46 Assessment of fetal genetic disorders

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INTRODUCTION

Since the first clinical use of fetal sex determination by amniocentesis in 1967, there has been a virtual explosion in both the availability and the application of prenatal diagnosis in the management of pregnancy. The rapid expansion of this field has been a result of numerous advances. The quality of ultrasonography has vastly improved, allowing detection of both gross and increasingly more subtle congenital anomalies and providing accurate guidance for diagnostic procedures including amniocentesis, chorionic villus sampling (CVS), fetal blood sampling, and fetal biopsy. The safety of these invasive methods improved due to higher resolution ultrasonography as well as progress in the development of the techniques themselves. Simultaneously, the “revolution” in molecular genetics has resulted in the identification of the genes and mutations responsible for many inherited disorders. This knowledge can, in many cases, be applied directly to prenatal diagnosis for pregnancies at risk for specific genetic diseases. All of these factors have contributed to the need for maternal–fetal medicine specialists to become experts in the field of prenatal diagnosis.

This chapter reviews the most common indications for prenatal diagnosis and the invasive methods currently used. The status of preimplantation diagnosis and of prenatal diagnosis using fetal cells or fetal DNA isolated from the maternal circulation is reviewed.

INDICATIONS FOR PRENATAL DIAGNOSIS

The most common reasons for referral for genetic counseling and prenatal diagnosis are summarized in Table 1. Currently, invasive prenatal testing is offered when the likelihood of detecting an abnormality exceeds the statistical risk of pregnancy loss related to the diagnostic procedure. Ethical issues regarding the somewhat arbitrary determination of when to offer prenatal diagnosis were recently considered and universal access to genetic diagnosis proposed (1); however, this practice is presently far from being adopted.

Maternal Age

The relationship between advancing maternal age and increasing incidence of chromosomal abnormalities due to meiotic nondisjunction is well established (2). The steadily rising risk with maternal age applies not only to trisomy 21 (Down syndrome), but to any trisomy or monosomy. The chance for a 35-year-old woman to have a live-born infant with a chromosomal abnormality is 1 in 192 (3). This risk approximates the stated 1 in 200 risk of procedure-related loss with second-trimester amniocentesis; thus, women are offered prenatal diagnosis for chromosome abnormalities when they will be 35 years of age or older at the time of delivery. Many chromosomally abnormal fetuses are lost during the course of pregnancy; therefore, the likelihood of having an aneuploid

fetus at the time of amniocentesis (usually 16–20 weeks of gestation) is greater than the risk at term (4). For example, women who will be 33 years old at the time of delivery have a risk of 1 in 200 of having a chromosomally abnormal fetus at the time of amniocentesis (4). For this reason, some practitioners advocate offering prenatal diagnosis to women who are 33 years of age or older, but no consensus has yet been reached.

Chromosomal Abnormalities

Couples with a previous trisomic child are thought to have approximately a 1% risk of recurrence (5) and are thus offered prenatal diagnosis. Siblings and second-degree relatives to an individual with Down syndrome may have a slightly increased risk for having a similarly affected child (6), though most studies have not demonstrated an increased incidence of Down syndrome in second- or third-degree relatives (7,8). Many people with an affected relative have a high degree of anxiety, which may justify prenatal testing.

Translocations and other chromosomal structural rearrangements can predispose to chromosomally abnormal progeny (see chapter 43 for a description of translocations). Couples in whom one partner carries a balanced reciprocal translocation are at high risk for recurrent pregnancy loss. At the time of prenatal diagnosis, approximately 10% to 12% of progeny have an unbalanced chromosome complement (9,10), while this risk declines to 6% of live-born offspring because of spontaneous abortion (11). Progeny of Robertsonian translocation carriers are at risk for trisomy or monosomy of the involved chromosomes, with the risk dependent on the specific chromosomes participating in the translocation (9,10). An extreme example is that of a Robertsonian 21/21 translocation carrier, whose progeny would all be predicted to be either trisomic (Down syndrome) or monosomic (lethal) for chromosome 21. Couples with a history of multiple pregnancy losses should be offered karyotypes to determine whether one member is a translocation carrier. Individuals known to be translocation carriers should be offered prenatal cytogenetic testing, as the fetal karyotype result may be normal, balanced, or unbalanced, with the proportion of each karyotype result varying, dependent on the specific translocation (10).

