



APEC Guidelines Prenatal Screening for Fetal Birth Defects and Aneuploidy

Approximately 2-4% of all infants are born with a major birth defect. (CDC, 2013) Birth defects are one of the leading causes of infant mortality, accounting for more than 20% of all infant deaths. (Mathews & MacDorman, 2012) Most congenital defects are idiopathic and occur in the absence of a family history. The most common structural malformations involve the cardiac (0.5-0.8% of all live births) and central nervous (0.2-0.4% of all live births) systems. (Cunningham et al., 2010) The genetics of most of these defects are multifactorial; therefore, antenatal screening for all birth defects is not possible. However, some disorders are amenable to prenatal screening and diagnosis. These include aneuploidies (Trisomy 21, Trisomy 18, etc.), Cystic Fibrosis and Sickle Cell Disease. In addition, certain ethnic groups, such as Ashkenazi Jews, have unique genetic considerations which are amenable to screening.

Counseling

All women who present for prenatal care before 20 weeks gestation should be offered screening for fetal birth defects and aneuploidy. Patients should be counseled on the difference between screening and diagnostic testing. The choice of tests depends on several factors including gestational age at the initial prenatal care visit, patient history, number of fetuses, and availability of nuchal translucency measurement. Regardless of which tests are offered, information provided about each test should include the purpose of the test, the detection and false-positive rates, and the limitations of testing. In addition, information on each test's risks and benefits should be given to the patient so she can make an informed decision.

Neural Tube Defects

Neural tube defects (NTD) occur in varying degrees of severity-anencephaly, encephalocele, and spina bifida-and affect approximately 3/1,000 pregnancies in Alabama. Most result from multifactorial inheritance in which there is both a genetic predisposition and a sum of environmental influences. Pregnancies complicated by an open fetal NTD are typically characterized by high maternal serum (MS) Alpha-Fetoprotein (AFP) levels. AFP is produced by the fetal liver and leaks through the fetal skin defect into the amniotic fluid and into the maternal circulation. A MSAFP cutoff of 2.5 MoM will detect more than 98% of fetuses with anencephaly and approximately 80-85% of those with open neural tube defects. (ACOG, 2003 reaffirmed 2013) Encephaloceles are often skin covered and are less reliably

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detected by MSAFP. Levels of MSAFP are approximately 40% lower in the serum of diabetic women therefore results from these women are typically analyzed differently. In addition, because women with insulin-dependent diabetes are at increased risk to have a fetus with a NTD, some laboratories use a lower threshold of 2.0 MoM. (ACOG, 2003 reaffirmed 2013) Women with a MSAFP above the laboratory threshold (≥ 2.5 MoM generally) should be referred for genetics counseling and a comprehensive ultrasound. A higher MSAFP threshold of 4.0 MoM is used for twin pregnancies.

Recommendations(ACOG, 2003 reaffirmed 2013)

- Preconception folic acid supplementation of 400 μg per day for low-risk women and 4 mg/day for high-risk (previous pregnancy with NTD, patient or partner with NTD) women reduces the risk of NTD by approximately 60-70%.
- All pregnant women should be offered second-trimester MSAFP screening for NTD or should have a careful anatomic assessment of the CNS anatomy.
- Women with elevated AFP should be referred for genetic counseling and offered a diagnostic test such as a targeted sonographic evaluation and potentially amniocentesis.
- The fetus with a NTD should be delivered at a tertiary care facility capable of managing all of the neonatal issues.

Fetal Aneuploidy and Conventional Screening Strategies

The American College of Obstetricians and Gynecologists has recommended that all pregnant women, regardless of maternal age, be offered prenatal assessment for aneuploidy. (ACOG, 2007 reaffirmed 2013) Aneuploidy refers to an abnormal number of chromosomes present in the fetus. The risk of aneuploidy increases with maternal age. Aging oocytes are at risk for missegregation of chromosomes into two copies. Once the missegregated oocyte is fertilized by a normal sperm, the resulting embryo has three chromosome copies or a trisomy. The two most common autosomal trisomies diagnosed in pregnancy are trisomy 21 (Down syndrome) and trisomy 18 (Edwards syndrome). The most common sex chromosome aneuploidies are Turner Syndrome (45 X) and Klinefelter Syndrome (47 XXY). Maternal age is not a factor in the development of Turner syndrome.

