Review Article

Prenatal Screening Methods for Aneuploidies

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Abstract

Aneuploidies are a major cause of perinatal morbidity and mortality. Therefore, it is the most common indication for invasive prenatal diagnosis. Initially, screening for aneuploidies started with maternal age risk estimation. Later on, serum testing for biochemical markers and ultrasound markers were added. Women detected to be at high-risk for aneuploidies were offered invasive testing. New research is now focusing on non-invasive prenatal testing using cell-free fetal DNA in maternal circulation. The advantage of this technique is the ability to reduce the risk of miscarriage associated with invasive diagnostic procedures. However, this new technique has its own set of technical limitations and ethical issues at present and careful consideration is required before broad implementation

Keywords: Biochemical screening, Cell-free fetal DNA, Non-invasive prenatal testing, Prenatal diagnosis, Trisomy

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Introduction

Screening is the process of surveying a population, using a specific marker or markers and defined screening cut-off levels, to identify the individuals in the population at higher risk for a particular disorder. Screening is applicable to a population; diagnosis is applied at the individual patient level.[1]

A couple may approach the doctor seeking preconception or early pregnancy genetic advice for a variety of reasons, including:

1. A possibly heritable condition in one (or both) of the couple
2. A history of infertility
3. History of recurrent pregnancy loss
4. A family history of one or more possibly heritable conditions
5. The couple is from a population group with a high frequency of certain genetic diseases
6. The couple is blood relatives (a consanguineous marriage)
7. Advanced age
8. The couple is anxious about reproductive risks, even though there is no specific indication that they are at increased risk.

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Aneuploidies are major causes of perinatal death and childhood handicap. Consequently, the detection of chromosomal disorders constitutes the most frequent indication for invasive prenatal diagnosis. Multiple marker screening uses a combination of maternal age and two or more biochemical tests, with or without an ultrasound examination, to produce a single result for risk of Down syndrome, trisomy 18, and open neural tube defects (ONTDs), which is used to offer options for clinical management. A screen is positive when the risk of one or more of the screened disorders falls above a designated risk cut-off. Counseling and further testing options are offered when a screen is positive.

Information was obtained through a literature search via PubMed and Google using key words like "aneuploidy screening", "non-invasive prenatal diagnosis" and "cell-free fetal DNA (cffDNA)". The internet search was accompanied by a detailed search of our library database. The articles were then reviewed and summarized in a comprehensive manner.

Criteria and Indication for Prenatal Diagnosis

When prenatal diagnosis is being considered in genetic

counseling, several basic factors must be examined, but the most important is whether the couple concerned actively wish for prenatal diagnosis; all too often it is suggested simply because it may be technically feasible and without adequate information. Because most prenatal diagnostic procedures involve a large amount of worry to the parents, and a significant morbidity and mortality to the fetus (with 100% mortality if the test proves abnormal and termination is requested), prenatal diagnosis should normally be carried out only if the general criteria summarized in [Table 1] are fulfilled. These are self-evident, but as in most clinical situations, cases of real doubt may occur.

Severity of disorder

This is beyond doubt in most of the disorders for which prenatal diagnosis is employed, including Down syndrome and other autosomal trisomies, ONTD and the rare neurodegenerative metabolic disorders. Other conditions may be more questionable, especially those where physical abnormalities (e.g., limb defects, cleft lip and palate) are likely to be accompanied by normal intellect and life expectancy. Albinism, which has few general health implications in northern climates, may, because of the likelihood of skin cancer, be a fatal disorder in tropical countries. Such variable categories are increasing, particularly as molecular analysis increasingly recognizes specific mutations with relatively mild clinical effects, and this may present difficult decisions, the outcome of which will vary from family to family, and between different societies.

