49 Second-trimester screening for fetal abnormalities

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INTRODUCTION

Screening tests to identify women at risk for fetal aneuploidy and fetal structural abnormalities are routinely offered to all women during pregnancy. A number of screening strategies are currently available, which utilize both biochemical analysis and ultrasonography to achieve high detection rates and determine whether further diagnostic testing is needed. Recently, first-trimester screening for fetal abnormalities has been utilized with increasing frequency as it offers women the opportunity of earlier testing and subsequent option for earlier diagnosis. Despite this change in practice, second-trimester biochemical and ultrasound screening remains a valuable resource in modern obstetric care as complementary to first-trimester screening or as the only screening in women who present for prenatal care after the first trimester of pregnancy.

SECOND-TRIMESTER SERUM MARKER SCREENING

Most second-trimester serum marker screening is now performed in conjunction with first trimester–combined screening protocols that utilize either an integrated, stepwise, or contingent approach. Currently, second-trimester maternal serum screening alone is available as a triple, quadruple, or penta-marker testing modality. Whether integrated with first-trimester serum marker testing or used in isolation for women who defer first-trimester screening or do not seek prenatal care until after 13 weeks of gestation, second-trimester serum marker screening remains a valuable tool in prenatal management.

Maternal Serum Alpha-Fetoprotein

Maternal serum alpha-fetoprotein (MSAFP) was the first maternal serum analyte utilized to detect fetal abnormalities, specifically, open neural tube and abdominal wall defects in cases of elevated MSAFP. In the mid-1980s, an association between a low MSAFP level and increased risk for Down syndrome was reported (1). MSAFP, along with other maternal serum analytes, was then incorporated into various screening protocols designed to modify maternal age–related risks for fetal aneuploidy.

Elevated MSAFP

Alpha-fetoprotein (AFP) is a normal fetal protein found in high concentrations in the fetal serum. It is present in amniotic fluid in normal pregnancies, presumably reaching the fluid through excretion in the fetal urine. From the amniotic fluid, AFP diffuses across the fetal membranes and can be detected in the maternal serum beginning at approximately 12 weeks of gestation (2). In cases of fetal anencephaly or open spina bifida, increased levels of AFP reach the amniotic fluid secondary to the concentration gradient from fetal serum to amniotic fluid. This increased AFP concentration can then be detected in the maternal serum. Ventral wall defects

(i.e., omphalocele or gastroschisis) can also result in similar elevations in MSAFP through this same mechanism. Other causes of elevated MSAFP are listed in Table 1.

MSAFP screening provides an effective means for selecting patients who are at increased risk for neural tube defects and other fetal disorders and identifies patients who warrant further evaluation with diagnostic ultrasound and/or amniocentesis. Additionally, elevated MSAFP in a patient with a normal fetal anatomic survey has been associated with adverse pregnancy outcomes (3). As a matter of fact, the most frequent pathologic reason for elevated MSAFP is placental disease. Although there is no consensus regarding the management of this patient population, women in this high-risk group for obstetric complications should be followed closely for the duration of the pregnancy for stillbirth, prematurity, intrauterine growth restriction, and neonatal death. In the presence of placenta previa and elevated MSAFP, placenta accreta should be ruled out, especially in patients with previous cesarean delivery.

Low MSAFP

The association between low MSAFP and fetal chromosomal abnormalities is well described in the literature and will be discussed in further detail later in this chapter. As with elevated MSAFP levels, a low MSAFP level is associated with a variety of fetal and maternal conditions (Table 2).

Ultrasound can be used in the initial evaluation of a patient with low MSAFP. The most common explanation for a low value is incorrect pregnancy dating and a gestational age less advanced than expected. Ultrasound will also identify missed abortion or molar pregnancy as a potential cause of low MSAFP. If ultrasound reveals a viable fetus at the anticipated gestational age, targeted genetic ultrasound for evaluation of Down syndrome should be performed. Patients should be counseled regarding the availability of amniocentesis as a diagnostic procedure. Low MSAFP in a patient with normal ultrasound and amniocentesis has not consistently been associated with adverse pregnancy outcomes and at this time warrants no further investigation.

