Non-invasive Prenatal Testing for Chromosomal Abnormality using Maternal Plasma DNA

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1. Introduction

Fetal genetic testing and aneuploidy diagnosis have until recently both needed invasive diagnostic sampling procedures carrying a small but significant risk of miscarriage. In 1997 the presence of cell-free fetal DNA (cffDNA) in the maternal circulation was reported. Fetal DNA comes from the placenta, can be detected from the first trimester of pregnancy onwards and is rapidly cleared from the maternal circulation after delivery. Maternal blood is therefore a reliable source of material for prenatal diagnosis. However, the cffDNA is mixed with a larger proportion of maternal cell-free DNA and current methodologies do not allow complete separation of fetal from maternal DNA in vitro. Therefore, the first applications of this phenomenon focused on the detection, or exclusion of, paternally-inherited fetal DNA sequences that are not present in the mother, such as Y chromosome sequences in pregnancies with a male fetus or rhesus D (RhD) sequences in women who are RhD-negative. Recently, DNA sequencing technologies have allowed very precise relative quantification of DNA fragments and so the detection of the extra material resulting from fetal chromosome trisomy within the maternal plasma DNA can now be performed in a robust manner.

This paper reviews the issues that underlie the decisions that maternity services and policy makers need to take in response to this new technology.

2. Conditions other than chromosomal abnormality

Fetal RhD typing using cffDNA to determine fetal blood group status from maternal blood has been used since 2000 to direct the management of pregnancies in RhD-negative women sensitised against RhD, which are therefore at risk of haemolytic disease of the fetus and newborn (HDFN). In the UK, cffDNA testing has replaced amniocentesis for fetal blood grouping. This is important because if the fetus is RhD-positive, amniocentesis for blood grouping risks boosting the antibody titre and so converting mild disease to severe. Determination of other fetal blood groups such as Kell, C, c and E using cffDNA has also been reported. The International Blood Group Reference Laboratory in Bristol reported the successful development of a high-throughput methodology using automated robotic techniques suitable for mass screening of all women who are RhD-negative. The use of cffDNA for fetal blood grouping has therefore moved from use in women with the antibodies, to routine antenatal care of RhD-negative women allowing antenatal administration of anti-D immunoglobulin (a blood product pooled from multiple donors) to be avoided in the 30–40% of RhD-negative women who are carrying RhD-negative babies.

Male fetal sex can be determined using cffDNA in maternal plasma by the identification of Y chromosome sequences (for example, DYS14 or SRY). Many studies have reported on the accuracy of non-invasive fetal sex determination using a variety of techniques, the most common being real-time polymerase chain reaction (PCR). In the UK, this is being used to determine fetal sex in women whose fetus is at risk of an X-linked disorder, where early identification of a male fetus indicates the need for an invasive diagnostic test to determine whether the affected X chromosome has been inherited. On the other hand, an invasive test is not required if the fetus is female. In pregnancies at risk of sex-linked diseases, non-invasive prenatal testing (NIPT) (with ultrasound) has been shown to reduce the use of invasive diagnostic testing by nearly 50% and has been approved by the UK Genetic Testing Network. A PCR-based sex prediction test can also be used in pregnancies at risk of congenital adrenal hyperplasia where early treatment of pregnancies with an affected female fetus can reduce the degree of virilisation of the external genitalia. This test can allow early cessation of dexamethasone treatment in pregnancies at risk of congenital adrenal hyperplasia where the fetus is found to be male.

Occasional diagnoses of single gene disorders have been reported by detecting or excluding the paternal allele inherited from an affected father with an autosomal dominant condition, such as Huntington’s...
disease. However, confirmation of achondroplasia in a fetus presenting de novo with short limbs in the third trimester has been reported and NIPT for achondroplasia and thanatophoric dysplasia was approved by the UK Genetic Testing Network in 2012 and is now part of established National Health Service (NHS) practice in the UK for pregnancies at risk of these conditions. For recessively inherited conditions, such as β-thalassaemia or cystic fibrosis, if the parents carry different mutations, exclusion of the paternal allele from the maternal plasma indicates that the fetus would be unaffected. If the paternal allele is present, an invasive test is required to determine whether the fetus has inherited the abnormal maternal allele and is thus affected. The possibility of using NIPT for virtually all single gene disorders (including X-linked and recessive disorders) was shown when digital PCR was able to detect small differences in the levels of mutant and wild-type alleles of disease-causing genes. The genotype of the fetus can thus be determined non-invasively. An alternative approach has also been implemented using massively parallel sequencing (MPS), targeting genomic regions involved in monogenic diseases.