Multiple-Marker Screening in the Second Trimester

The history of multiple-marker screening began in 1972 when Brock and Sutcliffe described the association of fetal anencephaly and spina bifida with elevations in maternal serum alpha-fetoprotein (MSAFP) levels (12). Subsequently, studies in the United States (13) and the United Kingdom (14) proposed the establishment of screening programs to detect pregnancies complicated by fetal open neural tube defects. During the 1980s, such screening programs became widespread in the United States, where the incidence of neural tube

Table 1 Indications for Prenatal Diagnosis

- Maternal age 33 or more at delivery
- Family history of chromosomal abnormality
- Parental translocation carrier
- Abnormal MSAFP or multiple-marker screening
- Family history of neural tube defect
- Single-gene disorder—family history or carrier detected by population screening
- Congenital malformation detected by ultrasonography
- Maternal desire

Abbreviation: MSAFP, maternal serum alpha-fetoprotein.

defects is 1 to 2 per 1000 live births (15). Elevated MSAFP values are associated not only with open neural tube defects, but also with other fetal abnormalities involving a break in skin integrity such as ventral wall defects (omphalocele, gastroschisis), teratoma, bladder exstrophy, or aplasia cutis. Numerous other fetal and placental abnormalities have been reported in association with elevated MSAFP levels (16,17). In addition, elevations in MSAFP unexplained by sonographic findings are associated with an increased risk of pregnancy complications, fetal growth restriction, and fetal death (17,18). The American College of Obstetricians and Gynecologists has proposed an algorithm for the evaluation of pregnancies with elevated MSAFP levels including ultrasonography, amniocentesis for amniotic fluid AFP, and acetylcholinesterase if the amniotic fluid AFP is elevated (15). Since MSAFP screening became the standard of care in the United States, elevated MSAFP has become a common indication for referral for prenatal diagnosis.

The clinical utility of MSAFP screening expanded substantially when an association was found between low MSAFP values and fetal chromosomal abnormalities (19,20). Using a combination of maternal age and MSAFP to identify pregnancies at high risk, approximately 20% of Down syndrome pregnancies in women younger than 35 years were detected in prospective trials (21,22). Subsequently, Down syndrome pregnancies were found to be associated with elevated maternal serum concentrations of human chorionic gonadotropin (hCG) (23) and decreased unconjugated estriol (uE_3) (24) as compared with normal controls. In a large prospective trial using maternal age in combination with maternal serum levels of AFP, hCG, and uE_3 to assign a risk of fetal Down syndrome of $>1:190$, 58% of Down syndrome pregnancies were identified, and 38 amniocenteses were performed for each confirmed case of Down syndrome (25). This trial included women ranging in age from 16 to 41 years. Since this publication in 1992, multiple-marker or “triple” screening came into more widespread use. Further applications of the triple screen have followed, with the recognition of a characteristic pattern of low values for AFP, hCG, and uE_3 in association with trisomy 18 (26,27), and a pattern similar to that for an increased risk for Down syndrome (low AFP, low uE_3 , high hCG) in association with Turner syndrome (45,X) (28,29). A newer maternal serum marker, dimeric inhibin-A, also measured in the second trimester, is approximately 2.06 times the median value in fetuses affected with Down syndrome (30). The detection rate for Down syndrome with the addition of inhibin-A is approximately 80%. Serum screening in the second trimester with the inclusion of

inhibin-A may be called the “quadruple screen.” It is important to note that inhibin-A is not used in the calculation of trisomy 18.

Multiple-marker screening allows the detection of pregnancies that would otherwise not be considered at increased risk for either neural tube defects or chromosomal aneuploidy. Older women may opt for multiple-marker screening to readjust their age-related risk of fetal Down syndrome and trisomies 18 and 13. A recent guideline from the American College of Obstetricians and Gynecologists suggests that all women be offered aneuploidy screening before 20 weeks of gestation, regardless of maternal age (31).

One of the main disadvantages of multiple-marker screening was its timing in the second trimester. An abnormal screen, typically obtained at 16–18 weeks of gestation, sets into motion a series of events including repeat screening in some cases (if AFP is high, *not* for increased risk of aneuploidy), ultrasonography to confirm dating, genetic counseling, and the offer to have amniocentesis for chromosomal analysis and amniotic fluid AFP measurement. At the completion of this evaluation, most patients are beyond 18 weeks and many are beyond 20 weeks of gestation. By this time, many pregnant women have begun to feel fetal movement and are readily recognized as pregnant by casual observers. In addition, the options for termination are induction methods (prostaglandin and/or urea instillation, prostaglandin suppositories) or dilatation and evacuation at an advanced gestational age. Either method is associated with significantly greater morbidity than first-trimester dilatation and curettage, as well as increased medical costs. For these reasons, the ability to perform screening in the first trimester provides a significant benefit over second-trimester screening.