Serum Screening for Trisomy 21

An association between low MSAFP levels and fetal chromosomal abnormalities was first described by Merkatz and colleagues in 1984 (1). The initial studies focused primarily on Down syndrome detection; however, further studies have shown an association with other chromosomal abnormalities. Combined maternal serum marker panels were subsequently developed to calculate a risk for Down syndrome in the fetus for a given pregnancy. Historically, maternal age 35 years or older at the time of delivery was used to identify women at the highest risk of having a child with Down syndrome, and only these women were offered genetic counseling and amniocentesis

Table 1 Findings Associated with Elevated Maternal Serum Alpha-Fetoprotein
Incorrect pregnancy dating
Multiple gestation
Fetal demise
Open neural tube defects
Ventral wall defects
Intestinal atresias
Skin or scalp defects
Congenital nephrosis
Cystic hygroma
Aneuploidy
Placental abnormalities or insufficiency
Partial molar pregnancy
Late pregnancy complications including stillbirth and low birth weight

Table 2 Findings Associated with Low Maternal Serum Alpha-Fetoprotein
Incorrect pregnancy dating
Missed abortion
Hydatidiform mole
Down syndrome and other aneuploidy

or chorionic villus sampling. With the introduction of biochemical serum screening for Down syndrome, the American College of Obstetricians and Gynecologists now recommends that all women should be offered invasive diagnostic testing or aneuploidy screening before 20 weeks of gestation, regardless of maternal age (4).

Triple-marker screening utilizing AFP, hCG, and unconjugated estriol was the first combined marker panel used to screen patients for trisomy 21. In the general population, triple-marker screening identifies approximately 60% to 70% of pregnancies affected by trisomy 21 with a 5% screen-positive rate (5). The quadruple screen was later developed when inhibin A was added to the triple-screen panel (6,7). Penta-screen testing is now available with the addition of invasive trophoblast antigen testing (8–10). These expanded panels increase trisomy 21 detection rate at the same 5% screen-positive rate (11) (Table 3).

Maternal serum screening for trisomy 21 can be performed anytime between 15 and 22 weeks, but is most accurate when performed between 16 and 18 weeks. Serum analyte levels can vary with gestational age, maternal weight, diabetic status, race, and number of fetuses; therefore, these variables are incorporated into an individual’s risk score calculation. Because the maternal age-related risk of trisomy 21 is the basis of any serum screening protocol, both the detection rate and the screen-positive rate increase with maternal age. Different laboratories have established their own reference values and screen-positive cutoffs that reflect an individual woman’s reported risk. Based on this modified risk, a woman may opt to pursue invasive diagnostic procedure such as amniocentesis or further modify her risk with genetic ultrasound, which will be detailed later in this chapter.

Serum Screening for Trisomy 18 and Other Fetal Aneuploidy

In counseling patients, it is important to emphasize that multiple maternal serum screening protocols were developed

Table 3 Sensitivity and Specificity of Prenatal Screening Tests for Down Syndrome		
Markers	Detection rate (%)	False-positive rate (%)
Age, AFP, hCG, uE3	72	5
Age, AFP, hCG,uE3, DIA	79	5
Age, AFP, hCG, uE3, DIA, ITA	83	5
Abbreviations: AFP, alpha-fetoprotein; hCG, human chorionic gonadotropin; uE3, unconjugated estriol; DIA, dimeric inhibin A; ITA, invasive trophoblast antigen.		

Table 4 Second-Trimester Serum Marker Levels in Various Fetal Chromosomal Abnormalities				
Genetic disorder	AFP	uE3	hCG	DIA
Down syndrome	↓	↓	↑	↑
Trisomy 18	↓	↓↓	↓↓	↔
Trisomy 13	↔	↔	↔	↔
Triploidy	↔	↓	↓ or ↑	?
Abbreviations: AFP, alpha-fetoprotein; hCG, human chorionic gonadotropin; uE3, unconjugated estriol; DIA, dimeric inhibin A.				

primarily to detect trisomy 21, and the patterns of serum markers observed in pregnancies affected by trisomy 18 and other fetal chromosomal abnormalities are different. In trisomy 18, MSAFP and unconjugated estriol are low; however, in contrast to Down syndrome, hCG value is low as well. Although these results are well correlated with trisomy 18, a positive second-trimester serum screen has poor sensitivity and poor prediction for trisomy 18 (12). By contrast, genetic ultrasound to evaluate for characteristic sonographic findings associated with this diagnosis is highly sensitive in identifying fetuses with the disorder (13,14). Ultrasound evaluation of fetuses with trisomy 18 will be discussed later in this chapter. Trisomy 13 is usually associated with normal levels of all three maternal serum markers, except in cases where open fetal defects are present. In fetal triploidy, all three biochemical markers are low. Table 4 summarizes the changes in serum marker levels in various fetal chromosomal abnormalities.