Treatment availability

Treatment may be clear-cut and satisfactory in some disorders that might otherwise be considered for prenatal diagnosis. Thus, in phenylketonuria, now detectable prenatally by molecular analysis, most children treated from birth have near normal health and intelligence, at least in countries where dietary treatment is available. In contrast, in galactosaemia, liver damage is occasionally present at birth and the long-term outlook for the infant is less clear. Whether prenatal diagnosis is undertaken here will probably depend on the attitudes and previous experience of the parents. In congenital adrenal hyperplasia the outlook with treatment for a second child is much better than for the first-born, in whom delayed diagnosis commonly results in death or serious morbidity, while treatment in utero is also a possibility.

Table 1: Criteria for prenatal diagnosis

Is the disorder sufficiently severe to warrant termination of the pregnancy?
Is treatment absent or unsatisfactory?
Is termination of an affected pregnancy acceptable to the couple concerned?
Is an accurate prenatal diagnostic test available?
Is there a significant genetic risk to the pregnancy?

Acceptability of termination

The acceptability of termination of pregnancy to a couple must be determined before any prenatal procedures are contemplated. In some cases, it is unacceptable on religious grounds or because of the prevailing attitude of the community; in others, it is a more personal ethical view. Acceptability may be a relative phenomenon. Thus, in the past many couples found fetal sexing by amniocentesis–with late termination of a male pregnancy which might be normal–unacceptable, whereas these same individuals may accept first-trimester termination, following chorionic villus sampling (CVS), of a definitely affected male pregnancy. Similarly, in some religious traditions, early termination may be allowable, while late termination is forbidden. It is essential to know the attitude of a couple before pregnancy occurs because this may well affect their decision whether or not to have further children. Unacceptability of termination should not be considered as automatically ruling out prenatal diagnosis. In rare instances, parents may feel that they will gain by being able to prepare for an affected child, although this is exceptional, especially when the risk of prenatal procedures is pointed out. Serious potential ethical problems arise if prenatal diagnosis is undertaken for a late-onset disorder and the pregnancy continues.

Feasibility of prenatal diagnosis

The feasibility of diagnosis is something that continues to change rapidly with scientific advances, so it cannot be too strongly stressed that the person giving genetic counseling must obtain accurate information on this point before suggesting the possibility to a couple, and must be satisfied that the technique is reliably applicable as a service rather than just as a research procedure. Failure to do this is as reprehensible as submitting a patient to some new surgical procedure without enquiring as to its benefit and mortality. This is especially relevant when using new molecular advances, where the boundary between research discovery and established techniques can be hard to define, especially for very rare disorders, or those where the gene has been recently isolated.

Current Screening Technologies

Nuchal translucency combined with biochemical markers (first trimester)

Nuchal translucency refers to the subcutaneous layer of fluid behind the fetal neck and lower cranium, which can be visualized on ultrasound. Increased nuchal translucency in the fetus is associated with increased risk of chromosomal abnormality and other diseases.[2]

Sonographic measurement of the thickness of the nuchal fold between 11 + 0 weeks and 13 + 6 weeks of pregnancy, together with maternal age and biochemical markers allows an individualized risk of aneuploidies such as trisomy 21, 13, and 18 to be calculated.

Prerequisites for an interpretable nuchal thickness measurement include operator qualification, choice of appropriate duration of investigation, and technical considerations. The Down syndrome detection rate in major studies ranges from 79% to 90% with a 5% false positive rate.[3] Table 2 showing inclusion of additional parameters such as measurement of the nasal bone, doppler assessment of the tricuspid valve, and the ductus venosus, and the facial angle allows individualized detection rates for trisomy 21 to be increased to up to 95%.[4,5] Two first trimester maternal serum biochemical markers emerged at the same time as NT was being investigated. These are PAPP-A and human chorionic gonadotropin (hCG) (free beta or total). PAPP-A is lower in Down syndrome pregnancies and hCG is higher.[6,7]

Second trimester biochemical screening

Traditionally at 16-20 weeks the concentration of maternal serum alphafetoprotein (MSAFP), unconjugated estriol, hCG in the "triple screen" and additionally inhibin A in the "quadruple screen" are measured and the composite risk for neural tube defect (NTD), trisomy 21and trisomy 18 is estimated. For a 5% false positivity rate the sensitivity of triple test is 70% and that of quadruple test is 75% [Table 2]. In foetuses with Down's syndrome, the MSAFP and estriol levels are decreased and hCG and inhibin levels are higher. In trisomy 18, levels of all the three markers are decreased.