3. Aneuploidy detection

In 1999, it was shown that pregnancies with Down syndrome fetuses had higher absolute concentrations of fetal DNA in maternal plasma or serum and, since then, a number of methods have been described for measuring fetal chromosome dosage using maternal plasma. These approaches have involved multiple techniques, including DNA methylation and circulating fetal RNA analysis and allelic ratio measurement. However, as these methods usually require polymorphic markers, no single marker or test is informative in all families; a limitation which makes broad application of these approaches challenging.

In 2007, Lo et al. and Fan and Quake reported an approach based on molecular counting by digital PCR, in which individual DNA molecules are counted. The core concept behind this approach is that the larger the number of molecules counted, the higher the analytical discrimination power of the technique. Furthermore, the number of DNA molecules in maternal plasma that need to be counted to detect a fetus with Down syndrome is inversely correlated with the percentage of fetal DNA. Part of the explanation for the success of this technique derives from the realisation that the proportion of cell-free DNA that is fetal is higher than was originally thought. However, the proportion of fetal DNA in the sample still has a strong effect on the reliability of maternal plasma DNA testing in general.

In 2008, the groups of Lo and Quake reported that maternal plasma MPS allows robust detection of fetal Down syndrome from maternal plasma. MPS with samples from pregnant women allows Down syndrome to be detected because of the existence of significantly more sequences from chromosome 21, despite the mixture of fetal DNA with maternal DNA. As MPS would allow virtually all DNA molecules in a plasma sample to be analysed (instead of being limited to DNA molecules having the binding sites of a specific pair of PCR primers), maternal plasma MPS offers a much more efficient method than digital PCR for maximising the amount of diagnostic information that can be obtained from a plasma sample.

3.1 Current technologies/services

Currently, two broad categories of MPS-based approaches are being used to provide plasma-DNA-based NIPT clinical services for patients:

3.1.1 Shotgun sequencing

In this approach, DNA molecules contained in a maternal plasma sample are sequenced at random. The proportional representation of DNA molecules sequenced from the chromosome of interest (for example, chromosome 21) is compared with those sequenced from elsewhere in the genome. The advantage of this approach is that the sequencing steps are essentially the same irrespective of the genomic locations of the chromosomal targets. Thus, this approach can potentially be applied for detecting chromosomal or genetic aberrations anywhere in the genome. This point is especially important for recent efforts to extend this approach to fetal karyotype or even fetal whole genome sequencing from maternal plasma, discussed below. One disadvantage of this approach is that genomic regions that are not directly relevant to NIPT are also analysed as the sequencing is random and so the
costs of sequencing and the information obtained are not focused. However, with the reduction in the cost of sequencing, it can be argued that the significance of this shortcoming is diminishing.

3.1.2 Targeted sequencing

In this approach, genomic regions containing the chromosomes at risk of the aneuploidy, as well as a selected group of reference regions, are selectively targeted for sequencing. To date, there are two different implementations of this approach, one involving targets that do not vary from individual to individual and another involving allelic ratio analysis for targets that are polymorphic within a population. The main advantage of the targeted sequencing approach is that the sequencing power can be concentrated on genomic regions of interest and the use of sequencing on areas not directly relevant to prenatal testing is minimised. One disadvantage of the targeted sequencing approach is that the targeting steps (for example, DNA hybridisation probes or PCR primers) need to be tailor-made for a particular test panel and changed when the number of test targets increase.

Whether targeted or shotgun sequencing gives more robust results will require future studies directly comparing these two approaches in the same cohort of patients.

3.1.3 Possible test refinements

The results of sequencing protocols can be analysed with the inclusion of additional clinical information, such as gestational age, maternal age or previous screening results, the proportion of fetal DNA in the sample and occasionally information from other sample types (e.g. a paternal DNA sample). Whether targeted or shotgun approaches are used, the cost of DNA sequencing is currently significant (although falling) and for that reason, mixing several patient samples together with a ‘molecular barcode’ for each sample before sequencing (so-called ‘multiplexing’) can be used. When considering such multiplexing approaches, test designers have to consider the number of sequence reads that would need to be obtained for the chromosomal region of interest, for example those from chromosome 21, such that an aneuploid fetus would be detected in a plasma sample with a particular percentage fetal DNA. With the current generation of DNA sequencers, a number of commercial laboratories are multiplexing up to 12 samples per shotgun sequencing reaction. In this regard, targeted sequencing protocols would allow the required number of reads for the chromosomal regions of interest to be generated at an even higher level of multiplexing, with the potential to reduce costs.