SECOND-TRIMESTER FETAL ANATOMY SURVEY

The American Institute of Ultrasound in Medicine first published guidelines for the performance of obstetric ultrasound in 1994 (15). These guidelines were updated in 2007 to reflect the minimum criteria for a completion of a fetal ultrasound examination (16). A standard obstetric sonogram in the second or third trimester includes an evaluation of fetal presentation, amniotic fluid volume, cardiac activity, placental position, fetal biometry, and fetal number, plus an anatomic survey. Fetal anatomy may be adequately assessed by ultrasound after approximately 18 weeks of gestational age. Minimum requirements in the standard examination of fetal anatomy include evaluating the cerebellum, choroid plexus, cisterna magna, lateral cerebral ventricles, midline falx, cavum septum pellucidum, upper lip, nuchal fold, four-chamber view of the fetal heart, outflow tracts (if technically feasible), stomach (presence, size, and situs), kidneys, bladder, umbilical

Table 5 American Institute of Ultrasound in Medicine
Minimum Elements of a Standard Examination of Fetal
Anatomy

Head, face, and neck
Cerebellum
Choroid plexus
Cisterna magna
Lateral cerebral ventricles
Midline falx
Cavum septum pellucidum
Upper lip
Chest
Four-chamber view of fetal heart
Abdomen
Stomach
Kidneys
Bladder
Umbilical cord insertion site into the fetal abdomen
Umbilical cord vessel number
Spine
Cervical, thoracic, lumbar, and sacral spine
Extremities
Legs and arms (presence or absence)
Sex
Medically indicated in low-risk pregnancies only for evaluation of multiple gestations

cord insertion site into the fetal abdomen, umbilical cord vessel number, cervical, thoracic, lumbar, and sacral spine, extremities, and genitalia (as medically indicated in low-risk pregnancies and in the evaluation of multiple gestations) (Table 5). The placental location, appearance, and relationship to the internal cervical os should be recorded and the umbilical cord should be visualized. The maternal cervix and adnexa should be examined.

A more detailed fetal anatomic examination may be necessary if an abnormality or suspected abnormality is found on the standard examination. Multiple gestations require the documentation of additional information including chorionicity, amnionicity, comparison of fetal sizes, estimation of amniotic fluid volume on each side of the membrane, and fetal genitalia.

Fetal anatomy may be adequately assessed after 18 weeks of gestation and is ideally performed before 20 weeks to optimize assessment of anatomy while at the same time allowing time for invasive testing if necessary or desired. Factors that may limit sonographic examination include equipment quality, sonographer experience, length of time spent on examination, fetal size, position, movement, fluid abnormalities, maternal abdominal scar, and increased maternal abdominal wall thickness.

Performing a complete fetal anatomy survey should be approached in a systematic way. The examination should ideally begin with imaging of the fetal head and proceed caudally. The entire uterus should be scanned in both a transverse and longitudinal direction to determine fetal situs, fetal position, amniotic fluid volume, and placental location. The fetal anatomy is then examined in a stepwise fashion. To begin, the fetal head, face, and neck are evaluated. The calvarium should be well mineralized and elliptical. The head shape may be flattened (dolichocephaly/increased anteroposterior distance) or rounded (brachycephaly/shortened

Figure 1 Axial view through fetal head at level of thalami and cavum septum pellucidum. The calipers show measurement of the biparietal diameter.

anteroposterior distance) as a normal variant or may suggest fetal aneuploidy (trisomy 21 or 18 associated with brachycephaly). A lemon-shaped head may be suggestive of a neural tube defect. The cranial sutures appear as hypoechoic areas between cranial bones and visualization excludes premature closure, craniosynostosis syndromes, and some skeletal dysplasias. The biparietal diameter is measured at the level of the thalami and cavum septum pellucidum (Fig. 1). The cerebellar hemispheres should not be visible in this transthalamic axial plane. The measurement is taken from the outer edge of the proximal skull to the inner edge of the distal skull. Head circumference is measured at the same level as the biparietal diameter, around the outer perimeter of the calvarium. The cavum septum pellucidum, midline falx, third ventricle, and frontal horns of the lateral ventricles can also be visualized in this view. The presence of a normal cavum suggests proper midline brain formation and makes diagnosis of holoprosencephaly or agenesis of the corpus collusum less likely. Next, a transventricular view should be obtained just superior to the transthalamic view (Figs. 2 and 3). This allows visualization of the lateral ventricles and bilateral choroid plexuses. The ventricle is measured through the atrium in an axial plane and is normally less than 10mm, and the choroid plexuses should fill the entire width of the lateral ventricles. The posterior fossa is then evaluated. This begins with obtaining a transcerebellar view to properly visualize the cerebellum, cerebellar vermis, cisterna magna, and nuchal fold (Fig. 4). Transcerebellar diameter provides a useful and accurate estimation of gestational age and is relatively spared in growth restriction (17,18). Thorough evaluation of the structures in the posterior fossa is crucial to exclude spina bifida, Dandy–Walker malformation or variant, occipital encephalocele, and cerebellar agenesis or hypoplasia. The nuchal fold can be measured in this transcerebellar view, with 5 to 6mm being the upper limit of normal. It is important to note that if fetal intracranial structures cannot be adequately visualized secondary to fetal positioning, transvaginal ultrasound may be utilized to complete this portion of the fetal anatomy survey.