Table 2: Detection rate of trisomy ten plotted against the type of screening parameters and tests used

First trimester	%
Maternal age	30-50
PAPP-A, hCG, MA	60-63
NT measurement and MA	74-80
Combined test (NT, PAPP-A, hCG, MA)	86-90
Combined test plus nasal bone, tricuspid flow, ductis venosus and facial angle	95
Second trimester	
Maternal age	30-50
2nd trimester double test (AFP, hCG, MA)	60
Triple test (AFP, hCG, E3, MA)	68
Quadruple test (AFP, hCG, E3, Inhibin A, MA)	79
Ultrasound (16-23 weeks) with anomaly screening	75
Invasive diagnostic testing	
Chorionic villus sampling	close to 100
Amniocentesis	close to 100

PAPP-A: Pregnancy associated plasma protein -A; MA: Maternal age; NT: Nuchal translucency; hCG: Human chorionic gonadotropin; AFP: Alphafetoprotein

Screening for NTD

Elevated MSAFP can identify 75-90% ONTDs, 95% anencephaly and 85% of ventral wall defects with a false positive rate of 5%. Raised MSAFP levels should be offered genetic counselling and targeted ultrasound for further evaluation.[8]

Integrated prenatal screening

In an effort to further improve performance, the first and second trimester screening tests have been combined into a process called IPS. IPS was based on the use of PAPP-A and NT in the first trimester and the quad screen in the second trimester, with results released when all the testing was completed.[9] This approach has been controversial, with some authors suggesting women had the right to know their results early and that it was unethical to withhold the first trimester results.[10] However, when IPS utilizes a quad screen in the second trimester, studies have shown a detection rate of 85% to 87% with an FPR of 0.8% to 1.5%.[6,11] The benefit of IPS over first trimester screening (FTS) is the achievement of a lower FPR and reduction of the number of invasive diagnostic procedures needed.

When NT is not available, IPS still can be offered, using PAPP-A in the first trimester and triple or quad screening in the second trimester. This approach has an 83% DR for a 4% FPR.[11] Given that timing is critical for serum analysis, accurate dating of the pregnancy is very important. Ultrasound dating should be performed if menstrual or conception dating is unreliable. For any abnormal serum screen (serum IPS, quad) calculated using menstrual dating, an ultrasound should be done to confirm gestational age.

Contingent screening

The concept of contingent screening has been suggested by Wright *et al.*[12] as an alternative to IPS. In contingent screening, the majority of women receive their result after FTS. Women at high risk (risk > 1/50) are offered invasive testing, and women at low risk (risk < 1/1500) require no further testing. A proportion of women with a risk between two cut-offs (1/50 and 1/1500) will go on to have second trimester screening and will receive a combined result.

Sequential screening

Sequential screening selects women for second trimester testing on the basis of their FTS results. Women found to be at high-risk on the basis of the FTS (e.g. risk ≥ 1 in 50) are offered invasive testing. Those with a risk lower than the cut-off are offered additional serum screening in the second trimester. The removal of screen positive affected cases in the first trimester decreases the

prevalence of Down syndrome in the second trimester and consequently lowers the PPV of second trimester serum screening.[13]

A substantial proportion of rural women with at-risk pregnancies go through their pregnancy period without significant modern antenatal care.[14] The current screening programs for aneuploidy require women to have multiple visits especially for contingent, integrated and sequential screening. This is inconvenient for the women as well as causes delay in diagnosis. Furthermore, expertise for NT is not available in all centers, thereby, limiting the detection of aneuploidies in FTS.