4. Results

To date, published data indicate extremely good results for trisomy 21 and trisomy 18 prediction when sequencing is successful. However, there is typically a single-digit percentage chance of no result due to the samples or sequencing results not meeting certain quality control criteria (which can vary from approximately 1–10% depending on the service provider), although a repeat sample will produce a result in the majority of such cases. Initially these tests were less reliable for trisomy 13 detection, but this has been evolving and subsequently better results have been reported for trisomy 13 as well, after optimisation of the algorithm. For trisomy 21 and 18, the detection rates in large series using different technologies have reported (after successful sequencing) sensitivity and specificity close to 100%. These analyses have been made both in freshly collected samples for this purpose but also in stored plasma samples, and the latter indicates considerable robustness to the system and analysis. In some parts of the world there is already considerable experience of using NIPT in mainstream clinical practice and test performance in clinical (i.e. not research) settings are consistent.

5. Possible sources of error

5.1 Early gestational age

The amount of cffDNA in maternal blood increases with gestational age. If the samples are taken too early in pregnancy, false-negative results with Y chromosome or RhD testing become more likely. For
aneuploidy testing, at the current depth of sequencing, many protocols allow detection only when the fetal DNA percentage is at least 4–5%. The commercially available aneuploidy tests based on MPS are generally stated to be for pregnant women from 10 weeks of gestation; a dating scan to establish gestational age before the sample is drawn is required. A number of sequencing protocols include a quality control step which involves measuring the fetal DNA percentage using either genetic or DNA methylation markers. Such precautions would be expected to reduce the chance of false-negative results due to an insufficient fetal DNA concentration.

5.2 Maternal obesity

The proportion of maternal plasma DNA that is fetal is affected by several maternal characteristics, including maternal weight. Increased maternal weight is associated with lower fetal DNA percentage, as has been well described in a study of over 22,000 samples. The reason is unclear, but could be high adipose cell turnover increasing maternal plasma DNA or increasing blood volume and so a dilutional effect. Regardless of the cause, since obesity is associated with significantly lower cffDNA fraction, which may predispose to less accurate NIPT results, this should be mentioned in counselling or patient information literature.

5.3 Multiple pregnancies

When a twin pregnancy is monochorionic (and so monozygotic), both fetuses will be affected or unaffected. Since the amount of cffDNA is approximately double that of a singleton pregnancy, cffDNA aneuploidy testing will not only be possible but probably more effective than in singletons. However, when twins are dichorionic, and so may be discordant, maternal plasma DNA testing would, in theory, not be as straightforward. Two studies reporting relatively small series of twin pregnancies have suggested that shotgun sequencing-based approaches are able to detect fetal trisomies in twin pregnancies. It is also possible to determine the zygosity of twin pregnancies using targeted sequencing from maternal plasma. In the event that the fetuses are shown to be dizygotic, this approach would also allow the fetal DNA percentage contributed by each fetus to be determined. This information will help ensure that the maternal plasma sample contains sufficient DNA from each fetus to yield a valid test result. The application of NIPT to twin pregnancies is still at a very early stage of development and there remain several unanswered questions, one of which lies in the scenario where one fetus in a dizygotic twin pregnancy miscarries; it is currently unknown how the DNA contribution from the miscarried fetus would change. The complexity introduced by twin pregnancies suggests that, prior to cffDNA testing, a good quality ultrasound scan would be a valuable first step in all pregnancies, to detect empty pregnancy sacs, for example, with fetal medicine counselling when one is suspected.

