v1: 4 September 2023

Research Article

Non-Invasive Prenatal Testing (NIPT) for Aneuploidy in a Setting with a High Consanguineous Rate – A Retrospective Cohort Review of 1,153 Cases

Peer-approved: 4 September 2023

Qeios, Vol. 5 (2023)
ISSN: 2632-3834

Badreldeen Ahmed[1], Mandy Abushma[2], Justin Konje[3,4]

1. Professor of Obstetrics and Gynaecology, Weill Cornell Medicine in Qatar, Doha, Qatar; 2. Assistant Professor of Obstetrics and Gynecology, Weill Cornell Medicine in Qatar, Doha, Qatar; 3. University of Leicester, Leicester, United Kingdom; 4. Professor of Obstetrics and Gynecology, Weill Cornell Medicine in Qatar, Doha, Qatar

Objective: To review the outcome of NIPT as a screening test for aneuploidy at a tertiary feto-maternal centre in a population with a high consanguinity rate and to investigate whether consanguinity is a factor in failure to generate results. Methods: A retrospective cohort study of the records of all the women who had NIPT at our centre over the six-year period 2015-2021 inclusive.
Results: Over the 6-year period, a total of 1,153 NIPTs were performed on 6 commercial brands. 216 (18.7%) were in consanguineous women. The gestational age at testing varied from 10-34 weeks, with 46 women being tested after 20 weeks. There were 20 true positives and 1 false negative. Results were not reported in 68 cases (5.9%); one of the brands (of the 4 most common) had a significantly (P<0.004) higher failure to generate a result rate (12.8% vs 3.9% and 3.2%). The failure to obtain a result was 8.5% in the consanguineous group, slightly higher (but not statistically significant) than 6.9% in the non-consanguineous group. There were 4 positive cases in low-risk women (who requested the test for assurance purposes), who would otherwise have had aneuploid fetuses, had they not requested testing.
Conclusion: For the first time, we showed that consanguinity does not appear to be a factor in failure to generate a result or very low cfFDNA. Further studies are required to confirm these important findings.

Corresponding author: Justin Konje, jck4@leicester.ac.uk

Introduction

Although aneuploidy complicates about 1 in 150 live births, [1] the number that complicates pregnancies overall is much higher. For example, of the 10-15%

 doi.org/10.32388/N2VRUA

clinically recognized pregnancies that result in miscarriages, [2] a significant proportion are associated with aneuploidy. Furthermore, about 50% of very early pregnancy losses have chromosome abnormalities. [3] Trisomies are the most frequently detected anomalies (61.2%), followed by triploidies (12.4%), monosomy X (10.5%), tetraploidies (9.2%), and structural chromosome anomalies (4.7%). [4]

Aneuploidy may be associated with structural abnormalities and/or learning disabilities even when there are no obvious structural abnormalities. A major rationale for prenatal diagnosis is the identification of pregnancies that are aneuploid, offering parents options including termination. Prior to 2011, this was done by measuring algorithms that included various risk factors (such as maternal age, personal, family or past obstetric history, and ultrasound markers) and combining these with biochemistry (maternal levels of various analytes), followed by invasive testing in the form of amniocentesis or chorionic villus sampling in high-risk cases. [5] This approach is associated with a false positive rate of up to 5%. Hence, a proportion of women who undergo invasive testing (with the associated risk of miscarriage) will be those with false positive results. [6][7]

Following the development of the technology to isolate cell-free fetal DNA from maternal circulation (cfFDNA), [8] non-invasive prenatal testing (NIPT) was introduced into clinical practice in late 2011 as a more reliable screening test for aneuploidy. [9] This was mainly for three aneuploidies (T13, T18, T21) and monosomy 45XO, which together constitute more than 60% of aneuploidies in pregnancies progressing beyond 10 weeks of gestation. [10][11] Increasingly, this practice is expanding to include sex chromosome aneuploidies, rare autosomal trisomies, and sub-microscopic copy-number variants. [12][13]

There have been several studies on the accuracy of NIPT as a screening test for aneuploidy, with reported sensitivities and specificities for these common aneuploidies of over 99%. [14][15] Among the reasons for false or discordant results are maternal weight (obesity is associated with a low cfFDNA fragment in maternal circulation), vanishing twin, fetal or maternal mosaicism, maternal malignancy, bioinformatics or human errors, and higher levels of homozygosity on the chromosomes tested when the single nucleotide polymorphisms (SNPs) between mother and baby are too similar to yield informative results – possibly from consanguinity, segmental uniparental disomy, or simply coincidence. [16] There have been, to the best of our knowledge, no extensive studies of the potential impact of consanguinity on NIPT.