The use of ultrasound in screening for chromosomal anomalies

At 18-20 weeks' gestation, all pregnant women should be offered a detailed ultrasound that meets previously established minimum standards.[15] Most major fetal anatomic abnormalities should be detected by this screen. In particular, the majority of ONTDs should be detected by this ultrasound.[16] In addition, ultrasound can detect "soft markers," which are features that increase the *a priori* risk of fetal aneuploidy but can also be variations of normal. When used alone, second trimester ultrasound soft markers do not effectively discriminate between unaffected fetuses and fetuses with Down syndrome, because of the high positive rate from the large number of potential markers.[17]

Ultrasound soft markers and anomalies identified in the 18-20 week ultrasound can be used to modify any *a priori* risk established by age or prior screening. In the absence of soft markers and anomalies, a reduction of risk can be applied.

Screening in twin pregnancies

The biochemical markers in twin pregnancies are on average twice that in singleton pregnancies. A "Pseudo risk" is calculated whereby the measured result (in Multiple of Median [MOM]) is divided by corresponding median MOM value. The risk is evaluated as for singleton pregnancy. This does decrease the sensitivity of the screening test compared to singleton pregnancy, however, remains a useful approach for evaluation.

Following points to be noted while ordering a screening test which have a significant impact on the screening performance–correct date of birth, gestation by USG, maternal weight, number of foetuses, chorionicity, natural/*in vitro* fertilization (IVF) conception, if ART date of embryo transfer/age of egg donor, maternal age, insulin dependent diabetes, family history of Down's syndrome.

CVS or Amniocentesis

An alternative to screening is invasive prenatal diagnosis by CVS or amniocentesis which directly assesses the chromosome constitution of the fetus through cells from the pregnancy. The advantage is the diagnostic certainty of detecting trisomy 21, 18, and 13. In addition, testing fetal cells and the amniotic fluid may allow for the detection of other chromosome abnormities, genetic conditions, or ONTDs [Table 2]. Although, this approach to the fetal testing is gold standard and gives definitive diagnosis, the chances of miscarriage (around 1%) and invasiveness makes it inconvenient to pregnant women.[18] Thus, the need for the non-invasive methods of detection of fetal cells led to detection of these fetal cells in the cervical mucus[19] and in maternal blood.

Non-invasive prenatal testing

The presence of cffDNA in the blood of pregnant women and its potential use in NIPT was first described in the 1990s.[20,21] Fetal DNA can be detected from the 4th week of gestation, though only reliably from 7 weeks, and the concentration increases with gestational age-from the 16 fetal genomes per ml of maternal blood in the first trimester to 80 fetal genomes per ml in the third trimester, with a sharp peak during the last 8 weeks of pregnancy. Fetal DNA is believed to originate from trophoblast cells, and comprises around less than 10% of the total cell-free DNA in maternal circulation during pregnancy.[22] Unlike cellular DNA, circulating cffDNA consists predominantly of short DNA fragments rather than whole chromosomes, of which 80% are <193 base-pairs in length. In contrast to fetal cells, cffDNA is rapidly cleared from the maternal circulation with a half-life of 16 min and is undetectable after 2 h of delivery.[23]

Different published clinical trials validated cell free DNA analysis to detect common aneuploidies with a high sensitivity and specificity [Table 3]. This led to the clinical availability of NIPT in high-risk pregnancies in the United States, beginning in late 2011.

Methods of detecting cff DNA

The basic principle in extracting the cffDNA is to take initially maternal plasma, separate cellular matter by centrifugation, followed by isolation and purification of all cell-free DNA, followed by exploiting the small differences between the fetal and maternal DNA sequences in order to make a specific fetal diagnosis.[23] The most common technique currently used for detection and identification of specific cffDNA sequence is polymerase chain reaction (PCR) with its different variants (nested PCR, real time PCR, digital PCR) and followed by DNA sequencing.