After the fetal head is evaluated, the fetal facial structures are examined. A range of genetic syndromes and disorders are associated with characteristic facial features, and thorough

Figure 2 Transventricular view of the fetal head showing the far lateral ventricle. Note the choroid plexus filling the width of the ventricle (white arrow).

Figure 3 Transventricular view of the fetal head with calipers showing measurement of the atria of the lateral ventricle.

evaluation of the fetal face can aid in supporting or excluding their diagnosis. Obtaining a combination of coronal, sagittal, and axial views is necessary to complete the facial evaluation. To begin, a fetal profile should be obtained in the sagittal plane (Fig. 5). This allows for proper visualization of the frontal bone, nasal bone, and fetal chin. Absent or shortened nasal bone may indicate fetal aneuploidy and will be discussed later in this chapter. Micrognathia has also been associated with a variety of genetic syndromes and disorders, making characterization of the fetal chin important. The fetal nose and lips should be imaged in coronal plane and the anterior palate should be visualized to exclude cleft lip and palate.

Next, the fetal spine should be imaged and fetal skeletal survey should be performed. It is important to note that any intracranial abnormality noted in the fetal anatomy survey may be the first indication of neural tube abnormality, and the presence of any such finding warrants comprehensive review. Again, a combination of coronal, sagittal, and axial views is necessary for evaluation. The skin overlying the fetal spine should be visualized and intact to exclude open spina bifida and any mass or tumor (Fig. 6). The ossification centers

Figure 4 Transcerebellar view of the fetal head with calipers showing the cerebellar diameter (cereb), cisterna magna (CM), and nuchal fold (NF) measurements.

Figure 5 Sagittal profile of the second-trimester fetus. Note the presence of the nasal bone (white arrow).

of each vertebral segment should align in a symmetrical fashion to exclude hemivertebrae and neural tube defect. Notation of the spinal curvature should be made. Overall evaluation of fetal bony structures may be completed at this time, with the evaluation of fetal ribs, clavicles, and extremities. Normal echogenicity and appearance aid in the exclusion of skeletal dysplasia. Documentation of fetal extremities as present or absent is the minimum requirement included in the guidelines for routine obstetric ultrasound examination; however, more complete evaluation may provide important diagnostic information. Movement and tone should be normal. Any absence, fracture, contracture, bowing, or hypomineralization of the bones should be considered abnormal. The length of each long bone (femur, humerus, radius, ulna, tibia, and fibula) should be measured and standard nomograms are available for comparison. The hands and feet should be examined. The hands should be noted to open freely and all five fingers should extend fully, as persistently clenched hands with overlapping digits is sonographic finding often associated with trisomy 18. The middle phalanx of the fifth digit may be imaged as its absence has been

Figure 6 Normal sagittal view of the fetal spine. The skin overlying the vertebral processes is well visualized and intact.

Figure 8 Fetal foot showing plantar surface at right angle with long bones of the lower extremity.

Figure 7 Fetal hand showing echogenic phalanges of four fingers, including the fifth digit (white arrow).

Figure 9 Apical four-chamber view of the fetal heart. Abbreviations: RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle.

associated with trisomy 21 (Fig. 7). Fetal toes should be visualized and the foot length can be measured. Sagittal views of the feet should be obtained so that the plantar surface is at a right angle to the lower extremity (Fig. 8). This allows exclusion of “rocker-bottom” appearance, which also has been associated with various genetic disorders.

The fetal thorax and visceral contents are imaged next. The diaphragm is examined for evidence of diaphragmatic hernia. Fetal lung echogenicity should be homogeneous to exclude mass or tumor such as cystic adenomatoid malformation. Any pleural effusion or evidence of pulmonary hypoplasia is considered abnormal. Fetal cardiac examination should then follow. American Institute of Ultrasound in Medicine practice guidelines for basic cardiac examination include a four-chamber view of the fetal heart and, if technically feasible, views of both outflow tracts. However, a more comprehensive cardiac evaluation may be needed to exclude some fetal cardiac abnormalities. To begin, cardiac rate and rhythm should be documented. Cardiac size should be measured and is expected to be approximately one-third of the fetal thorax. The cardiac apex should point to the left at an angle of 45 ± 20 degrees relative to the midline. Normal situs should be established