Qatar is a small Middle Eastern country with an indigenous population of 300,000, although the total population is about 2.8 million (85% of the population are expatriates). Consanguinity rates among Qataris are reported to be up to 50%. [17] NIPT was introduced in our services in Qatar in 2015, and we have been offering this to our patients, most of whom are indigenous Qataris or from other Middle Eastern countries with similar consanguinity rates. The aims of this study were to (a) review the outcome of NIPT as a screening test for aneuploidy in our centre and (b) investigate whether consanguinity may have an impact on the NIPT.

Subject and Methods

This was a retrospective cohort study of all the records of the women who underwent NIPT at the Feto Maternal Centre, Doha, between 2015 when it was introduced and 2021. The records of the women were reviewed for variables, which included:

1. Demographic – age, nationality, consanguinity status, weight at the time of the NIPT, and height from which BMI was calculated.
2. Pregnancy specific – gestational age at test, CRL, number of fetuses, and outcome (normal or abnormal karyotype).
3. NIPT specific – fetal fraction (cfFDNA percentage), interval from receiving the sample to result, commercial brand, risk of aneuploidy (mainly T13, T17, and T21) and others (specified), and identified karyotype of the fetus if available.
4. Confirmatory invasive testing (amniocentesis/CVS) if done.

The records of those who did not have NIPT were excluded from the review.

All these variables were entered into an anonymised spreadsheet and analysed. IRB approval for the study was exempt, as it is an anonymised review of records. In our centre, women are offered screening for aneuploidy from 10 weeks of gestation based on risk factors which include age, past obstetric history, family or family history, structural anomalies in this pregnancy, or on request. Collected blood samples for the NIPT are processed, and the women are informed as soon as these are available. Those with a high-risk test (>1:150) are counselled and offered invasive

testing (amniocentesis or chorionic villus sampling depending on the gestational age). When the pregnancy ends, the outcome is linked to the antenatal records, including any postnatal testing for aneuploidy.

There was no need to calculate a sample size, as this was a study reviewing all the records over a defined period of time.

Results are presented as the mean and standard deviation (where data are normally distributed – normality tested by the Kolmogorov test) or median and ranges (where the data are not normally distributed). Sensitivity and specificity of NIPT were determined, and correlations between cfFDNA percentage and maternal BMI and gestational weight calculated. Comparisons were also made for rates of un-interpretable (low fetal fraction DNA percentage) in consanguineous and non-consanguineous pregnancies.

Results

Over the 6-year period, a total of 1,153 non-invasive tests were performed at the centre; 660 were Qataris, 220 were Caucasians (from Europe, North America, and Australasia), 165 were of other Arab nationalities, and 108 were of other non-Arab nationalities. **Table 1** shows the demographics of this cohort. Six commercial brands were employed during this period with no preference, but the most commonly used ones were Harmony (55.7%), Verifi (29.6%), and Nifty (8.1%).

Characteristic	Mean (range)
Age (years)	34 (21-46)
BMI (Kg/M^2)	28 (17.1-63.1)
Ethnicity	
Qatari	660 (57.2%)
Other Arab	165 (14.3%)
South Asians (Pakistanis, Indians, Bangladesh, Sri Lanka)	108 (9.0%)
Others (Europeans, Americans, Australasia, Japan, Singapore Hong Kong, Filipinos)	220 (19.4%)
Commercial Brand	
Harmony	642 (55.7%)
Verifi	341 (29.6%)
Nifty	93 (8.1%)
Microgen	60 (5.2%)
Illumina	16 (1.4%)
Panorama	1 (0.09%)

Table 1. Demographic characteristics of the women who had NIPT

Table 2 shows the indications for NIPT. The most common indications were advanced maternal age (56.3%) and patient request (35.6%). The gestational ages for the test varied from 10 to 34 weeks. **Figure 1** shows the relationship between cfFDNA and gestational age. As expected, there was a significantly direct relationship between the increase in cfFDNA and gestational age (R=0.364; P<0.05). Out of the 1,153 cases, 83 (7.2%) had low or insufficient results; 68 (5.9%) were insufficient to report on (i.e., no result), and 15 (1.3%) were low (below 4%). The BMI in 18 of these was greater than 40 kg/m^2. There was a statistically significant inverse relationship between cfFDNA and BMI, as shown in **Figure 2** (R= -0.67; P<0.001).