5.4 Placental mosaicism.

There is good evidence that the source of the cffDNA is the placenta. It is known from chorionic villus sampling (CVS) that abnormal cell lines can be present in the placenta that are not present in the fetus (in approximately 1% of CVS samples), a phenomenon often called ‘confined placental mosaicism’. As there will be cases with trisomic placental cells but a normal fetus, this is likely to be one of the reasons why invasive testing confirmation will be required before termination of pregnancy. Only with time will data from a sufficient number of cases be collected to assess the frequency of the potential for placental mosaicism to give inaccurate results. The most likely explanation for the so-called ‘false’ (discordant) positives is confined placental mosaicism: i.e. NIPT is detecting mosaic abnormal colonies of cells in the placenta.

5.5 Maternal conditions

Maternal chromosomal abnormalities, including mosaicism or malignant disease, could be very rare causes of discordant results.

6. Comparison of cffDNA tests with current practice

6.1 Comparison with invasive testing (karyotyping/microarray)

Invasive testing and karyotyping provide some information about all the chromosomes and if that is the aim of the non-invasive test, then sequencing material from all 46 chromosomes should be the target.
After invasive procedures, the potential advantage of assessing the whole genome is that one obtains ‘as much information as possible’ having undertaken the risk of the procedure. That debate has extended over many years in relation to whether accurate testing for chromosome 21, such as PCR or fluorescence in situ hybridisation (FISH) should be used alone or whether karyotyping should also be offered. That discussion is now being extended in the opposite direction (of obtaining more detailed information) with microarray comparative genomic hybridisation (CGH) used to examine DNA rather than karyotyping. The recent reports of fetal whole genome sequencing, using shotgun MPS from maternal plasma, follow this philosophy. There are clearly advantages and disadvantages of any level of detail.

### 6.1 Advantages of testing

One advantage of testing as widely as possible is the possibility of detecting a disease of potentially serious consequences serendipitously. Thus, shotgun MPS has been used to detect microdeletions, duplications, translocations and other chromosomal aberrations. In one case, a fetal microdeletion as small as 300 kb was detected. Although the technology is remarkable, if such a public health approach is undertaken, one could argue for screening for many more conditions at birth than is currently the policy. With non-invasive fetal whole genome sequencing and molecular karyotyping now a technological reality, the implications for the individual later in life need to be considered: for example, whether they wished to know about a diagnosis such as Huntington’s disease.

### 6.1.2 Disadvantage of testing

The major disadvantage of screening nonspecifically for chromosomal aneuploidies (including but not limited to Down syndrome) is that women who are pregnant will frequently be informed of findings of uncertain significance. Indeed, that risk already applies to karyotyping where it is clear that the use of the traditional criteria for a screening test would not suggest the introduction of karyotyping if it was being considered for the first time now. National screening policies require precise information about the condition under investigation (such as Down syndrome) and for the natural history to be known, among other criteria. Both karyotyping and microarray analysis could be described as being nonspecific ‘screening’ tests for conditions of very variable and, at times, unknown significance. This potential criticism also applies to ultrasound scanning. However, once a genetic concern has been raised, detailed, specific ultrasound examination will have an important role.

### 6.2 Comparison with screening tests

The combined test (nuchal translucency measurement with serum protein markers) has until now been the recommended NHS method of screening for chromosomal abnormalities, with a Down syndrome detection rate of nearly 90% for a 3% positive rate. The results of maternal plasma MPS have already been shown to be more effective than any of the previous screening tests with a sensitivity and specificity for detecting Down syndrome both approaching 100% as mentioned earlier. In England, the implementation of combined screening has greatly reduced the number of invasive procedures, leading to savings in the costs of invasive testing and in the loss of pregnancies as a result of invasive procedure related miscarriages. More accurate screening using maternal plasma MPS could therefore continue that process and even lead to better identification of cases in which invasive testing is considered. Maternal plasma MPS will be much better than maternal serum screening in the second trimester, which is still offered for women who book late. It is also expected that invasive procedures will still be used before termination of pregnancy after positive sequencing results for the foreseeable future. Although the false-negative rate is very low indeed, it is also possible that some women may request invasive procedures despite being at very low risk.

### 7. Options for implementation

#### 7.1 International policy statements

The American College of Obstetrics and Gynecology published an opinion statement in 2012 in which they suggested that at that time maternal plasma DNA testing should not be offered to ‘low-risk’ women. However, they suggested the indications for considering maternal plasma DNA testing to include:
maternal age ≥ 35 years, increased risk of aneuploidy on ultrasound, previous pregnancy with trisomy, positive screening test or parental balanced translocation giving increased risk to fetal trisomy. The advice in the opinion statement was therefore to use maternal plasma DNA testing in some form of contingent approach offering testing to higher-risk pregnancies.