Trisomy 21

Trisomy 21 occurs in approximately 1 in 800 live births overall. A number of different screening strategies can be used to determine a women's risk for trisomy 21. Traditionally, a maternal age of ≥ 35 has been considered as a threshold at which to offer invasive prenatal diagnosis but only 20% of all infants with trisomy 21 are born to women 35 or older. Thus, 80% of fetuses with trisomy 21 would not

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be identified by utilizing an age based screening method. Women less than age 35 can be screened with various strategies in the first and second trimester.

First trimester (10-13.6 weeks GA) screening for trisomy 21 combines nuchal translucency (NT) measurement with two maternal serum analytes: free or total β - human chorionic gonadotropin (hCG) and pregnancy-associated plasma protein A (PAPP-A). HCG is higher and PAPP-A is lower in the maternal serum with a pregnancy complicated by fetal trisomy 21. Procedures for NT measurement require specific training, standardization, use of appropriate ultrasound equipment, and ongoing quality assessment and should be limited to settings and individuals with active certification status. (ACOG, 2007 reaffirmed 2013) The combination of ultrasound plus the two serum markers has a detection rate of 85-90% at a screen positive rate of 5%. (Platt, 2010)

Second trimester (14-22 weeks GA) screening utilizes a combination of four markers (quad screen) combined with maternal age to detect pregnancies at increased risk for trisomy 21. The markers include hCG, estriol, alpha-fetoprotein (AFP), and inhibin A. In pregnancies complicated by fetal trisomy 21, hCG and inhibin serum levels are higher than normal while estriol and AFP are lower. The quad screen has a sensitivity rate of 80-85% for Down syndrome fetuses at a 5% screen positive rate. (Platt, 2010)

Integrated or Sequential screening tests that combine both first and second screening modalities can provide detection rates as high as 95% at a 5% screen positive rate. Even without the NT, combined first and second trimester serum screening has a detection rate of close to 90% at a screen positive rate of 5%. ACOG has stated that the ideal screening test for patients who present in the first trimester is a combination of first and second trimester tests, either with or without the NT. (ACOG, Practice Bulletin #77, 2007; reaffirmed 2013)

Trisomy 18

Trisomy 18 is a lethal aneuploidy occurring in approximately 1 in 6000 fetuses, and of fetuses >90% with trisomy 18 spontaneously abort or die in utero. The median age at death for the 5-10% fetuses surviving to term is 10 days after delivery. As with trisomy 21, the risk of pregnancy complicated with trisomy 18 is greater with increased maternal age.

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First trimester screening for trisomy 18 combines NT with PAPP-A and hCG, as with trisomy 21. In trisomy 18 the NT is enlarged while the PAPP-P and hCG maternal serum level are low. The combination is combined with maternal age risk and detects approximately 90% of trisomy 18 fetuses at a false positive rate of < 1%.(Platt, 2010)

Second trimester (14-22 weeks GA) screening for trisomy 18 utilizes three maternal serum analytes: hCG, estriol, and AFP with a detection rate of 60-70% at a screen positive rate of <1%. (Platt, 2010) In trisomy 18 all three analytes, hCG, estriol, and AFP, are typically decreased. Inhibin does not add meaningfully to screening algorithms for trisomy 18.

| Screening test | Test type | Screen for | Detection Rate |
|---------------------------------------|--|--|----------------|
| 1st trimester (10-13.6 weeks)combined | PAPP-A, hCG, and nuchal translucency | Trisomy 21 Trisomy 18 | 85-90% |
| 2nd trimester (14-22 weeks) | MSAFP | Neural tube defect | 80-85% |
| 2 nd trimester quad screen | AFP, hCG, estriol, and inhibin-A | Trisomy 21 Trisomy 18 Neural tube defect | 80-85% |
| Integrated screen | 1 st trimester PAPP-A and hCG plus NT ultrasound and 2 nd trimester quad | Trisomy 21 Trisomy 18 Neural tube defect | 95% |
| Serum Integrated screen (blood only) | 1 st trimester PAPP-A and hCG and 2 nd trimester quad | Trisomy 21 Trisomy 18 Neural tube defect | 85-88% |

Recommendations (ACOG, 2007 reaffirmed 2013)

- Screening and invasive diagnostic testing for aneuploidy should be available for all women who present for prenatal care before 20 weeks of gestation.
- Information about the detection and false-positive rates, advantages, disadvantages, and limitations of aneuploidy testing should be available to and discussed with patients so they can make an informed decision.
- Screen positive results should prompt referral for comprehensive ultrasound and amniocentesis.
- First-trimester screening using both NT measurement and biochemical markers is an effective screening test for Down syndrome in the general population.
- Procedures for NT measurement require specific training, standardization, use of appropriate ultrasound equipment, and ongoing quality assessment and performance should be limited to settings and individuals with active certification status.