Indication	No (%)	Positive
Advanced maternal age (>=35 years)	655 (56.8%)	16 (2.4%)
Patient request	410 (35.6%)	4 (0.9%)
Abnormal NT	17 (1.5%)	5 (29.4%)
Structural abnormality	7 (0.6%)	1 (14.3%)
Past obstetric history (aneuploidy)	25 (2.2%)	0
Unknown/not available in records	39 (3.4%)	0

Table 2. Indications for NIPT

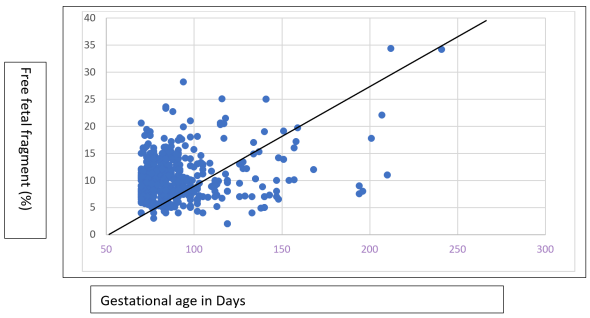

Figure 1. Changes with gestational age with cell free fetal DNA percentage (R=0.364; R^2=0.132; P<0.05)

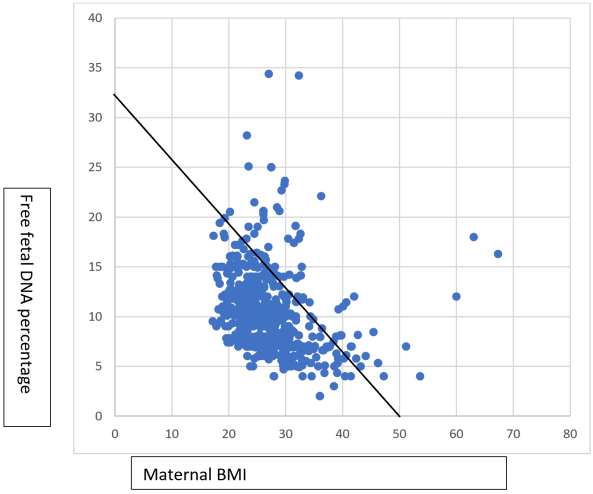

Figure 2. Relationship between maternal BMI and cell free fetal DNA percentage R=-0.67; R^2=0.449; P<0.001)

Table 3 shows the comparison between the various commercial brands. There were no differences in the demographics and cell-free fetal DNA in these. The time to reporting results was shortest with Nifty, but this was not statistically different from that with the others. One patient in the cohort had a false negative test on Verifi, and her BMI was 63.1 kg/m^2. One of the commercial brands (Verifi) had more low-fragment/no result cases (55 or 12.8%) compared to the others (Harmony - 25 or 3.9% and Nifty - 3 or 3.2%). This difference was statistically significant (P<0.05) when these were compared. There were no differences in the weights of the women tested by each of the commercial brands.

doi.org/10.32388/N2VRUA

	Harmony	Verifi	Nifty	Microgen	Illumina
N (%)	642	341	93	60	16
Age (years)	35 (23-44)	34 (21-47)	35 (22-45)	33.4 (20-41)	35.2 (29-44)
GA @ test	12.3 (10.0-34.3)	11.5 (10-27)	11.2 (10-26)	10.4 (10-24)	11.1 (10.2-19.5)
Feta fraction (%)	11.13 (5.1-34.4)	8.6 (1-21)	9.35 (3.49-34)	7.08 (7-11.6)*	8.4 (4.5-18.9)
Time to report (Days)	6 (2-15)	5 (2-12)	4 (3-11)	5 (3-12)	4 (3-11)
Fraction <4%/NA	0	14 (4.4%)	1	0	0
++No result	25 (3.9%)	41 (12.8%)	2 (2.2%)	0	0
Positive (True)	10 (1.6%)	5 (1.6%)	2 (2.2%)	2 (3.3%)	0
False positive	0	0	0	0	0
False negative	0	1 - T21**	0	0	0

Table 3. Comparisons between different Commercial Brands

** the only reading that was over 7 with Microgen*
*** high BMI 63.1*
++ P<0.0036 for the "No result" difference between the various brands.

Table 4 shows the comparisons between the consanguineous and non-consanguineous groups. Out of the cohort of 1,153, 216 (18.4%) were in consanguineous marriages, of which 17 were non-Qataris (12 from other Arab countries, 4 from India, and 1 from Pakistan). The consanguinity rate for the Qataris was 30.2% (119/660). Of the 83 with low or insufficient results, 18 (9.0%) were in the consanguineous group, compared to 63 (6.9%) in the non-consanguineous group (P>0.05). There were 3 cases in the consanguineous group with low (two below 1%) and too low to measure (one) cfFDNA. In the non-consanguineous group, 6 had low readings (1<1%, 1 was 1%, 3 were 2%, and 1 was 3%). The range of cfFDNA was similar in both groups, although it was greater (but not statistically significant) in the non-consanguineous than in the consanguineous groups; values above 20% were reported in 1.5% versus 1.9% respectively.