In February 2013, guidance from Canada recommended that maternal plasma MPS for trisomies 21, 18 and 13 should be an option available to women at increased risk as an alternative to amniocentesis, but that invasive test confirmation of positive results should be undertaken before termination of pregnancy. It was stated that although maternal plasma DNA testing appears very promising for screening purposes, studies in average-risk pregnancies as well as lower test cost were required before it could replace the current maternal screening approaches.

In April 2013, the International Society for Prenatal Diagnosis published a position statement which concluded that maternal plasma DNA testing may be considered in women classified as high-risk by previous screens or assessed as high-risk as a result of maternal age, the presence of an ultrasound finding suggestive of trisomy 21, 18 or 13, family history of a chromosome abnormality that could result in full trisomy 21, 18 or 13, and history of a previous trisomy 21, 18 or 13 affected pregnancy. They also concluded that local economic considerations and access to sonography, invasive testing and counselling resources should be considered when deciding on the use of maternal plasma MPS.

7.2 Options for implementation within the NHS

7.2.1 Contingent (step-wise) screening

The use of maternal plasma MPS within a contingent approach would mean continuing combined testing and then to offer the maternal plasma DNA test to the subgroup (perhaps 20%) identified as higher risk. The cut-off to offer maternal plasma MPS could be set to manage the numbers and resulting costs and this approach could significantly reduce the invasive procedure numbers (by greatly reducing the false-positive rate). However, the sensitivity of this approach would depend on the false-negative rate chosen for the first step and so many cases of chromosomal abnormality would still be missed than would have been the case if maternal plasma DNA testing had been implemented within the primary test.

7.2.2 Maternal plasma DNA testing as part of the combined test.

Another possible use of maternal plasma DNA testing is to replace the protein assays in the combined test (i.e. human chorionic gonadotrophin (hCG) and pregnancy-associated plasma protein A (PAPP-A)) and instead use the maternal plasma DNA test results with the scanning information (such as nuchal translucency) and other clinical information such as maternal age. Indeed, one of the published algorithms can use the risk derived from the combined test with the maternal plasma MPS data. Some would argue that the information from the hCG and PAPP-A assays are useful in relation to predicting pre-eclampsia or fetal growth restriction and that this might make these assays in pregnancy still worthwhile. However, a new use (independent of Down syndrome screening) of an existing test should require a re-analysis of the cost-effectiveness. If the serum protein markers were no longer needed using this policy, the potential cost saving of the biochemistry services could help pay for the maternal plasma MPS test.

7.2.3 Maternal plasma MPS as the primary testing method

Another possible use of this technology could be for maternal plasma MPS to be used as the primary screening test for women who wish to know about chromosomal abnormalities. This approach is already occurring in the private sector when women are paying for the test, although they will require an initial scan. The use of maternal plasma DNA testing as the first test has the advantage of a very high detection rate with a very low false-positive rate, such that the number of affected pregnancies missed and the number of invasive procedures could both be greatly reduced. However, the cost of the maternal plasma MPS test as implemented within the NHS is not yet known. The combined test in the NHS costs approximately £100 per pregnancy and before its NHS implementation about 10–20% of pregnant women in England were prepared to pay more than £100 for the combined test in the private sector. A
similar proportion is likely to choose to pay for maternal plasma MPS as their primary test if the price falls to a similar rate.

The health economics analysis of this implementation policy might include spending the resources currently used for the combined test on maternal plasma MPS but that would be conditional on stopping both the nuchal translucency scan and serum biochemistry (such as PAPP-A and hCG) measurements, which some would argue against, as discussed above. The major, and probably decisive, advantage of this implementation approach is that the false-negative rate will be very low and so, in women who want to know about Down syndrome, few affected pregnancies would not be detected.