Multiple-Marker Screening in the First Trimester

Multiple biochemical marker screening may also be offered in the first trimester. Investigations into the first-trimester biochemical maternal serum marker screening began in the 1980s. Two useful markers emerged, free beta human chorionic gonadotropin ($\text{f}\beta\text{hCG}$) and pregnancy-associated plasma protein A (PAPP-A). While maximum detection rates for each of these markers are at 14 completed weeks for $\text{f}\beta\text{hCG}$ and 9 completed weeks for PAPP-A (32,33), used together (along with maternal age) at 11 to 13 weeks of gestation yields a detection rate for Down syndrome of approximately 67% with a false positive rate set at 5%. Better detection rates are obtained by incorporating a sonographically acquired measurement of the sonolucent area just under the skin line on the posterior fetal neck, the nuchal translucency (NT). Using *only* first-trimester NT screening and maternal age (without biochemistry) has yielded a Down syndrome detection rate of 73% at the same 5% false positive rate. Today, first-trimester “combined” screening employs maternal age, maternal serum analytes, and an NT, with or without the presence or absence of the fetal nasal bone to arrive at an individual’s risk for fetal Down syndrome. In one large prospective study, in women ≥ 35 years old, the detection rate was 89.8% for fetuses with trisomy 21 with a false positive rate of 15.25%, while 100% of fetuses with trisomy 18 were detected (34). For those women < 35 years old, first-trimester “combined” screening had a detection rate for trisomy 21 of

60% with a false positive rate of 5%. In pregnancies complicated by fetal trisomies 13 and 18, free β hCG and PAPP-A are decreased, while those pregnancies complicated by fetal sex chromosome abnormalities demonstrate a normal free β hCG and PAPP-A is low (35–37).

Measurements of the NT may be taken from 10 to 14 weeks of gestation. In a prospective screening study of 1273 women mostly of advanced maternal age, Nicolaides et al., observed an NT of ≥ 3 mm in 86% of the trisomic and 4% to 5% of the normal fetuses. An NT of ≥ 3 mm was associated with a 12-fold increase in fetal aneuploidy and was independent of maternal age (38). This study was confirmed by Chitty et al. in a group of patients who were not considered at risk for fetal aneuploidy (39). As the gestational age increases, so does the NT. Gestational age-specific multiples of the median have been derived and are used commonly.

There are several other terms in use today that describe various combinations of first-trimester screening with second-trimester aneuploidy screening, including “integrated” screening and “sequential” screening. The “combined” first-trimester screen includes an NT, PAPP-A, and a β hCG. “Integrated” and “sequential” screenings both combine elements of first- and second-trimester screening and differ when the first-trimester screening results are reported. In an “integrated” test, an NT is obtained along with a PAPP-A plus a quadruple screen. The results are provided to the patient after all tests are completed. In a “stepwise sequential” test, the combined first-trimester screen is used plus a quadruple screen with results given after each test. In a “contingent sequential” test, the combined first-trimester screen is performed, and only those with a risk between 1:30 and 1:1500 have a quadruple screen (40). Not all centers offer, and not all women opt for “combined” first-trimester screening.

Family History of Single-Gene Disorders

A family history of a single-gene disorder is another common indication for referral for prenatal genetic counseling. Fetal risk assessment is dependent on many factors, such as the distance of the relationship between the affected family member and the individual seeking counseling, as well as the frequency of the disease gene in the population. Couples with a previously affected child may be obviously at higher risk for recurrence, while the risk declines with increased distance from the affected relative ($1^\circ > 2^\circ > 3^\circ$). For example, parents of a child with an autosomal recessive disorder have a 25% risk of recurrence with each pregnancy. By contrast, the offspring to the siblings of an affected individual have a risk calculated as follows: the risk of the unaffected sibling being a carrier (2/3), times the risk that the partner is a carrier (the population carrier frequency if the family history is negative), times the risk of having an affected offspring given that both parents are carriers (1/4). Thus, for cystic fibrosis, with a carrier frequency of 1 in 25 Caucasian Americans, the risk for an unaffected sibling of an affected individual to have an offspring with cystic fibrosis is $2/3 \times 1/25 \times 1/4 = 1/150$. Carrier testing of the sibling, when available, may refine these odds.