	Consanguineous N=212	Non-Consanguineous N=941
Age (years)	35 (21-46)	35.3 (20-4
BMI	29 (19-44.3)	32 (20-63.1)
Mean % fFDNA	7.92 (2-26.4)	8.73 (1-34.4)
Positive results N(%)	5 (2.4)	14 (2.2)
Insufficient or Not measured fFDNA N (%)	18 (8.5)	65 (6.9)

Table 4. Comparisons between consanguineous and non-consanguineous cases*

** P>0.05 for the comparisons between groups for each of the variable*

Table 5 shows the details of the high-risk tests that were confirmed by karyotyping. There were 10 cases of trisomy 21, 6 cases of trisomy 13, and 4 cases of trisomy 18. The sensitivity of the cfFDNA was >99% for three trisomies, while the specificity was 100 for T18 and T13 but >99% for T21. The percentage of fetal DNA fragment was less than 4% in one case (T18). Maternal age was the most common indication for testing in this group, with 5 of them having an abnormal NT as well. Patient request was an indication in 4 cases. There were no other aneuploidies or sex chromosomal abnormalities in this cohort.

Gestational age range (weeks)	Number	Indication for test
21^{+0}-24^{+6}	32	Patient request - 15 Maternal age - 13 Fetal abnormalities (including echogenic bowel) - 4
25^{+0}-29^{+0}	9	Patient request - 7 Maternal age (39 and 40 years) - 2 Fetal abnormality - 1
30^{+}	5	Patient request - 3 Maternal age (39 and 40 years) - 2

Table 5. Breakdown of NIPT in women over the age of 20^{+6} weeks

Table 6 shows the indications for testing in this group. There were 46 cases, most of whom were tested for patient request (25) and maternal age (17). There were 4 cases tested for minor fetal structural abnormalities, and one with echogenic fetal bowel. The echogenic bowel was tested at 22^{+4} weeks and ended as an IUFD at 28 weeks. There were no positive or false negative tests in this group.

No	ff%	Age (years)	GA (weeks)	BMI	Indication	Commercial Brand	NIFT (High risk for)	Karyotype
1	2	45	12W3D	39.5	Age & NT	Verifi	T18	47XX+18
2	6.2	36	11W1D	38.5	Age & NT	Harmony	T21	47XY+21
3	7	35	10W3D	28.3	Age	Microgen	T18	47XY+18
4	7	35	13w5d	30	Age & NT	Microgen	T21	47XY+21
5	7	41	12w1d	NA	Age & NT	Verifi	T21	47XY+21
6	6	42	12w6d	31.2	Age & NT	Verifi	T21	47XX+21
7	10.1	39	10w	30.1	Age	Harmony	T21	47XY+21
8	6.4	45	12w2d	32.5	Age	Nifty	T13	47XX+13
9	4.9	42	13w2d	35.7	Age	Harmony	T13	47XY+13
10	6	41	12w4d	32.5	Age	Harmony	T13	47XX+13
11	8.6	35	13w2d	24.3	Request	Harmony	T18	47XX+18
12	10.1	40	10w0d	25.5	Age	Harmony	T13	47XY+13
13	6.2	44	11w0d	33.4	Age	Harmony	T13	47XY+13
14	16	43	12w0d	30.2	Age	Verifi	T13	47XY+13
15	10.4	40	12w0d	19.0	Age	Harmony	T21	47XY+21
16	18	29	14w0d	63.02	Request	Verifi	False -ve T21	47XX+21@birth
17	7	33	21w0d	32.7	Request	Verifi	T18	47XX+18
18	7.8	29	14w2d	29	Request	Harmony	T21	47XY+21
19	7.9	37	13w6d	26.9	Age	Harmony	T21	47XY+21
20	14.1	39	13w4d	26.9	Age	Nifty	T21	47XY+21
21	87.1	9	13w2d	31.2	Age	Verif	T21	47XY+21

Table 6. Details of cases with high risk results

Discussion

From our cohort of 1,153 cases tested over a 6-year period, results were not obtained in 5.9% of cases (i.e., we obtained a result in 94.1% of cases). There were 20 positive results, all of which were confirmed on invasive testing, resulting in a sensitivity of >99% and a specificity of 99.9%. There was one false negative result in a patient with a BMI of 63, and there were no false positives. The most common indication for testing was advanced maternal age. The commercial brands used for the screening performed equally with no differences in time to result. These results are in keeping with previously published studies. [14][16][18][19][20][21][22] The average time taken for results was about 5 days, well within the recommendation that results should be available within 7-10 working days (NHS). [23] The prevalence of the combined aneuploidies (T21, T18, and T13) was 1.7% (20/1,153), much higher than would have been expected. This could possibly be due to the highly selected population – much older and based on affordability. Our proportion of cases in which results were not available (5.9%) falls within the range of 0-9% in the literature. [16][24][25][26][27][28]

