pregnancies.

REFERENCES

1. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol.* 2017;50(3):302-14.
2. Hui L, Lindquist A, Poulton A, Kluckow E, Hutchinson B, Pertile MD, et al. State-wide utilization and performance of traditional and cell-free DNA-based prenatal testing pathways: the Victorian Perinatal Record Linkage (PeRL) study. *Ultrasound Obstet Gynecol.* 2019.
3. Lo YM, Chiu RW. Genomic analysis of fetal nucleic acids in maternal blood. *Annu Rev Genomics Hum Genet.* 2012;13:285-306.
4. Wright D, Wright A, Nicolaides KH. A unified approach to risk assessment for fetal aneuploidies. *Ultrasound Obstet Gynecol.* 2015;45(1):48-54.
5. Hartwig TS, Ambye L, Werge L, Weiergang MK, Norgaard P, Sorensen S, et al. Non-Invasive Prenatal Testing (NIPT) in pregnancies with trisomy 21, 18 and 13 performed in a public setting - factors of importance for correct interpretation of results. *Eur J Obstet Gynecol Reprod Biol.* 2018;226:35-9.
6. Cuckle H, Benn P, Pergament E. Cell-free DNA screening for fetal aneuploidy as a clinical service. *Clin Biochem.* 2015;48(15):932-41.

7. Benachi A, Letourneau A, Kleinfinger P, Senat MV, Gautier E, Favre R, et al. Cell-free DNA analysis in maternal plasma in cases of fetal abnormalities detected on ultrasound examination. *Obstet Gynecol.* 2015;125(6):1330-7.
8. Lee TJ, Rolnik DL, Menezes MA, McLennan AC, da Silva Costa F. Cell-free fetal DNA testing in singleton IVF conceptions. *Human reproduction (Oxford, England).* 2018;33(4):572-8.
9. Rolnik DL, Wertaschnigg D, Benoit B, Meagher S. Sonographic detection of fetal abnormalities before 11 weeks of gestation. *Ultrasound Obstet Gynecol.* 2019.
10. Salomon LJ, Alfirevic Z, Audibert F, Kagan KO, Paladini D, Yeo G, et al. ISUOG updated consensus statement on the impact of cfDNA aneuploidy testing on screening policies and prenatal ultrasound practice. *Ultrasound Obstet Gynecol.* 2017;49(6):815-6.
11. Norton ME, Biggio JR, Kuller JA, Blackwell SC. Society for Maternal-Fetal Medicine (SMFM) Consult Series #42: The role of ultrasound in women who undergo cell-free DNA screening. *Am J Obstet Gynecol.* 2017;216(3):B2-B7.
12. Vora NL, Robinson S, Hardisty EE, Stamilio DM. Utility of ultrasound examination at 10-14 weeks prior to cell-free DNA screening for fetal aneuploidy. *Ultrasound Obstet Gynecol.* 2017;49(4):465-9.

13. Mack LM, Lee W, Mastrobattista JM, Belfort MA, Van den Veyver IB, Shamshirsaz AA, et al. Are First Trimester Nuchal Septations Independent Risk Factors for Chromosomal Anomalies? *J Ultrasound Med*. 2017;36(1):155-61.
14. Magnus MC, Wilcox AJ, Morken NH, Weinberg CR, Haberg SE. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. *BMJ*. 2019;364:l869.
15. Hassold T, Chiu D. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. *Hum Genet*. 1985;70(1):11-7.
16. Grande M, Solernou R, Ferrer L, Borobio V, Jimenez JM, Bennasar M, et al. Is nuchal translucency a useful aneuploidy marker in fetuses with crown-rump length of 28-44 mm? *Ultrasound Obstet Gynecol*. 2014;43(5):520-4.
17. Scholl J, Chasen ST. First trimester cystic hygroma: does early detection matter? *Prenatal diagnosis*. 2016;36(5):432-6.
18. Australian Institute of Health and Welfare 2018. Australia's mothers and babies 2016—in brief. Perinatal statistics series no. 34. Cat. no. PER 97. Canberra: AIHW.
19. Multiple Pregnancies Following Assisted Conception: Scientific Impact Paper No. 22. *BJOG*. 2018;125(5):e12-e8.

20. Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH. Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11-13 weeks. *Prenat Diagn.* 2011;31(1):90-102.
21. Syngelaki A, Hammami A, Bower S, Zidere V, Akolekar R, Nicolaides KH. Diagnosis of fetal non-chromosomal abnormalities on routine ultrasound examination at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol.* 2019;54(4):468-76.

FIGURE LEGENDS

Figure 1. Flowchart demonstrating the local protocol for cDNA provision.

cfDNA: Cell-free DNA screening; TV: transvaginal.

The dotted box encompasses situations when cfDNA screening would not be performed.

Figure 2: Potential reasons for change in management by age group.

* $p < 0.001$ when compared to age < 35 years;

** $p = 0.011$ when compared to age < 35 years;

+ $p = 0.020$ when compared to age < 35 years.

TABLES

Table 1. Characteristics of the study population.

	No sonographic findings n= 5609	Sonographic findings n= 598	p-value	All N = 6207
Maternal age (years), mean (SD)	33.3 (4.5)	34.1 (5.0)	< 0.001	33.4 (4.6)
Age < 35 years, n (%)	3674 (65.5)	345 (57.7)	< 0.001	4019 (64.8)
Age 35 to 39.9 years, n (%)	1538 (27.4)	180 (30.1)	0.164	1718 (27.7)
Age ≥ 40 years, n (%)	397 (7.1)	73 (12.2)	< 0.001	470 (7.5)
Weight (Kg), median (IQR)	64.0 (56.0 to 74.0)	62.0 (55.0 to 72.0)	0.240	63.6 (56.0 to 74.0)
Height (cm), mean (SD)	163.7 (7.0)	163.5 (7.2)	0.614	163.7 (7.0)
BMI (Kg/m ²), median (IQR)	23.6 (21.2 to 27.2)	23.4 (21.1 to 26.8)	0.362	23.6 (21.2 to 27.1)
Parity				
Nulliparous, n (%)	2850 (50.8)	292 (48.8)	0.357	3142 (50.6)
Parous, n (%)	2759 (49.2)	306 (51.2)		3065 (49.4)
Conception				
Spontaneous, n (%)	4804 (85.6)	525 (87.8)	0.153	5329 (85.9)
IVF, n (%)	805 (14.4)	73 (12.2)		878 (14.1)
Own egg, n (% of IVF)	709 (88.1)	64 (87.7)	0.919	773 (88.0)
Egg donor, n (% of IVF)	96 (11.9)	9 (12.3)		105 (12.0)
Twin pregnancy, n (%)	113 (2.0)	64 (10.7)	< 0.001	177 (2.9)
Gestational age (weeks), median (IQR)*	11.0 (10.6 to 11.5)	9.9 (9.6 to 10.7)*	< 0.001	10.9 (10.4 to 11.3)

Categorical variables given as absolute number and percentage. Continuous variables given as mean and standard deviation when normally distributed and as median and interquartile range when non-normally distributed. Hypothesis testing was performed with independent-samples t test for normally distributed variables, Wilcoxon rank sum test for non-normally distributed variables, and chi-squared for categorical variables.

* Gestational age defined according to previous ultrasound or crown-rump length on pre-cfDNA ultrasound examination, except in cases of *in vitro* fertilization, in which the transfer date was used. Excludes cases of miscarriage.

Table 2. Test characteristics of cell-free fetal DNA testing.

cfDNA screening test characteristics	Age < 35 years n = 4,019 (64.7%)	Age 35–39.9 years n = 1,718 (27.7%)	Age ≥ 40 years n = 470 (7.6%)	TOTAL n = 6,207
Primary indication				
Maternal age or choice	3849 (95.8)	1,585 (92.3)	399 (84.9)	5,833 (94.0)
Other	55 (1.4)	44 (2.5)	25 (5.3)	124 (2.0)
High-risk screening result*	3 (0.1)	2 (0.1)	0 (0.0)	5 (0.1)
Previous pregnancy with aneuploidy	52 (1.3)	42 (2.4)	25 (5.3)	119 (1.9)
Test not performed	115 (2.8)	89 (5.2)	46 (9.8)	250 (4.0)
Fetal fraction, median (IQR)	10.0 (7.0–13.0)	10.0 (7.0–13.0)	9 (6.0–11.0)	10.0 (7.0–13.0)
No-call result	62 (1.5)	34 (2.0)	20 (4.3)	116 (1.9)
Test failure	15 (0.4)	7 (0.4)	9 (1.9)	31 (0.5)
Low fetal fraction	7 (0.2)	4 (0.2)	7 (1.5)	18 (0.3)
Technical failure	8 (0.2)	3 (0.2)	2 (0.4)	13 (0.2)

cf-DNA: cell-free DNA, IQR: interquartile range, *pertains to first trimester maternal serum screening combined with nuchal translucency, or second trimester screening result.