Table 3: Clinical trials validated cffDNA analysis for detection of fetal aneuploidies

Studies	Trisomy 21		Trisomy 18		Trisomy 13	
	Sensitivity % (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Palomaki *et al*., 2011[40]	98.6 (95.9-99.7)	99.8 (99.4-99.9	-	-	-	-
Palomaki *et al*., 2012[41]	-	-	100 (93.9-100)	99.7 (99.3-99.9)	91.7 (61-99)	99.1 (98.5-99.5)
Bianchi *et al*., 2012[42]	100 (95.9-100)	100 (99.1-100)	97.2 (85.5-99.9)	100 (99.2-100)	78.6 (49.2-99.9)	100 (99.2-100)
Dan *et al*.; 2012[43]	100	99.96	100	99.96	-	-
Norton *et al*.; 2012[44]	100 (99.5-100)	99.97 (99.8-99.99)	97.4 (86.5-99.9)	99.93 (99.75-99.98)	-	-
Ashoor *et al*.; 2012[45]	100	100	98	100	-	-
Sparks *et al*.; 2012[46]	100	99.2	100	100	-	-
Ashoor *et al*. T 13; 2012[47]	-	-	-	-	80 (49-94.3)	99.9 (99.7-100)
Nicolaides *et al*.; 2012[26]	99	99.9	99	99.9	-	-

CI: Confidence Interval

NIPT for fetal aneuploidy

One of the applications of NIPT that appears to be close to clinical implementation is a test for fetal-chromosome abnormalities, notably Down syndrome. This testing is envisaged as being available to all women in the first trimester of pregnancy and would potentially replace current screening and diagnostic methods. Recently, NIPT by analysis of cffDNA in maternal blood has shown promise for highly accurate detection of common fetal autosomal trisomies.[24] Analysis of cffDNA has been validated in several clinical studies utilizing next generation DNA sequencing technology.[25] Clinical studies have primarily included women identified by prior screening, with maternal age and biochemical and/or sonographic testing in the first or second trimester of pregnancy, to be at high-risk for aneuploidies.

In a recently published article of NIPT of fetal trisomies in a routinely screened first trimester population showed that NIPT with a chromosome-selective sequencing approach is highly accurate for fetal aneuploidy detection with very low FPR. The estimated trisomy risk score was >99% in all cases of trisomy 21 and trisomy 18 and <1% in 99.9% of the euploid cases.[26]

There is less confidence in NIPT as a screen for trisomy 13 due to technical issues and the infrequency of the condition. Detection rates between 79% and 92% have been reported, meaning between 8 and 21 out of 100 pregnancies with affected fetuses will be missed. The false-positive rate may be about 1%, so 1 out of 100 unaffected pregnancies may be positive for trisomy 13, so confirmatory testing is recommended.

Positive result

NIPT is highly sensitive and specific for trisomies 21 and 18; positive results are "near diagnostic". However, false positives have been reported so at this time it is recommended that positive results be followed with confirmatory testing by CVS or amniocentesis.[27] Confirmatory testing can also provide important information about the cause of the trisomy; specifically, CVS or amniocentesis will identify cases of Down syndrome that are due to a 21 chromosome translocation as opposed to the more common trisomy 21. This has important recurrence risk implications for the parents and other family members.

Fetal anatomic ultrasound can also be a helpful tool for pregnancies that test positive on NIPT, looking for additional ultrasound findings that support the diagnosis.

Negative result

Even though NIPT is highly sensitive and specific, it is important to remember that it is not 100%. There are false-negative results, so a negative result cannot absolutely rule out an affected fetus. A laboratory may provide a risk score, allowing the clinician to quantify risk for trisomy.

"Unreportable" or "no-call" result

Depending on the laboratory, 0.5-7% of women who undergo NIPT will not get a result, often because there is an insufficient amount of fetal DNA in the sample (low fetal fraction) due to various clinical reasons which may include high maternal weight or early gestational age.[27] A laboratory may decline to report results near the cut-off. In any case, the clinician must determine, in conjunction with the NIPT laboratory and the patient, whether to draw another sample later in the pregnancy, revert to conventional serum or ultrasound screening, move on to invasive testing, or decline any further testing.