Carrier screening is currently widely available for some recessive conditions, including sickle cell anemia and Tay-Sachs disease. Carrier screening has become available for other relatively common genetic conditions, creating another group

of candidates for prenatal diagnosis. In 2001, the American College of Obstetricians and Gynecologists and the American College of Medical Genetics issued guidelines that recommended cystic fibrosis carrier screening be offered to Caucasian couples who are planning a pregnancy or seeking prenatal care, and be made available to patients, regardless of ethnic background (2001 guideline). Cystic fibrosis is more common in Caucasians and in Ashkenazi Jews than in other populations, and the screening test detects a higher percentage of carriers in this population than in other groups. Due to the fact that defining a single ethnicity in many people in the United States is becoming more difficult, the guidelines for carrier screening from the American College of Obstetricians and Gynecologists were updated in 2005 and state that it is reasonable to offer cystic fibrosis carrier screening to all pregnant patients, provided that women are aware of their a priori carrier risk and the test limitations (41).

Sonographic Findings

As the resolution and therefore the sensitivity of ultrasonography improve, an increasing number of referrals for prenatal diagnosis are generated because of sonographic fetal abnormalities. Some of the more frequent findings include fetal heart defects, ventral wall defects, central nervous system abnormalities, gastrointestinal anomalies, renal anomalies, choroid plexus cysts, and nuchal fold thickening. These may be found in isolation, in conjunction with chromosomal abnormalities or as part of a genetic syndrome. As technical skills in ultrasonography as well as the resolution of the images improve, fetal abnormalities will be more frequently identified. As a result, our understanding of developmental abnormalities and what may represent normal variation will expand and thereby modify the genetic counseling engendered and what is offered in terms of prenatal diagnosis.

Not only have the indications for referral for prenatal diagnosis have expanded over the last four decades, but also the methods available to prenatally detect genetic disorders have also expanded. In addition to amniocentesis, invasive diagnostic methods currently include CVS, fetal blood sampling, and fetal biopsy for specific indications. Samples obtained by these techniques are used for cytogenetic analysis (karyotype, fluorescence in situ hybridization (FISH), array comparative genomic hybridization), molecular DNA diagnosis, and/or biochemical evaluation, dependent on the specific disorder being investigated. Each invasive procedure has risks and benefits to be considered when offering diagnostic testing.

INVASIVE PRENATAL DIAGNOSTIC METHODS

Midtrimester Amniocentesis

The use of amniocentesis to prenatally detect chromosomal abnormalities was first reported in 1967 (42). Since that time, amniocentesis has become a widely accepted method for prenatal diagnosis of chromosomal abnormalities, inherited diseases, and some congenital infections. Despite about experience of three decades with midtrimester amniocentesis, determination of the risk of procedure-related pregnancy loss has been difficult. Risk estimates have ranged from 0.5% to 1% in large, prospective, multicenter trials (43–45). Total loss rates following invasive procedures are more readily accessible,

encompassing the “background” fetal loss rate plus the procedure-related loss rate. A systematic review by Mujezinovic et al. showed a pooled pregnancy loss within 14 days after amniocentesis of 0.6% and rising to 0.9% before 24 weeks of gestation. The total pregnancy loss rate was 1.9% (46).

While fetal loss resulting from amniocentesis is considered the most serious risk of the procedure, other fetal and maternal risks must also be considered. Fetal-needle puncture resulting in scars at birth has been reported (47), but is quite rare. Amniocentesis performed under continuous ultrasound guidance allows sampling of fluid while avoiding fetal parts, thus minimizing the risk of such an injury. Two large prospective trials found an association between midtrimester amniocentesis and neonatal respiratory difficulty (43,44), while a study from the National Institute of Child Health and Human Development (NICHD) of the U.S. National Institutes of Health (43) did not corroborate this finding. An increased incidence of orthopedic postural deformities in association with amniocentesis was reported in the initial United Kingdom Medical Research Council (MRC) study (44), but was not found in two other large trials (42,45).