Categorical data expressed as absolute number (percentage of each subgroup).

Table 3. Findings that could possibly lead to change in management following ultrasound prior to cf-DNA screening by maternal age when compared with age less than 35 years.

Ultrasound Finding	Age < 35 years n = 4,019 (64.7%)	Age 35–39.9 years n = 1,718 (27.7%)		Age ≥ 40 years n = 470 (7.6%)		TOTAL N = 6,207
	n (%)	n (%)	Odds ratio (95% CI)	n (%)	Odds ratio (95% CI)	n (%)
Gestational age < 10 weeks	160 (4.0)	68 (4.0)	0.99 (0.74–1.33)	17 (3.6)	0.91 (0.54–1.51)	245 (4.0)
Miscarriage	79 (2.0)	62 (3.6)	1.87 (1.33–2.62)*	34 (7.2)	3.89 (2.57–5.88)*	175 (2.8)
Demised (“vanishing”) twin	20 (0.5)	16 (0.9)	1.88 (0.97–3.64)	7 (1.5)	3.023 (1.27–7.19)*	43 (0.7)
Fetal edema / increased nuchal thickness	77 (1.9)	28 (1.6)	0.85 (0.55–1.31)	10 (2.1)	1.11 (0.57–2.17)	115 (1.9)
Structural abnormality	9 (0.2)	6 (0.3)	1.56 (0.56–4.39)	5 (1.1)	4.80 (1.60–14.36)*	20 (0.3)
Any finding	345 (8.6)	180 (10.4)	1.25 (1.03–1.51)*	73 (15.5)	1.96 (1.49–2.58)*	598 (9.6)

cf-DNA: cell-free DNA. CI: Confidence interval

* Statistically significant at $p < 0.05$ when compared to age < 35 years (reference group).

Categorical data expressed as absolute number (percentage of each subgroup).

Table 4. Findings that can potentially lead to change in management following ultrasound prior to cf-DNA screening by mode of conception.

Ultrasound Finding	Spontaneous n = 5,329 (%)	IVF n = 878 (%)	Odds Ratio (95% CI)
Gestational age < 10 weeks	227 (4.3)	18 (2.1)*	0.47 (0.29–0.76)*
Miscarriage	154 (2.9)	21 (2.4)	0.82 (0.52–1.31)
Demised (“vanishing”) twin	30 (0.6)	13 (1.5)*	2.66 (1.38–5.11)*
Fetal edema / increased nuchal thickness	97 (1.8)	18 (2.0)	1.13 (0.68–1.88)
Structural abnormality	17 (0.3)	3 (0.3)	1.07 (0.31–3.66)
Any finding	525 (9.9)	73 (8.3)	0.83 (0.64–1.07)

cf-DNA: cell-free DNA. CI: Confidence interval. IVF: *In vitro* fertilization

* Statistically significant at $p < 0.05$ when compared to spontaneous conception.

Categorical data expressed as absolute number (percentage of each subgroup).

Supplementary Table 1. Structural abnormalities detected at the pre-cf-DNA ultrasound.