Other clinical uses of NIPT

Sex determination was the first application of NIPT. Because the Y chromosome is absent in the genome

of the pregnant woman, detection and measurement of fetal-derived paternally inherited DNA was the first focus of researchers in the prenatal screening field. While, detection and identification of fetal sex-linked or sex limited conditions is considered a legitimate medical reason for NIPT testing of sex, the more common use of pre-conception and prenatal technology for sex-determination is based on preference.[28] It is mainly helpful for sex-linked disease, such as haemophilia, Duchenne muscular dystrophy, X-linked mental retardation, adrenoleukodystrophy, Alport's syndrome, X-linked severe immunodeficiency, retinitis pigmentosa.[23]

NIPT allows for faster determinations of the Rh factor status of the mother and fetus.[29] It lessens misdiagnosis (where both the mother and fetus are Rh D negative), and the unnecessary exhaustion of medical resources.

Technical difficulties

There are a number of technical and clinical obstacles to achieving high diagnostic accuracy:[23]

1. It is important to emphasize that complete fetal genotyping is not conceivable using cffDNA in the maternal circulation and that the genetic information derived from cffDNA is entirely restricted to the specific DNA sequence (or chromosome) detected.
2. False negatives can be the result of failure to extract or detect sufficient material, due to individual variability in the amount of total cell-free DNA and the small proportion of fetal versus maternal cell-free DNA.
3. False positives can be the result of either technical issues, such as contamination, or clinical abnormalities such as the presence of a non-identical vanishing twin.

Ethical issues

Widespread clinical implementation of NIPT is likely to have significant societal consequences. One issue will be the degree of equity as to which groups will have access.

NIPT may be costly and only covered by some types of medical insurance policies. If so, it could be yet another technology disproportionately available to the affluent. Indeed, a consequence of these inequalities could be the perception that NIPT constitutes a contemporary form of eugenics with the affluent, educated, or other selected groups having a greater ability to determine the genetic characteristics of their children.[30]

The ethical implications of sex-selection are well documented. Sex selective breeding and sex selective abortion are most commonly associated with the nations of India and China, whose overpopulations concerns have led to growth control policies, generally targeting girl children.[31]

Professional society statements

Professional societies are beginning to make statements about the use of NIPT. American College of Obstetricians and Gynecologists (ACOG) recommends offering aneuploidy screening or invasive testing to all women, regardless of age. The ACOG and Soceity of Maternal Fetal Medicine (SMFM) both say that cffDNA testing can be offered to pregnant women at increased risk for trisomy 13, 18, or 21. Women age 35 and older, women with a history of a child with trisomy, and women carrying a fetus that shows abnormalities on an ultrasound are at increased risk. The cffDNA test should not be offered to low-risk women or women carrying multiple fetuses because it has not been sufficiently tested in these groups.

A patient with positive result should be referred for genetic counselling and offered invasive prenatal testing for confirmation of test results. cffDNA does not replace the accuracy and diagnostic precision of prenatal diagnosis with CVS or amniocentesis, which remain an option for women.[32]

International society of prenatal diagnosis

ISPD recognizes that NIPT can be helpful as a screening test for women who are at high-risk for Trisomy 21 with suitable genetic counseling. A positive test should be confirmed through invasive testing.

National society of genetic counselors

NSGC recognizes NIPT as an option for aneuploidy assessment in pregnancy: Peer-reviewed data currently supports NIPT only as a screening tool for select populations. While abnormal NIPT results have a high positive predictive value, NIPT results should not be considered diagnostic at this time, and any abnormal results should be confirmed through a conventional prenatal diagnostic procedure, such as CVS or amniocentesis.[33]

Rapid aneuploidy detection

Newer molecular cytogenetic techniques have been introduced which provide rapid results. They allow a rapid diagnosis of the common aneuploidies

1. Quantitative fluorescence polymerase chain reaction (QF-PCR): Highly polymorphic short tandem repeats on chromosomes 13, 18, 21, X and Y are identified using fluorescently labeled primers and PCR. After amplification, electrophoresis and automated analysis of fluorescence intensity of the alleles in a genetic analyzer analysis is performed.