with fetal liver on the right and stomach on the left. A four-chamber apical view should be obtained with all chambers being approximately equal in size (Fig. 9). There should be no pericardial fluid or collection. An intact intraventricular and intra-atrial septum excludes the presence of ventricular septal defect and atrial septal defect respectively. The outflow tracts, aortic arch, ductal arch, and inferior and superior vena cava should be identified. An echogenic intracardiac focus (EIF), most commonly seen in the left ventricle, can occur in up to 5% of normal pregnancies (19). It is typically caused by a specular reflection secondary to mineralization within the papillary muscles (20,21). Because it is seen in approximately 13% to 18% of pregnancies complicated by Down syndrome, presence of an EIF has traditionally been utilized by some investigators as a sonographic marker to modify a woman's a priori risk of fetal aneuploidy (22–24). However, more recent literature suggests that when an EIF is seen as an isolated finding in an otherwise normal fetal anatomy survey, it is more likely to be a normal variant (25,26). This will be further discussed later in the chapter.

Abdominal circumference should be determined at the skin line on a transverse view at the level of the junction of the

Figure 10 Appropriate plane for the measurement of abdominal circumference. Abbreviations: UV, umbilical vein; SP, spine; ST, stomach.

umbilical vein, portal sinus, and fetal stomach (Fig. 10). The umbilical vein should curve away from the fetal stomach; otherwise, persistence of the right umbilical vein is suspected. The abdominal circumference is the most sensitive fetal growth parameter and is used in conjunction with other fetal biometric measurements to determine an overall estimated fetal weight. Abdominal viscera identifiable on ultrasound include the liver, spleen, gallbladder, stomach, and bowel. The liver occupies most of the upper abdomen and should be located on the right. The gallbladder appears as a tear-shaped organ in the right upper quadrant at the inferior edge of the liver. The fetal stomach should be located on the fetal left below the level of the diaphragm. An absent or small stomach that remains unchanged throughout the course of sonographic evaluation may suggest esophageal atresia, tracheoesophageal fistula, or a diaphragmatic hernia. The appearance of the fetal bowel varies with gestational age. If fetal anatomy survey is performed in the second trimester, the bowel should appear uniformly echogenic and fill the majority of the fetal abdomen. Hyperechoic bowel is associated with aneuploidy, infection, ischemia, intrauterine bleeding, and other bowel abnormalities. In the third trimester, more prominent loops of hypoechoic large bowel may be seen. More dilated loops of bowel may reflect atresia or obstruction. The umbilical cord should be identified at its insertion site into the fetal abdomen. As the umbilical vein enters the abdomen, it should course superiorly where it enters the liver to communicate with the portal vein. The umbilical vein then continues into the ductus venosus and then inferior vena cava and right atrium. The ventral abdominal wall adjacent to the umbilical cord insertion should be visualized and intact to exclude ventral wall defects including omphalocele and gastroschisis.

The genitourinary tract should next be examined for evidence of fetal anomaly. The kidneys should be identified bilaterally and appear as hypoechoic paraspinal structures. Identification of the anechoic urine-containing renal pelvis assists in their visualization (Fig. 11). Abnormal findings include enlargement or absence of the kidneys, renal masses or cysts within the parenchyma, hyperechogenicity, and dilation of the renal pelvis, calices, or ureter. When the kidneys cannot be easily identified, color or power Doppler imaging of the

Figure 11 Transverse view through fetal abdomen at the level of fetal kidneys that appear as hypoechoic paraspinal structures. The white arrows indicate the central anechoic renal pelvis.

renal arteries can be utilized to confirm congenital absence of one or both kidneys or presence of a pelvic kidney. The bladder is an anechoic urine-filled structure located in the anterior midline of the fetal pelvis. The bladder may empty and fill during the course of the fetal anatomy survey and care should be taken to exclude bladder exstrophy or evidence of lower obstructive uropathy. The two umbilical arteries should be identified as they course around the fetal bladder to confirm the presence of a three-vessel cord. Attempt at fetal gender assignment should be made in order to exclude ambiguous genitalia. Female gender is confirmed by the identification of the labia, which may appear as three parallel linear echoes. Male gender is assigned if both the penis and scrotum are seen.

SECOND-TRIMESTER GENETIC ULTRASOUND

The genetic ultrasound is a targeted examination for fetal aneuploidy, specifically trisomy 21. The goal of genetic ultrasound is to further modify the a priori age or serum screening risk for trisomy 21 and thus improve the selection of pregnant women who may be candidates for invasive prenatal diagnosis. First characterized in the 1990s, the combination of second-trimester serum screening with identification of ultrasound markers on genetic ultrasound improved the detection rate of fetuses affected with Down syndrome (27). At the same time, it identified patients at decreased risk of carrying a pregnancy complicated by Down syndrome if the genetic sonogram was normal (28,29). With the increased use of first-trimester screening, the prevalence of fetal Down syndrome in the second trimester has decreased (30). For this reason, interpretation of second-trimester sonographic markers of Down syndrome must be interpreted in the context of first, second, and/or integrated screening results in order to achieve the most accurate risk assessment of a potentially affected fetus.