8. Consequences for existing services

Whichever of the possible implementation strategies are adopted, it can be assumed that the need for prenatal cytogenetics will continue to fall, as invasive procedures are used in a better-focused way. In addition, genetic testing will continue to move from cytogenetics to molecular genetics and, pending the results of the Evaluation of Array Comparative genomic Hybridisation in prenatal diagnosis of fetal anomalies (EACH) study, this will especially be the case for CGH microarrays. These changes may release resources that could be used for maternal plasma MPS testing. However, the reduction in numbers of invasive procedures will make it harder for fetal medicine trainees to become skilled in the techniques. The changes will also mean the workload of trained fetal medicine practitioners undertaking amniocentesis/CVS will reduce and the ratio will shift towards more CVS and away from amniocentesis. It seems likely that invasive procedures will be undertaken in fewer, larger centres than at present. The workload of biochemical screening laboratories may be reduced and these services may no longer be required at the current level. Ultrasound scanning will continue to be clinically important as its value for predicting nonchromosomal fetal abnormalities and pregnancy complications seems compelling. However, the previous abandonment of the use of what used to be called ‘soft markers’ to adjust screening risk will be further consolidated. Resources for the education and training of health professionals offering this testing and pre-test information and discussion with the patient will be required.

9. Cost/availability

The cost of sequencing has been falling. The ability to multiplex many patient samples into a single sequencing run has the potential to reduce the cost of an individual test significantly. However, intellectual property costs may increase the price of these tests. It seems likely that this aspect will influence the initial implementation by the NHS and public health services in other countries. Cost savings from the reduction in other diagnostic tests could be used to fund these developments, as has already been demonstrated in reductions in invasive tests for sex-linked diseases.

At present it seems that laboratories offering these services are in limited geographical areas. In Europe, one of the first laboratories providing MPS-based aneuploidy testing was established in Germany. Considering the number of tests that may now be requested and the potential difficulties of transporting human blood samples, consideration of the geographical distribution of services is required. The UK needs to establish a maternal plasma MPS aneuploidy testing service, which at least in the private sector may initially be for women who are reluctant to have invasive testing after positive combined test screen results or fetal structural anomalies have been found.

10. Ethical aspects

As with many new advances, these technological developments are not without ethical issues. Gaining reassurance in a pregnancy will become much safer for many women by removing the risks associated with invasive tests. In addition, testing for information/preparation only (when termination of pregnancy would not be chosen whatever the result) will be easier and perhaps a more common choice. However, by removing the need to discuss the miscarriage risk, maternal plasma DNA testing may provide an
apparent ease of access that could compromise informed decision making by increasing the likelihood that the remaining implications of test outcomes would not be considered adequately. Excellent communication between women and health professionals is essential to understanding the implications of the apparently ‘simple, safe blood test’ offered to detect Down syndrome or other conditions. Moreover, the potential simplicity of NIPT raises the prospect of such tests being offered on a direct-to-consumer basis, perhaps via the internet or mail order as has already happened in the USA for fetal sexing. Consideration will need to be given to the potential commercialisation of this technology and whether and how regulation is implemented.

Discussion of the complete range of ethical issues is beyond the scope of this paper but on a global perspective, an important issue relates to fetal sex determination. The high degree of accuracy of NIPT as early as 7 weeks of pregnancy carries a number of socio-ethical implications, such as the selective termination of fetuses according to sex. Other issues subsequent to the removal of miscarriage risk include the possibility that women may seek prenatal diagnosis for an increasing number of conditions or for paternity testing.

11. Opinion

NIPT using maternal plasma DNA is available for several conditions. Obstetricians have used NIPT to guide management of women who are RhD-negative and whose fetuses are at risk of HDFN for many years and guidelines should already reflect this change in practice. Fetal sex determination for clinical indications in pregnancies at high sex-linked genetic risk is also established practice. Testing for aneuploidy and especially Down syndrome by maternal plasma MPS is now available commercially but while the test result is much more accurate than existing screening strategies, it is still not a diagnostic assay. Hence, the term ‘non-invasive testing’ is currently used.

Detection of Down syndrome by maternal plasma DNA testing will alter the way that prenatal diagnosis and screening is delivered in the UK, both in the NHS and the private sector. It will have significant implications for some existing services such that clinical biochemistry serum screening laboratories and cytogenetic and molecular genetics laboratories can expect a fall in the number of samples. Since women are already accessing these tests, all obstetricians should have knowledge of the counselling issues involved. In addition, major NHS health policy decisions are required. In time, this technology is likely to become the primary screen for chromosomal abnormalities in pregnancy. This will enhance the information available to pregnant women while greatly reducing the loss of uncomplicated pregnancies as a result of miscarriage caused by unnecessary invasive procedures.

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