As expected, the fetal fraction of DNA increased with gestational age, as shown in Figure 1. This rise is similar to what has been reported by others. There

was also an inverse relationship between maternal weight and the fraction of fetal DNA. In this series, there were 15 (1.3%) cases in which the fetal fraction was <4%, and for these cases, rates were reported. Most guidelines recommend that the fetal fraction should be at least 4% for risk to be assigned to the patient. [29][30][31][32] However, there is considerable data from commercial providers reporting results with a fetal fraction of 1.6% or more, [33] 1.9% or more, [34] 2.2% or more, [35] and 6.4% or more. [36] The data from the low fetal DNA fraction in our small series is in line with that from commercial providers and supports the recommendation by Fiorentino et al. [37] that the widely adopted minimum acceptable measured FF value of 4% may not be applicable to all cfDNA methods, and as such, each commercial provider should choose a value based on their own limit of detection. Interestingly, none of our cases where results could not be reported for one reason or another opted to have an invasive test (which is the standard recommendation after a failed repeat test. [38]

Several reasons have been advanced for false or failure to generate results. These include low fetal fraction [16][39][40][41] and noisy data because the DNA analysed was inherently less informative, making it difficult for the analytical algorithm to generate a risk with high confidence. [41][42][43] In some cases, the DNA of either the mother or fetus was not interpretable as a result of missing pre-analytics information, such as multiple pregnancies or egg donor pregnancies, vanishing twin pregnancies, fetal or maternal mosaicism. [16][39][40][41] A higher level of homozygosity on the chromosomes tested (especially when the SNPs between the mother and fetus are too similar to yield informative results) as a consequence of consanguinity has been suggested as another possible reason. [16] This potential cause has not been investigated previously. In our series, the consanguinity rate was 30.7% in the Qataris, allowing us the opportunity to investigate this factor. Although the rate of failure to get a result was higher in the consanguineous group than the non-consanguineous group (8.5% vs. 6.9%), this was not statistically significant. While consanguinity is a plausible cause of failure to obtain a result, our data do not support this possibility. However, we acknowledge that our numbers are small (albeit the largest published with such a high consanguinity rate), and therefore, caution should continue to be exercised in discounting this as a possible reason for failure to obtain results until data on larger numbers are published.

Of those with a positive result in our series, abnormal NT was an indication in 5 cases, all of whom were above the age of 35 years. In those with an abnormal NT and age below 35 years, none were positive on NIPT. Although it is recommended that invasive (diagnostic) testing be offered to those with an abnormal NT, [38] our women opted for NIPT despite counselling. While the results are reassuring (i.e., there was no false positive/negative), it is important to emphasize during counselling that NIPT is a screening rather than a diagnostic test. With more data, this may well become the option to offer women, as it reduces the risk of fetal loss from invasive testing. Interestingly, 4 of our positive results were in women who had requested the test because they perceived their *a priori* risk as high. Without testing, they would have delivered aneuploid babies. Therefore, it would seem reasonable to consider offering NIPT to every woman (as recommended by the American College of Obstetricians and Gynecologists). {m/44/} Such an approach would have significant cost implications where insurance does not exist. Hopefully, with wide adoption of NIPT, the cost will fall and make this available.

There have been very few studies in which NIPT has been performed in the late second and third trimesters. In fact, the NHS screening recommends that this should be performed only up to 20^{+6} weeks. [23] We were surprised by the numbers tested above 20^{+6} weeks in our series. Interestingly, most of them were either for maternal age or patient request, in contrast to the findings by Bajka et al. [16] where most cases above 20 weeks were for structural abnormalities. None of the tests in our series were abnormal. As expected, the cfDNA fraction was higher than that in the early gestations. It is uncertain why there were requests for late testing in other centres, bearing in mind that terminations at this late gestation would not be an option as they are not allowed in the country after 140 days. We speculate that one possible reason for this may be the need to ascertain the gender of the fetus, as there is considerable pressure on women to know the gender of the fetus prior to birth. While acknowledging that women are at liberty to ask for the test at any gestational age from 10 weeks, there must be recognition that a high-risk test requiring confirmatory invasive testing may be problematic for both the clinician and the woman. We therefore feel

that extreme caution should be exercised in this regard, and perhaps more emphasis should be placed on counselling in early pregnancy and making this option available.