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This document should not be construed as dictating an exclusive course of treatment or procedure to be followed.

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- Serum integrated (first and second trimester) screening is a useful option in pregnancies where NT measurement is not available.
- Women found to be at higher risk of aneuploidy should be referred for genetic counseling and testing.
- NTD screening should be offered in the second trimester to women who elect only first trimester screening for aneuploidy. This can be either in the form of MSAFP or careful sonographic evaluation of CNS anatomy.

Cell Free DNA

All of the early studies investigating cell free DNA focused on a population at increased risk for aneuploidy. Thus, given the limited data on the general obstetric population, ACOG did not recommend its use in the general population. Since that time, additional data on the performance of cell free DNA in the general population have been published. While the sensitivity and specificity are similar, the positive predictive value of an abnormal result is lower in this population. It is therefore even more imperative that the non-diagnostic nature of this testing be emphasized to these patients should they undergo this testing. In addition, several recent studies have demonstrated that cell free DNA testing may not detect all of the chromosome abnormalities conventional screening and prenatal diagnosis would.

Analysis of maternal plasma as a means of assessing the risk of common chromosome aneuploidy became available clinically in late 2011. This technique is able to estimate the risk of aneuploidy based on the measurement of placental DNA fragments in the maternal circulation. Circulating cell free fetal DNA, which comprises approximately 5-20% of the cell-free maternal DNA, is thought to be derived from the placenta and is cleared from the maternal blood within hours after childbirth. Different technologies are utilized to assess the fetal DNA composition, but all appear to perform similarly, especially for chromosomes 21, 18 and the sex chromosomes. There is more variability in assessment of chromosome 13. The technology can detect trisomy 13, trisomy 18, and trisomy 21 as early as the 10th weeks of pregnancy. Detection rates for fetal trisomy 18 and trisomy 21 are > 98% with a less than 0.5% false-positive rate; detection rates for trisomy 13 are >90%.

(ACOG, 2012)

Indications for Considering the Use of Cell Free Fetal DNA

- Maternal age \geq 35 at delivery.
- Fetal ultrasound findings indicating an increased risk of aneuploidy.
- History of prior pregnancy with a trisomy.
- Positive test result for aneuploidy, including first trimester, sequential, or integrated screen or a quadruple screen.
- Parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21.

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This technology has evolved rapidly and there has been active marketing to physicians and to patients. While it is a very useful technique with high sensitivity and specificity, there are numerous limitations of the testing which must be recognized as these tests are being offered. Most important is the fact that these are NOT DIAGNOSTIC tests--false positive, as well as false negative results do occur. All patients with positive test results should be referred for genetic counseling and diagnostic testing.

Recommendations(ACOG, 2012)

- At the present time, given the cost and testing limitations, offering cell free DNA testing should be limited to women at increased risk for fetal aneuploidy. Given the lack of cost effectiveness data, the use of cell free DNA testing is not recommended in the general obstetric population.
- Use of cell free DNA for detection of chromosomal microdeletions is not recommended as its performance has not been prospectively evaluated.
- Data on the performance of cell free DNA in multiple gestations is limited and its use is not recommended.
- Cell free DNA testing should not be part of routine prenatal laboratory assessment but should be an informed patient choice after pretest counseling.
- Pretest counseling should include a review that cell-free DNA testing is not a diagnostic test, although it has high sensitivity and specificity. The test will only screen for common trisomes and, at the present time, gives no other genetic information about the pregnancy. Patients should be counseled that the false positive and false negative results occur.
- If a fetal structural anomaly is identified on the ultrasound examination, prenatal diagnosis should be offered.
- A patient with a positive test result should be referred for genetic counseling and prenatal diagnosis for confirmation of test results recommended. Patients should be counseled that a positive result on cell free DNA does not mean that the fetus is affected.
- For patients who desire a diagnostic test, amniocentesis or CVS should be offered rather than cell free DNA.