Other complications from midtrimester amniocentesis include chorioamnionitis, leakage of amniotic fluid, and vaginal bleeding. The incidence of chorioamnionitis is <1 per 1000 procedures (48). Amniotic fluid leakage occurs in 1% to 2% of patients (42,44), but is usually self-limited, with re-accumulation of fluid and a normal pregnancy outcome in most cases (49). The incidence of vaginal bleeding after second-trimester amniocentesis is also approximately 1% and is positively correlated with the number of needle insertions required (42).

An advantage of midtrimester amniocentesis over CVS is the ability to measure amniotic fluid levels of AFP, a sensitive marker for open neural tube defects and ventral wall defects. Normal values for amniotic fluid AFP levels in the first trimester have not been clearly established. Detection of acetylcholinesterase in the amniotic fluid is a useful adjunct to amniotic fluid AFP. Today, high-resolution ultrasound imaging with a qualified sonographer is more sensitive than MSAFP, which has an 80% sensitivity. High-resolution sonographic visualization approaches the sensitivity of amniotic fluid AFP to detect open neural tube defects. In practice, ultrasound imaging may detect some closed neural tube defects missed by AFP testing. In a study evaluating neural tube defect detection, standard ultrasound improved the sensitivity of MSAFP by correcting the errors in gestational age (50). In this study, moreover, ultrasound detection of neural tube defects was 100% (50).

Chorionic Villus Sampling

Due to the desire for prenatal diagnosis in time to allow safer termination options, increased patient privacy, and decreased emotional stress, earlier diagnostic measures were being sought. The technique of CVS entered the realm of clinical practice in the 1980s. Early attempts to obtain trophoblastic tissue had been described in the 1960s and 1970s, which were fraught with high failure and complication rates (51,52). The ability to perform ultrasound guidance (53) and the development of a thin, malleable catheter for transcervical sampling (54) led to a resurgence of the technique and the

naissance of first-trimester genetic diagnosis. Since the publication of these groundbreaking articles, numerous investigations, including large prospective trials to determine the risks and benefits of CVS, have been undertaken.

CVS is usually performed between 10 and 14 weeks of gestation, but can be performed transabdominally in the first trimester as well as throughout later gestation. Cytogenetic, molecular DNA analysis, and/or biochemical methods can be applied to villus tissue. These assays can detect chromosomal abnormalities, specific gene defects or linkage to at-risk loci, and/or evidence of enzymatic activity abnormalities in pregnancies at known risk for particular inherited conditions. In all of these respects, CVS generally provides the same type of information available through amniocentesis. There are several instances in which these two procedures are not equivalent in their ability to provide diagnostic information. For example, CVS is not useful in detecting a fetus with an open neural tube or ventral wall defect. Admittedly, the sonogram performed at the time of CVS might identify such a fetus; no information regarding AFP level or the presence of acetylcholinesterase, however, can be obtained through CVS. In fragile X testing, the methylation status may not yet be established in chorionic villi at the time of sampling, and an amniotic fluid sample may be needed if ambiguous results are obtained from villus tissue. On the other hand, some assays can be performed using chorionic villi but not amniocytes. The prenatal diagnosis of forms of osteogenesis imperfecta that are based on collagen studies cannot be completed on amniotic fluid samples (55). Thus, in contemplating the preferred method for prenatal diagnosis in a given pregnancy, both the couples' wishes and the ideal means to obtain the desired information must be considered.

The same issues that faced early laboratory investigations of cultured second-trimester amniocytes were confronted by early investigators of chorionic villus cells, including maternal contamination and chromosomal mosaicism. Maternal contamination from decidual cells poses a threat to long-term chorionic villus cell cultures. Careful dissection of the villi using a microscope with 40× magnification will minimize this risk.

Chromosomal mosaicism refers to two (or more) cell lines in the same tissue, organ, or individual. An example would be a villus sample with both a normal (e.g., 46,XX) cell line and a trisomic (47,XX,+21) cell line. Because the placenta arises from different late progenitor cells than the embryo, mitotic errors that result in chromosomal nondisjunction can be limited to cells destined to become chorionic villi (56). This circumstance is identified as mosaicism by the laboratory at the time of CVS and is found in about 1% of chorionic villus samples (55). By contrast, mosaicism is seen in 0.25% of midtrimester amniotic fluid samples (57).