Limitations and Strengths

There are several limitations to the study. The main one is the small sample size. It is possible that with larger numbers, differences that are not statistically significant may indeed become significant. It would have been interesting to analyse the data based on the platforms used for the NIPT to potentially compare the failure to generate a result with each platform. Another limitation is that our data comes from a private provider where cost is a crucial factor in the care offered. Our participants were therefore highly selected based on their ability to pay and were indeed much older. This could partly explain the higher prevalence of aneuploidies. A more representative population could potentially yield different results. A major strength of the study is that this is the largest and, to the best of our knowledge, the only study that has investigated this possibility.

Conclusion

While we have presented our experience with NIPT in general, the study yielded a total of 20 true positive results and 1 false negative. There were no differences in outcomes between the consanguineous and non-consanguineous groups, providing some (albeit not robust) evidence against consanguinity possibly leading to homogeneity in DNA between the mother and fetus and resulting in failure to generate a result. While this observation is important, it is based on a small cohort, and more data are required to confirm these findings.

Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. ^*Carlson, L. M., & Vora, N. L. (2017). Prenatal diagnosis: screening and diagnostic tools. Obstetrics & Gynecology Clinics of North America, 44(2), 245–256. https://doi.org/10.1016/j.ogc.2017.02.004*
2. ^*Gardner, R. J. M., Sutherland, G. R., & Shaffer, L. G. (2012). Chromosome Abnormalities and Genetic Counselling (4th ed.). Oxford University Press.*
3. ^*Warburton, D. (2000). Cytogenetics of reproductive wastage: from conception to birth. In M. H. F. L. Mark (Ed.), Medical Cytogenetics (pp. 213–246). Marcel Dekker.*
4. ^*Eiben, B., Bartels, I., Bähr-Porsch, S., Borgmann, S., Gatz, G., et al. (1990). Cytogenetic analysis of 750 spontaneous abortions with the direct-preparation method of chorionic villi and its implications for studying genetic causes of pregnancy wastage. American Journal of Human Genetics, 47, 656–663.*
5. ^*Fox, C. E., & Kilby, M. D. (2016). Prenatal diagnosis in the modern era. The Obstetrician and Gynaecologist, 18, 213–219.*
6. ^*Niemimaa, M., Suonpää, M., Perheentupa, A., Seppälä, M., Heinonen, S., Laitinen, P.,... Ryynänen, M. (2001). Evaluation of first trimester maternal serum and ultrasound screening for Down's syndrome in Eastern and Northern Finland. European Journal of Human Genetics, 9, 404–408.*
7. ^*Kagan, K. O., Maier, V., Sonek, J., Abele, H., Luthgens, K., Schmid, M.,... Hoopmann, M. (2019). False-positive rate in first trimester screening based on ultrasound and cell-free DNA versus first trimester combined screening with additional markers. Fetal Diagnosis and Therapy, 45, 317–324. https://doi.org/10.1159/000489121*
8. ^*Lo, Y. M. D., Corbetta, N., Chamberlain, P. F., Rai, V., Sargent, I. L., Redman, C. W. G., & Wainscoat, J. S. (1997). Presence of fetal DNA in maternal plasma and serum. The Lancet, 350, 485–487.*
9. ^*Chitty, L. S., & Bianchi, D. W. (2013). Non-invasive prenatal testing: the paradigm is shifting rapidly. Prenatal Diagnosis, 33, 511–513.*
10. ^*Carlson, L. M., & Vora, N. L. (2017). Prenatal Diagnosis: screening and Diagnostic Tool. Obstetrics & Gynecology Clinics of North America, 44, 245–256. Ref for initial NIPT.*
11. ^*Norton, M. E., Jacobsson, B., Swamy, G. K., Laurent, L. C., Räzinkov, A. C., Brar, H.,... Dukes, K. (2015). Cell-free DNA analysis for noninvasive examination of trisomy. New England Journal of Medicine, 372, 1589–1597.*
12. ^*Shaw, J., Scotchman, E., Chandler, N., & Chitty, L. S. (2020). Preimplantation genetic testing: non-invasive prenatal testing for aneuploidy, copy-number variants and single gene disorders. Reproduction, 160.*
13. ^*Benn, P. C., & Pergament, E. (2013). Non-invasive prenatal testing for aneuploidy: current status and future prospects. Ultrasound in Obstetrics & Gynecology, 42, 15–33.*