Approximately 25% of fetuses with Down syndrome have major characteristic structural abnormalities that can be identified on second-trimester ultrasound (31). For the majority of Down syndrome fetuses, no major fetal malformation will be present on sonographic evaluation. Over the past 20 years, numerous sonographic markers have been associated

Table 6 Sonographic Aneuploidy Markers of the Genetic Sonogram

Nuchal fold thickening (>5–6 mm)
Absent or hypoplastic nasal bone
Short humerus
Short femur
Pyelectasis
Echogenic bowel
Echogenic intracardiac focus
Choroid plexus cyst(s)
Short ear length
Wide iliac angle
Sandal gap toes
Hypoplastic middle phalanx of the fifth digit
Clinodactyly

with fetal Down syndrome, which, when present, convey an increased risk for fetal aneuploidy. As with any screening modality, a useful sonographic marker Down syndrome screening should be present in a high proportion of Down syndrome pregnancies, not commonly seen in normal fetuses and amenable to imaging during a standard sonographic examination. The marker should be easily seen at a gestational age that does not preclude subsequent diagnostic testing by amniocentesis and option for pregnancy termination.

At the time of genetic ultrasound, thorough investigation for abnormal fetal biometry, fetal structural anomalies, and markers for fetal aneuploidy is performed. In this way, second-trimester ultrasound can be combined with serum biochemistry to determine which women would most benefit from amniocentesis. Additionally, a normal sonogram may be an indication to defer amniocentesis, even when a woman is at increased risk for aneuploidy based on serum screening (32). Conversely, abnormal findings on a genetic sonogram may prompt invasive testing even when a woman has normal serum biochemistry. Markers of aneuploidy include increased nuchal fold thickness, absent or hypoplastic nasal bone, shortened long bones, pyelectasis, hyperechoic bowel, EIF, choroid plexus cysts, short ear length, wide iliac angle, sandal gap toes, hypoplastic midphalanx of the fifth digit, and clinodactyly (Table 6).

When considered in isolation, each marker, except thickened nuchal fold or absent nasal bone, has a low to moderate sensitivity for trisomy 21 and may not necessarily increase the risk of aneuploidy, especially in low-risk patients (maternal age <35 with normal serum analyte screening) (31). The risk of fetal aneuploidy increases when two or more sonographic markers are seen in low-risk patients or when isolated markers are seen in high-risk patients.

An increased nuchal fold (>5–6mm) is a highly specific marker for Down syndrome. Approximately 40% to 50% of second-trimester fetuses with Down syndrome have a thickened nuchal fold with a false-positive rate of 0.1% (33–35). To obtain this measurement, the fetal head is imaged in a transverse plane similar to that for measuring the biparietal diameter (Fig. 4). The thalami and the upper portion of the cerebellum should be in the plane of the image. The measurement is then made from the outside of the occipital bone to the distal surface of the skin edge.

The fetal nasal bone has been demonstrated to be hypoplastic or absent in up to 60% of Down syndrome

Figure 12 Sagittal profile of second-trimester fetus with absent nasal bone as indicated by circle.

pregnancies imaged in the second trimester and only about 1% to 2% of unaffected pregnancies (Fig. 12) (36,37). Initially observed at the time of first-trimester evaluation of nuchal translucency, hypoplastic or absent nasal bone ossification is being used with increased frequency as a second-trimester screen for Down syndrome (37–42). Numerous studies have been performed to determine the optimal definition of nasal bone hypoplasia as an ultrasonographic marker for aneuploidy in the second trimester. In one series, expressing multiples of the median (MoM) for the nasal bone length at each gestational age was the most efficient definition of nasal bone hypoplasia (43). Nasal bone smaller than 0.75 MoM had a sensitivity and specificity of 49% and 92% respectively. Another series showed the positive likelihood ratio for fetal trisomy 21 increased from 17.1 with a nasal bone length of 0.7 MoM or less to 74.1 with a nasal bone length of 0.5 MoM or less (44). In the hands of experienced operators, nasal bone evaluation may be the best single ultrasound marker for second-trimester risk assessment. It is important to keep in mind that there is variability in the prevalence of this finding depending upon ethnicity, and this should be incorporated in to patient counseling if this finding is present on ultrasound evaluation. In general, absent nasal bone in the second trimester is seen in approximately one-third of Down syndrome fetuses (40).