Abnormalities at pre-cf-DNA ultrasound	CRL pre-cf-DNA scan (mm)	cf-DNA	Ultrasound 12–14 weeks	Outcome
Features consistent with holoprosencephaly, fourth ventricle dilatation, polydactyly	42.4	No	N/A	TOP, postnatal karyotype indicated T13
Suspected cardiac abnormality and bilateral pleural effusion	45.8	No	Nuchal translucency > 99 th centile (11.2 mm), Complex cardiac anomaly (hypoplastic left heart)	Miscarriage 16 weeks
Single umbilical artery and Increased nuchal translucency (4.9 mm)	35.5	No	Miscarriage detected	Miscarriage (first trimester)
Suspected cardiac abnormality, single umbilical artery and Increased nuchal translucency (3.7 mm)	34.0	No	Nuchal translucency > 99 th centile (4.9 mm), atrioventricular septal defect, aberrant right subclavian artery.	No genetic testing. TOP
Suspected encephalocele	43.3	No	Occipital encephalocele with herniation of the cerebellum and midbrain through the posterior skull defect.	No genetic testing. TOP
Acrania	50.1	No	N/A	No genetic testing. TOP
Acrania	42.0	No	N/A	No genetic testing. TOP
Suspected large omphalocele and increased nuchal translucency (6 mm)	41.0	No	Nuchal translucency > 99 th centile (8.8 mm) omphalocele, ventricular septal defect	CVS indicated T18. TOP
Radial ray deformity with univentricular heart	40.1	No	N/A	CVS indicated T18. TOP
Suspected cardiac abnormality, single umbilical artery and subcutaneous edema	32.5	Yes (normal)	NT > 95 th centile (3 mm), single umbilical artery	Amniocentesis showed normal karyotype

Suspected brain abnormality, abnormal posterior fossa (increased intracranial translucency), likely dilatation of the fetal cerebral ventricles	31.5	No	Severe brain abnormalities, early signs of ventriculomegaly, holoprosencephaly	CVS showed normal karyotype. TOP
Radial ray deformity and subcutaneous edema	44.0	No	Complex cardiac abnormality (double outlet right ventricle or common arterial trunk) and Radial ray deformity	CVS indicated T18, TOP
Suspected large omphalocele and subcutaneous edema	37.9	No	Omphalocele, cardiac anomaly, hydrops	CVS indicated T18. TOP
Lumbosacral spina bifida with omphalocele, bladder exstrophy and fixed flexion deformities of the lower limbs with polydactyly. Possible amniotic rupture syndrome, body stalk anomaly or OIES complex	38.8	No	N/A	TOP
Multiple dilated loops of bowel within the fetal abdomen	49.3	No	N/A	CVS indicated T21. TOP
Pentalogy of Cantrell	30.6	No	N/A	TOP
Acrania	47.3	No	N/A	TOP
Suspected large omphalocele, lumbar sacral kyphosis, increased nuchal translucency (3.8 mm)	46.6	No	Absent nasal bone, Omphalocele, Horseshoe kidney, Cardiac abnormality.	CVS indicated T18. TOP
Omphalocele, prominent fetal bladder, increased nuchal translucency (4.8 mm)	54.3	No	Nuchal translucency > 99 th centile (4.1 mm) Omphalocele, Cardiac Abnormality (ventricular septal defect and tricuspid regurgitation).	Abnormal chromosomal microarray (2 copy number losses). TOP
Bladder and kidneys could not be identified	39.7	Yes (normal)	Bilateral renal agenesis	Normal microarray. TOP

TOP: termination of pregnancy; N/A: not available; CVS: chorionic villous sampling; T13: Trisomy 13; T18: trisomy 18; T21: trisomy 21; OEIS: omphalocele, exstrophy, imperforate anus and spinal defects.

Supplementary Table 2. Logistic regression coefficients and adjusted odds ratios for the presence of ultrasound findings that could lead to change in management.

Variable	Regression coefficient	Adjusted Odds Ratio (95% CI)	P value
Intercept	-4.771	–	–
Maternal age (years)	0.049	1.050 (1.028–1.072)	<0.001
Maternal weight (Kg)	-0.004	0.996 (0.990–1.003)	0.267
Maternal height (cm)	0.007	1.007 (0.987–1.028)	0.461
Nulliparity	-0.046	0.955 (0.770–1.185)	0.674
IVF pregnancy	-0.352	0.703 (0.533–0.928)	0.013

Nagelkerke R²: 0.01. IVF: *In vitro* fertilization

PD_5788_PRENIP Figure 1.png

PD_5788_PRENIPT Figure 2.png

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Author/s:

Brown, I;Fernando, S;Menezes, M;Costa, FDS;Ramkrishna, J;Meagher, S;Rolnik, DL

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