14. ^a, ^bZhang, Y., Xu, H., Zhang, W., & Liu, K. (2022). Non-invasive prenatal testing for the detection of trisomy 13, 18, and 21 and sex chromosome aneuploidies in 68,763 cases. Frontiers in Genetics, 13. https://doi.org/10.3389/fgene.2022.864076
15. ^Dai, R., Zhang, H., Li, L., Jian, Y., Liu, R., & Zhang, H. (2021). Analysis of 17428 pregnant women undergoing non-invasive prenatal testing for fetal chromosome in Northeast China. Medicine, 100(6). https://doi.org/10.1097/MD.0000000000025005
16. ^a, ^b, ^c, ^d, ^e, ^f, gBajka, A., Bajka, M., Chablis, F., & Burkhardt, T. (2021). Audit of the first >7500 non-invasive prenatal aneuploidy tests in a Swiss genetic centre. Archives of Gynecology and Obstetrics, 303. https://doi.org/10.1007/s00404-021-06203-7
17. ^Oniya, O., Neves, K., Ahmed, B., & Konje, J. C. (2019). A review of the reproductive consequences of consanguinity. European Journal of Obstetrics & Gynecology and Reproductive Biology, 232, 87–96.
18. ^Comas, C., Echevarría, M., Rodriguez, M. A., Prats, P., Rodriguez, I., & Serra, B. (2014). Initial experience with non-invasive prenatal testing of cell-free DNA for major chromosomal anomalies in a clinical setting. Journal of Maternal-Fetal & Neonatal Medicine. https://doi.org/10.3109/14767058.2014.947590
19. ^Fairbrother, G., Johnson, S., Musci, T. J., & Song, K. (2013). Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18 and 13 in a general screening population. Prenatal Diagnosis.
20. ^Lau, T. K., Cheung, S. W., Lo, P. S. S., Jiang, F., Li, Y., Jian, H.,... Shang, X. (2014). Non-invasive prenatal diagnosis of common fetal chromosomal aneuploidies by maternal plasma DNA sequencing. Journal of Maternal-Fetal & Neonatal Medicine. https://doi.org/10.3109/14767058.2011.635730
21. ^Warsof, S. L., Larion, S., & Abuhamad, A. Z. (2015). Overview of the impact of non-invasive prenatal testing on diagnostic procedures. Prenatal Diagnosis, 35, 972–979.
22. ^Lau, T. K., Chen, F., Pooh, R. K., Jiang, F., Li, Y., Jian, H., Li, X., Chen, S., & Shang, X. (2012). Noninvasive prenatal diagnosis of common fetal chromosomal aneuploidies by maternal plasma DNA sequencing. Journal of Maternal-Fetal and Neonatal Medicine. https://doi.org/10.3109/14767058.2011.635730
23. ^a, ^bHill, M., Wright, D., Daley, R., Lewis, C., McKay, F., Mason, S., Lench, N., Howarth, A., et al. (2014). Evaluation of non-invasive prenatal testing (NIPT) for aneuploidy in an NHS setting: A reliable accurate prenatal non-invasive diagnosis (RAPID) protocol. BMC Pregnancy and Childbirth, 14, 229. https://doi.org/10.1186/1471-2393-14-229
24. ^Judah, H., Gil, M. M., Syngelaki, A., Akolejar, R., & Nicolaides, K. H. (2021). Cell-free DNA testing of maternal blood in screening for trisomies in twin pregnancy: Updated cohort study at 10–14 weeks and meta-analysis. Ultrasound in Obstetrics & Gynecology, 58, 178–189. https://doi.org/10.1002/uog.23648
25. ^Gill, M. M., Accurti, A., Santacruz, B., Plana, M. N., & Nicolaides, K. H. (2017). Analysis of cell-free DNA in maternal blood in screening for aneuploidies: Updated meta-analysis. Ultrasound in Obstetrics & Gynecology, 50, 302–314.
26. ^Nicolaides, K. H., Musci, T. J., Struble, C. A., Syngelaki, A., & Gil, M. M. (2014). Assessment of fetal sex chromosome aneuploidy using directed cell-free DNA analysis. Fetal Diagnosis and Therapy, 35, 1–6.
27. ^Revello, R., Sarno, L., Ispas, A., Akolekar, R., & Nicolaides, K. H. (2016). Screening for trisomies by cell-free DNA testing of maternal blood: Consequences of a failed result. Ultrasound in Obstetrics & Gynecology, 47, 698–704. https://doi.org/10.1002/uog.15851
28. ^Cirigliano, V., Ordoñez, E., Rueda, L., Syngelaki, A., & Nicolaides, K. H. (2017). Performance of the neoBona test: A new paired-end massively parallel shotgun sequencing approach for cell-free DNA-based aneuploidy screening. Ultrasound in Obstetrics & Gynecology, 49, 460–464.
29. ^Rose, N. C., Barrie, E. S., Malinowski, J., Jenkins, G. P., McClain, M. R., & LeGrave, D. (2022). Systematic evidence-based review: The application of noninvasive prenatal screening using cell-free DNA in general risk pregnancies. Genetics in Medicine, 24, 1379–1391.
30. ^Hyett, J. (2014). Non-invasive prenatal testing for Down syndrome. Australian Prescriber, 37, 51–55.
31. ^McKie, F. L., Allen, S., Morris, R. K., & Kilby, M. D. (2017). Cell-free DNA-based non-invasive prenatal testing of aneuploidy. The Obstetrician and Gynaecologist, 19, 211–218. https://doi.org/10.1111/tog.12388
32. ^Monni, G., Zoppi, M. A., Luculano, A., Piras, A., & Arras, M. (2014). Invasive or non-invasive prenatal genetic diagnosis? Journal of Perinatal Medicine, 42, 545–548.
33. ^Taneja, P. A., Snyder, H. L., de Feo, E., Kruglyak, K. M., Halks-Miller, M., Curnow, K. J., & Bhatt, S. (2016). Noninvasive prenatal testing in the general obstetric population: Clinical performance and counseling considerations in over 85,000 cases. Prenatal Diagnosis, 36, 237–243.