Down syndrome fetuses may have a shorter humerus and femur length than expected based on biparietal diameter measurement. An observed-to-expected ratio of ≤0.90 or a biparietal diameter-to-femur ratio of >1.5 has a reported likelihood ratio of 1.5 to 2.7 when present as an isolated finding (45,46). A short humerus is more strongly associated with Down syndrome (sensitivity of 50%, false-positive rate of 5%) when compared with shortened femur length (sensitivity 40–50%, false-positive rate of 7%) (47–49). As with nasal bone ossification, there is variability in these long bone lengths between different ethnic groups, making these markers difficult to use as isolated findings or in a low-risk population.

Fetal pyelectasis, defined as a renal pelvis anterior–posterior diameter at least 4 mm, is present with increased frequency in Down syndrome fetuses as compared with second-trimester euploid fetuses (Fig. 13) (50). As an isolated marker, the likelihood ratio ranges from 1.5 to 1.9; however, the sensitivity

Figure 13 Transverse view at the level of fetal kidneys with calipers showing bilateral renal pyelectasis.

Figure 14 Fetal abdomen with increased echogenicity of the fetal bowel as indicated by white arrow.

for this marker is low (17–25%) with a false-positive rate of 2% to 3%, making this marker difficult to use as an isolated finding or in a low-risk population (49–51).

Increased echogenicity of the fetal bowel, defined as fetal bowel at least as echogenic or brighter than the surrounding bone, is a subjective marker for Down syndrome and is dependent on the machine and the operator performing the ultrasound (Fig. 14). Sensitivity of this marker ranges anywhere from 3% to 27% with a low false-positive rate of <1% (35,49,52–54). The finding of hyperechoic bowel can also be seen with fetal cystic fibrosis, cytomegalovirus infection, swallowed bloody amniotic fluid, and severe intrauterine growth restriction. This differential diagnosis list must be kept in mind when counseling patients regarding this finding.

EIFs occur in up to 5% of normal pregnancies and in approximately 16% to 18% of Down syndrome gestations (Figs. 15 and 16) (19). The risk does not seem to vary if the isolated focus is in the right or left ventricle; however, the presence of bilateral or multiple unilateral EIFs, especially in the right ventricle, may increase the risk for Down syndrome (55–57). Because an EIF is one of the most common sonographic markers of aneuploidy to be seen among normal fetuses, much recent

Figure 15 Apical four-chamber view of fetal heart showing single echogenic focus in the left ventricle (white arrow).

Figure 16 Apical four-chamber view of fetal heart showing multiple echogenic foci [two in the left ventricle (white arrows) and one in the right ventricle (yellow arrow)].

literature has focused on its clinical utility as a screen for Down syndrome. Recent literature has shown EIF as an isolated finding, and no additional maternal risk factor probably does not increase the risk of fetal aneuploidy and does not require invasive testing (25,26,58).

Like the EIF, the presence of a choroid plexus cyst is a sonographic finding, which can also be seen in euploid fetuses (Fig. 17). Again, there has been debate in the literature regarding an association between isolated choroid plexus cyst in a low-risk woman and fetal aneuploidy, with most researchers identifying this finding as a benign variant when noted in isolation (26,59).

Other minor sonographic markers associated with Down syndrome include short ears, altered foot-to-femur ratio, widened iliac wing angle, sandal gap between the first and second toes, hypoplastic fifth middle phalanx of the hand, and clinodactyly. These markers are inconsistently used because of variable operator experience and possibly high false-positive rates.

Different methods have been proposed to interpret the presence or absence of sonographic markers identified at

Figure 17 A single sonolucent choroid plexus cyst in the far lateral ventricle (white arrow).

the time of genetic sonogram (60). These include summing up the scores of individual markers to obtain a “Sonographic Scoring Index” (49), multiplying the likelihood ratios of individual markers (61), calculating a negative likelihood ratio in the absence of any sonographic markers (32), and using multivariate analysis to identify independent sonographic predictors (62,63). A standardized methodology in applying soft markers as a screening test for Down syndrome will aid in patient counseling regarding the findings. Additionally, the development of uniform and reproducible sonographic and measurement standards will further enhance the clinical utility of the genetic sonogram. Regardless of its interpretation, the genetic sonogram plays a useful role in identifying fetuses at risk for Down syndrome or reducing the risk for Down syndrome, if normal, in high-risk women who wish to avoid amniocentesis. The reported detection rates of Down syndrome after genetic sonography when combining multiple ultrasound markers range from 50% to 90% (64–66). It is important to combine the genetic sonogram with first- and/or second-trimester maternal serum screening, as these results establish a woman’s baseline or a priori risk. Additionally, combining maternal serum screening with second-trimester fetal ultrasound evaluation is more effective than either modality alone at identifying fetuses at risk for aneuploidy (67,68).