Frequently, when mosaicism is found by CVS, the fetus is chromosomally normal (58). If, however, a mitotic error occurred in a very early progenitor cell, both the villus and the embryonic descendants of this progenitor could contain the abnormality. In such a case, a chromosomal abnormality would often be revealed both at CVS and at follow-up amniocentesis. More often, follow-up amniocentesis in the midtrimester usually has normal cytogenetic results (59), with normal fetal outcomes. Though a normal karyotype on

amniocytes provides presumptive evidence for a chromosomally normal fetus, it is not a guarantee, as mosaicism can be limited to only some fetal tissues.

As for any prenatal diagnostic method, patients are concerned about the risk of procedure-related pregnancy loss. Initial studies to assess the safety of first-trimester CVS were descriptions of clinical experience, which described total fetal loss rates from 2% to 7% (60,61) and were unable to address the procedure-related loss rate. A large proportion of the total loss rate represents the naturally occurring spontaneous abortions characteristic of an earlier gestational age. In addition, chromosomally abnormal fetuses are viable in greater proportions at 10 to 12 weeks than at 16 to 20 weeks, the time of midtrimester amniocentesis. These and other issues were subsequently addressed in several multicenter trials of CVS.

Similar rates of pregnancy loss were found between CVS and midtrimester amniocentesis in a large randomized Canadian trial (62), in which the observed difference in loss rates between eligible women with a sonographically viable fetus at 9 to 12 weeks in the CVS and midtrimester amniocentesis groups was 0.6%, which was not statistically significant.

The MRC European trial of CVS (63) was less optimistic about the safety of the procedure. This multicenter, prospective study compared pregnancy outcomes after randomization with either first-trimester CVS or midtrimester amniocentesis. The study concluded that CVS "reduces the chances of a successful pregnancy outcome by 4.6% in comparison with second-trimester amniocentesis." One explanation for the increased loss rate in the MRC trial may be related to operator experience; the European study included a larger number of centers, with individual centers contributing fewer patients.

A prospective, nonrandomized trial conducted by the NICHD Collaborative Group (46) also compared pregnancy outcomes in patients undergoing first-trimester transcervical CVS with a group of patients undergoing midtrimester amniocentesis. This study showed total loss rates of desired pregnancies of 7.2% in the CVS group and 5.7% in the amniocentesis group. These rates included elective terminations for chromosomal and sonographic abnormalities, as well as spontaneous losses. After correction for differences in gestational age and maternal age between the two groups, the total excess loss rate in the CVS group was 0.8% when compared with the amniocentesis group. While this difference was not statistically significant, some centers currently quote a slightly higher procedure-related loss rate for CVS than for second-trimester amniocentesis. Many patients, nonetheless, would consider the benefits of early diagnosis in choosing to have CVS including the greater safety of pregnancy termination at an earlier gestational age. The systematic literature review cited earlier estimates a total pregnancy loss rate of 0.7% within 14 days, 1.3% before 24 weeks of gestation, and an overall pregnancy loss rate of 2% with CVS (46).

In addition to the pregnancy loss rate, the question of an increased risk of fetal limb abnormalities in association with CVS has undergone scrutiny. Firth and coworkers (46) reported an unusually high incidence of oromandibular-limb hypogenesis syndromes in pregnancies undergoing CVS at 56

to 66 days of gestation (8–9 weeks). None of the three large trials (61–63), in which CVS was usually performed at 10 to 12 weeks of gestation, found such an association. This led Firth et al. to hypothesize that an earlier gestational age at the time of CVS may predispose to limb reduction defects (64).

Subsequently, Burton and colleagues (65) reported their experience in which four transverse limb reduction defects were identified during follow-up after CVS in 436 pregnancies, an incidence far greater than the reported incidence of limb malformations of 1 in 1500 to 2000 live births (66). The procedures were performed at 9.6 to 11.3 weeks of gestation, thus the limb abnormalities cannot be explained by early timing of the CVS. This report has prompted speculation regarding the technique used in the single center involved, particularly since their spontaneous loss rate was considerably higher than that of other centers (67). A survey conducted in Taiwan (68) also found an association between CVS and limb defects, with a 100-fold increased incidence of severe limb defects after CVS exposure as compared with the general population. However, in this series, 87% of the cases involving limb defects in which the timing of CVS was known were performed prior to 63 days (9 weeks) of gestation. In an effort to objectively evaluate the potential etiologic role of CVS in the development of limb reduction defects she first described, Firth and colleagues reported a series of 75 infants who were exposed to CVS in utero and had limb reduction defects at birth (69). The authors found a strong correlation between increasing severity of the defects and earlier gestational age at the time of CVS, with the most severe defects following CVS at 9 weeks of gestation or less. These studies and others support postponement of CVS until 9 completed weeks of gestation to minimize the risk of fetal injury.