QF-PCR has the advantage that it is less expensive and allows automation and simultaneous processing of larger number of samples than FISH. The sensitivity and specificity of QF-PCR for targeted aneuploidies is 95.65% and 99.97%.[34]

There is a residual risk of a chromosome aberration when interphase FISH or QF-PCR shows a normal result. This risk was estimated to be overall 0.9% for all indications for invasive prenatal diagnosis in a meta-analysis of 12 studies, and in 0.4% of all invasive tests the chromosome aberration was deemed to have clinical significance.[35]

2. Multiplex ligation dependent probe amplification (MLPA): It is a PCR based technique that utilizes the amplification and quantification of the probes instead of nucleic acids. MLPA is a new PCR-based technology that discriminates between copy numbers of specific sequences of DNA. MLPA uses two-part probes of unique length that, when hybridized to adjacent target sequences on genomic DNA, can be joined together by the enzyme DNA ligase. This then allows all target sites to be amplified using a single primer pair that is complementary to the two free ends common to all probes. The products are run on a capillary electrophoresis system and separated by size. This method is not being used routinely and is still under investigation for its potential role.

Though they provide rapid results, RAD techniques are unable to detect structural chromosomal aberrations apart from aneuploidies. They appear suitable for prenatal diagnosis in women undergoing invasive testing for aneuploidies alone. For women with risk factors such as structural malformations on ultrasound or family history of chromosomal translocations, a full cytogenetic karyotype analysis is required.

Preimplantation genetic screening

PGS is performed on oocytes or a blastomere obtained from a preimplantation embryo. The goal is to identify de-novo aneuploidy in embryo (s) of couples known or presumed to be chromosomally normal to allow selection of only those embryos with normal karyotypes for transfer. A high percentage of preimplantation embryos are aneuploid, indicating a potential etiology for the relatively low implantation efficiency of both natural conceptions and IVF. Theoretically, avoiding transfer of aneuploid embryos will reduce the risk of pregnancy failure and improve the probability of conceiving a viable pregnancy. The primary populations targeted for this approach are women with the highest risk of having aneuploid embryos, such as women over age 35 or who have had multiple IVF failures or recurrent pregnancy loss. However, PGS has also been promoted to support embryo selection in patients with low probability of having aneuploid embryos.

Early PGS protocols examined chromosomes 13, 18, 21, X, and Y, (which are sometimes compatible with viable pregnancies), thus, screening for aneuploid syndromes (i.e. trisomy 13, 18, 21, Turner [45, X], and Klinefelter [47, XXY]). This probe combination led to a reduction in the incidence of aneuploid syndromes but did not result in any statistically significant improvement of implantation rates. Current protocols involve the combination of up to five probes in a single experiment and also investigate up to 15 chromosomes in two sequential FISH rounds.[36] Preliminary data obtained with such protocols have demonstrated a doubling in implantation rates and a significant increase in pregnancies per retrieval for women of advanced maternal age and/or couples who have had recurrent miscarriage.[37]

While it is generally accepted that PGS succeeds in reducing miscarriage rates and the incidence of aneuploid syndromes, there are conflicting data concerning the efficacy of PGS in raising implantation and birth rates. Recently, Mastenbroek *et al*. reported the results of a large, multicentre, randomized, double-blind trial demonstrating that a planned one-cell biopsy with FISH for nine chromosomes is not an effective means of improving pregnancy outcomes for women 35-41 years of age. Given these findings, PGD for aneuploidy screening should not be performed solely because of advanced maternal age. Adequately powered randomized trials are needed to assess whether the same is true when this procedure is used for recurrent unexplained miscarriage and recurrent implantation failure; its use for these conditions should be restricted to research studies pending evidence of effectiveness.[38]

Future

Prenatal screening and diagnosis are routinely offered in antenatal care, and are considered to be important in managing pregnancy and allowing women to make informed choices about the continuation of pregnancies affected by developmental abnormalities.[39] The feasibility of prenatal diagnosis is something that continues to change rapidly with scientific advances. Validation studies of NIPT are underway in "low risk" women and results should be available within a few years. It is expected that labs will continue to explore the number of conditions that can be detected using circulating cffDNA. As the sensitivity and specificity in the general population are better established, it is likely that NIPT will become a diagnostic test for fetal chromosomal aneuploidy for routine use in all pregnancies.

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