Finally, obstetricians must keep in mind that although ultrasound is a low-risk and non-invasive screening tool for the evaluation of the fetus and intrauterine environment, it is not always a benign process. Pregnant women referred for targeted ultrasound because of the finding of a marker have high anxiety, with reported anxiety levels higher than women referred for ultrasound evaluation based on advanced maternal age alone and comparable with women with abnormal maternal serum screening (69,70). This further supports the need for standardization in the approach to performing and interpreting sonographic aneuploidy markers and the patient counseling that accompanies this evaluation.

SECOND-TRIMESTER ULTRASOUND TO DETECT TRISOMY 18 AND 13

After Down syndrome, trisomy 18 is the second most common aneuploidy among live-born fetuses, occurring in

approximately 1 in 8000 live births (71). While most of the literature detailing second-trimester ultrasound has focused on the detection for fetal structural anomalies and sonographic markers for Down syndrome, several ultrasound markers, such as choroid plexus cysts and clenched hands, have been shown to be associated with trisomy 18 (72–77). The vast majority of trisomy 18 fetuses have sonographically detectable abnormalities in the second trimester (78,79). In one series of 8763 women with singleton pregnancy and serum screening significant for increased risk trisomy 18, 15% had an abnormal genetic sonogram, including 1240 of pregnancies with normal fetuses and 50 of those with trisomy 18 (80). If the genetic sonogram was normal, a woman’s risk of having a fetus with trisomy 18 was reduced by approximately 90%. The most commonly identified abnormality was a cardiac defect, followed by skeletal, central nervous system, facial, and abdominal abnormality. Most fetuses (81%) with trisomy 18 had an ultrasound marker present. Specifically, choroid plexus cysts and clenched hands were seen in large percentage of affected fetuses (52% and 36% respectively). These ultrasound markers were almost always seen in conjunction with one or more structural anomalies or other markers.

Trisomy 13 is the third most common of the autosomal trisomies to result in a live-born neonate. Prevalence of this disorder ranges between 1 in 5000 to 1 in 20,000 and it is characterized by multiple severe anomalies resulting in neonatal death (81). Severe craniofacial and midline defects are more common and severe in this trisomy when compared with the abnormalities seen in trisomy 18 or 21 fetuses. As with trisomy 18 fetuses, second-trimester sonographic examination can show many of the anomalies characteristic of trisomy 13. In one series of 28 fetuses with trisomy 13, major structural malformations were seen in 23 cases (82.1%) and minor anomalies were detected in 16 cases (57.1%) (82). Among major anomalies, the most common (64.3%) were central nervous system (ventriculomegaly, holoprosencephaly, posterior fossa cysts) and facial (midline clefts, microphthalmos, protrusio bulbi, hypertelorism). Cardiovascular anomalies were the next most common abnormality detected (53.6%) followed by renal anomalies (42.9%) and polydactyly (7.1%). Ultrasound markers for fetal aneuploidy were also detected (57.1%), but almost always in association with a major structural defect (87.5%).

THREE-DIMENSIONAL SONOGRAPHY

In recent years, the use of three-dimensional (3D) ultrasound has emerged as an important imaging tool for the diagnosis of fetal abnormality. The 3D ultrasound may be utilized to further identify the nature, severity, and location of fetal defects first identified on standard ultrasound evaluation (83). It is a complementary tool that may be used to expand upon the 2D ultrasound examination. Specific applications include improved visualization of fetal facial features in the evaluation of suspected abnormal findings including micrognathia, cleft lip and palate, tumors extending to the face, and markers of trisomy 21 (84–88). The 3D ultrasound has proven useful in the assessment of limb malformation and prenatal diagnosis of skeletal abnormalities, including rib anomalies and abnormal cranial sutures (89–91). It also has been utilized to localize the severity and extent of fetal neural tube defects (92). Other

applications include spatial presentation of blood flow and vasculature and, more recently, the ability to reconstruct a 3D rendered image of the fetal heart (93,94).

FETAL MAGNETIC RESONANCE IMAGING

Fetal magnetic resonance imaging (MRI) has emerged as a useful complementary imaging modality to further elucidate abnormalities noted at the time of fetal ultrasound evaluation. MRI provides superior soft tissue resolution in comparison with ultrasound regardless of fetal position, gestational age, maternal body habitus, oligohydramnios, or skill of operator (95). Brain imaging is the most common application for MRI of the fetus and use of MRI for diagnosing central nervous system abnormalities is widely reported. It has been utilized in cases of unexplained hydrocephalus, suspected agenesis of the corpus collusum, abnormalities of the posterior fossa, and neural tube defects (96,97). MRI has also been utilized in fetal imaging to study lung and chest anatomy, cervical masses, and urogenital abnormalities (98–101). MRI also has been shown to play a role in assessing abnormal placentation, including cases of suspected placenta accreta, increta, and percreta (102–105).

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