Operator experience may explain the lack of an association with fetal limb defects reported by the majority of U.S. centers. A publication by the World Health Organization reported an incidence of approximately 6 limb reduction defects per 10,000 CVS cases (70), which is consistent with the background rate of this congenital anomaly (65). At this time, most centers feel that patients should be informed of the possible association, made aware of their options, and allowed to select a method of prenatal diagnosis most consistent with their medical and psychosocial needs.

Chorionic villus tissue may be obtained by one of three approaches: transcervical, transabdominal (71,72), or transvaginal (73). The transcervical method is currently the most widely used, although some claim that transabdominal sampling has a lower risk of infection and is easier to perform due to the technique's similarity with amniocentesis. Several prospective randomized trials compared transabdominal and transcervical methods. Two of these studies, a collaborative one through the NICHD (74) and a single-center study by Brambati and colleagues (75), found no difference in the success of sampling, fetal loss, delivery, or neonatal outcome between groups. A third randomized trial (76) showed a significantly greater total fetal loss rate after transcervical CVS (10.9%) when compared with transabdominal CVS (6.3%) and midtrimester amniocentesis (6.4%). The reasons for the differences in fetal loss rates are unclear. In all the three studies, an increased incidence of minor symptoms were found in the groups undergoing transcervical CVS.

Common symptoms following CVS include cramping, spotting, and vaginal bleeding. Minor cramping is reported by 16% to 22% of women after transabdominal or transcervical CVS (74,78); 19% to 32% reported spotting after transcervical procedures, versus 4% after transabdominal CVS (74,78); vaginal bleeding as heavy as a menstrual period was reported by up to 6% to 7% of patients after transcervical CVS and only 1% to 2% of women undergoing transabdominal CVS (63,74). Amniotic fluid leakage is uncommon after CVS, occurring transiently in 1.1% and for several weeks in 0.7% of cases in the initial NICHD study (78), and less frequently in other reports. Of those women who continued to leak fluid, 20% (3 of 14 patients) went on to lose the pregnancy.

A significant concern is the risk of infection. For this reason, transcervical CVS is contraindicated in the presence of a genital tract infection, including active herpes genitalis. Case reports of septic abortion and septic shock following CVS have been published (77,79); however, this complication was not reported in any of the large CVS trials.

Fetal Blood Sampling

Though amniocentesis or CVS is usually sufficient for prenatal diagnosis, fetal blood sampling can provide rapid and accurate analysis for a multitude of disorders (Table 2). For prenatal cytogenetic analysis, amniocentesis and CVS are the most widely used methods. However, patients occasionally present at late gestational ages with compelling indications for rapid fetal karyotype, such as severe intrauterine growth retardation, oligohydramnios, hydrops fetalis, or major fetal anomalies. Though CVS or amniocentesis may provide diagnostic information, the quality of a rapid cytogenetic analysis from direct preparations of villus tissue may be poor, and the time required for culture of amniocytes or villi may significantly alter obstetric management. Under such circumstances, fetal blood sampling to obtain a fetal lymphocyte karyotype, which is usually available in 2 to 3 days, may be indicated. A clinical example would be a patient in the mid-third trimester of pregnancy presenting with preterm labor, severe fetal growth retardation, and a fetal cardiac defect detected by ultrasound. A rapid diagnosis of trisomy 18 would allow that patient to undergo counseling and afford her the option of foregoing tocolysis, antepartum surveillance, cesarean section for fetal

indications, and neonatal intervention after birth. Patients may also be offered fetal blood sampling to provide rapid diagnosis at the time limits of legal pregnancy termination.

An ever-increasing number of inherited conditions can be diagnosed through molecular, cytogenetic, and biochemical techniques, as the gene alterations, chromosomal locations (for linkage analysis), and/or enzymatic derangements relevant to specific disorders are identified. As a result, fetal blood has become infrequently required for the prenatal diagnosis of coagulopathies, hemoglobinopathies, and other heritable diseases. Historically, some of the first applications of prenatal diagnosis were to detect hemoglobinopathies. This was done initially using fetoscopically obtained fetal blood, a procedure associated with higher rates of morbidity and fetal mortality (80,81). Later, knowledge of specific gene defects and advancements in molecular technology allowed diagnosis using DNA extracted from amniocytes (82), and subsequently chorionic villi, methods that are now applied for the detection of numerous inherited conditions.