34. ^McCullough, R. M., Almasri, E. A., Guan, X., Geis, J. A., Hicks, S. C., Mazloom, A. R., Deciu, C., Oeth, P., Bombard, A. T., & Saldivar, J. S. (2014). Non-invasive prenatal chromosomal aneuploidy testing - Clinical experience: 100,000 clinical samples. PLoS One, 9, e109173.
35. ^Zhang, H., Gao, Y., Jiang, F., Fu, M., Yuan, Y., Guo, Y., Zhu, Z., Lin, M., Liu, Q., Tian, Z., Zhang, H., Chen, F., Lau, T. K., Zhao, L., Yi, X., Yin, Y., & Wang, W. (2015). Non-invasive prenatal testing for trisomy 21, 18 and 13 - Clinical experience from 146,958 pregnancies. Ultrasound in Obstetrics & Gynecology, 45, 530–538.
36. ^Dar, P., Curnow, K. J., Gross, S. J., Hall, M. P., Stosic, M., Demko, Z., Zimmermann, B., Hill, M., Sigurjonsson, S., Ryan, A., Banjevic, M., Kolacki, P. L., Koch, S. W., & Strom, C. M., & Rabinowitz, M., & Benn, P. (2014). Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. American Journal of Obstetrics and Gynecology, 211, 527.e1–17.
37. ^Fiorentino, F., Bono, S., Pizzuti, F., Mariano, M., Polverari, A., Duca, S., Sessa, M., Baldi, M., Diano, L., & Spinella, F. (2016). The importance of determining the limit of detection of non-invasive prenatal testing methods. Prenatal Diagnosis, 36, 304–311.
38. a, bChan, N., Smet, M.-E., Sandow, R., da Silva Costa, F., & McLennan, A. (2018). Implications of failure to achieve a result from prenatal maternal serum cell-free DNA testing: A historical cohort. BJOG: An International Journal of Obstetrics and Gynaecology, 125, 848–844. https://doi.org/10.1111/1471-0528.15006
39. a, bGil, M. M., Revello, R., Poon, L. C., et al. (2016). Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test. Ultrasound in Obstetrics and Gynecology, 47, 45–52. https://doi.org/10.1002/uog.15783
40. a, bSamura, O., & Okamoto, A. (2020). Causes of aberrant non-invasive prenatal testing for aneuploidy: A systematic review. Taiwanese Journal of Obstetrics and Gynaecology.
41. a, b, cCuckle, H. (2017). cfDNA screening performance: accounting for and reducing testing failures. Ultrasound in Obstetrics and Gynaecology, 49, 689–692.
42. ^Snyder, M., Simmons, L. E., Kitzman, J. O., Coe, B. P., Hendure, J., & Gammil, H. S. (2015). Copy-number variation and false positive prenatal aneuploidy screening results. New England Journal of Medicine, 372, 1639–1645.
43. ^Hill, M., Wright, D., Lewis, R., McKay, F., Mason, S., Lench, N., et al. Evaluation of non-invasive prenatal testing (NIPT) for aneuploidy in an NHS setting: a reliable accurate prenatal non-invasive diagnosis (RAPID) protocol. https://www.acog.org/clinical/clinical-guidance/practice-bulletin/articles/2020/10/screening-for-fetal-chromosomal-abnormalities

Declarations

Funding: No specific funding was received for this work.
Potential competing interests: No potential competing interests to declare.