Cystic Fibrosis

Cystic fibrosis (CF) is a serious life-long illness typically associated with abnormal respiratory, pancreatic, and gastrointestinal function. CF is caused by mutations in the CF transmembrane regulator (CFTR), located on chromosome 7. Prenatal and preconception carrier screening for CF was first introduced into routine obstetric practice in 2001.(ACOG, 2011) CF is more common among the non-Hispanic white population with an incidence rate of 1 in 2,500. Since it is becoming increasingly difficult to assign a single ethnicity to affected individuals, it is reasonable to offer CF carrier screening to all

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patients. (ACOG, 2011)The sensitivity of the screening test varies among different ethnic groups therefore screening is most efficacious in non-Hispanic white and Ashkenazi Jewish populations.

| Racial or ethnic group | Detection Rate (%) | Carrier Rate Before Testing | Approximate Carrier Risk after Negative Test Result |
|------------------------|--------------------|-----------------------------|---|
| Ashkenazi Jewish | 94 | 1/24 | 1/380 |
| Non-Hispanic white | 88 | 1/25 | 1/200 |
| Hispanic white | 72 | 1/58 | 1/200 |
| African American | 64 | 1/61 | 1/170 |
| Asian American | 49 | 1/94 | 1/180 |

Recommendations(ACOG, 2011)

- While CF carrier screening should be offered to all couples in which at least one partner is Caucasian, it is reasonable to offer screening to all women of reproductive age, regardless of race or ethnicity. Although a long-standing recommendation from ACOG, many insurance providers do not cover asymptomatic carrier screening. Patients should be counseled regarding the availability of this testing, but should be advised about the potential limited coverage. Currently, Medicaid does not reimburse for this testing.
- If the patient has been previously screened for CF, the CF results should be documented but the test should not be repeated.
- Complete analysis of the CFTR gene by DNA sequencing is not appropriate for routine carrier screening.
- Newborn screening panels that include CF screening do not replace maternal carrier screening.
- For couples in which both partners are carriers, genetic counseling is recommended.
- For couples in which both partners are unaffected but one or both has a family history of CF, genetic counseling is recommended.
- If a women’s partner has CF or apparently isolated congenital bilateral absence of the vas deferens, the couple should be referred for genetic counseling.

Sickle Cell Disease

Sickle cell anemia affects approximately 70,000 people in the United States, mainly African Americans and some Hispanic Americans. (Morrison & Parrish, 2010)Individuals of African, Southeast Asian and Mediterranean decent and those with a family history of a hemoglobinopathy should be

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screened for sickle cell disease with a hemoglobin electrophoresis. A sickle prep or a Sickle dex is not an adequate screening test because it does not detect other important hemoglobin variants. Patients with Hgb S-S, Hgb S-C, and Hgb S-β Thal have sickle cell disease, are considered high risk, and should be referred for genetic counseling and to a Maternal Fetal Medicine specialist for care. Approximately 2 million Americans are carriers of sickle cell trait. While Sickle Cell trait is considered a benign condition during pregnancy, it is associated with twice the rate of urinary tract infections. Patients with sickle cell trait should be screened for UTIs each trimester or more often as needed.

Carrier Screening in Jewish Individuals

Patients of Ashkenazi Jewish descent or with a positive family history should be offered carrier screening for Tay-Sachs, Canavan disease, Cystic fibrosis, and Familial dysautonomia. (ACOG, 2005 reaffirmed 2010) Carrier screening before conception is optimal. When only one partner is of an at risk ancestry, that individual should be screened first. If that individual is found to be positive, screening should be offered to the non-at risk partner and the couple referred for genetic counseling. There are now several commercial laboratories that offer comprehensive screening panels for these and other disorders that may offer an economic alternative to patients. Prior to ordering any of these tests, providers should familiarize themselves with the limitations of the screening tests and the mutations tested for as many of these panels screen for a limited number of mutations for each particular disorder. As with CF carrier testing, many insurance companies do not cover routine screening in asymptomatic patients.

Benchmarks

Aneuploidy screening performed in >50% of women with prenatal care prior to 20 wk.

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