Other specific applications of fetal blood sampling for diagnostic and treatment purposes are considered throughout this text. Examples include fetal evaluation for viral infections, erythroblastosis fetalis, alloimmune thrombocytopenia, and assessment of fetal acid–base status. Fetal blood sampling may be performed either by cordocentesis or fetal intrahepatic vein sampling (83). The techniques and complications of fetal blood sampling are discussed at length in chapter 53 and therefore will not be considered here.

NONINVASIVE METHODS FOR PRENATAL DIAGNOSIS
Preimplantation Genetic Screening and Diagnosis

Preimplantation genetic techniques have transformed our existing ideas of ascertaining genetic abnormalities in offspring. Preimplantation genetic screening (PGS) refers to the technique used to assess for the possible existence of embryonic aneuploidy. Preimplantation genetic diagnosis (PGD) refers to the technique used to rule out specific genetic conditions, by analyzing the embryonic DNA. Development of the polymerase chain reaction (PCR) (84) revolutionized molecular genetics. PCR permits millions of copies of a targeted DNA segment to be amplified from a small DNA sample in a matter of hours. This technology has been combined with in vitro fertilization in an effort to provide earlier information by PGD. For couples at risk for an inherited disorder, results obtained from amniocytes or chorionic villi may present them with a difficult decision of whether or not to continue the pregnancy. Many women would prefer to completely avoid carrying affected fetuses (85), and some would prefer a technology that allows testing of embryos prior to implantation. The technology for PGD is quite expensive, however, a factor that must be considered at a time when resources available for health care are being allocated.

The technology involved in preimplantation diagnosis includes in vitro fertilization, embryo or polar body biopsy, PCR amplification of the DNA sequences of interest, and molecular genetic techniques using the amplified DNA to determine the presence or absence of a specific allele(s) in the embryo. Only those embryos with genetic material predictive of an unaffected fetus are implanted, thus avoiding the need for pregnancy termination.

Table 2 Indications for Fetal Blood Sampling	
Chromosomal	
Karyotype	
Mosaicism	
Intrauterine infection	
Toxoplasmosis	
Rubella	
Varicella	
Cytomegalovirus	
Human parvovirus B19	
Red cell isoimmunization	
Platelet isoimmunization	
Fetal hematologic indices	
Hemoglobinopathies	
Immunodeficiency syndromes	
Assessment of fetal acid–base status	

Methods to obtain the cell(s) required for analysis include biopsy of one to several cells of the 2- to 3-day embryo, biopsy of the trophectoderm of the 5- to 6-day blastocyst, and polar body biopsy (removal of the nonfunctioning haploid product of meiosis I) (86). Each of these methods has benefits and drawbacks, including the degree of technical difficulty, variations in pregnancy rates, and errors in diagnosis due to the hazards of single-cell analysis. An analysis of various embryo biopsy techniques concluded that biopsy of a quarter of the embryo on day 3 after fertilization (approximately the 12-cell stage) may be the "most feasible" for PGS (87). Preimplantation diagnosis from polar body biopsy is in use for PGD or PGS. This technique has been performed for aneuploidy screening, X-linked disorders, and maternal translocations. It may also be used in autosomal recessive conditions where both parents are carriers of a particular condition, and an oocyte spared of the mutant gene is used for fertilization. When polar body biopsy is used, the paternal genetic component cannot be evaluated.

Of central importance is the accuracy of diagnosis. When basing a diagnosis on DNA amplified from a single cell, problems such as contamination, PCR failure, and PCR error are of paramount importance. Navidi and Arnheim (88) considered these sources of error mathematically and suggested that blastomere analysis may be preferable in this regard to polar body analysis, while a combination of the two results in a significantly lower risk of misdiagnosis.

The clinical use of preimplantation diagnosis was first described in 1990 by Handyside and colleagues (89) for sex selection in pregnancies at risk for X-linked diseases. Since that time, this technology has been applied for the specific diagnosis of many disorders, including cystic fibrosis (90), Duchenne muscular dystrophy (91), and alloimmune thrombocytopenia (92). It is also being used to evaluate or screen for aneuploidy employing FISH, in the form of chromosome-specific probes to rapidly determine the copy number of a certain chromosome. As has been done with X- and Y-chromosome-specific probes for sex determination (93), PGS utilizes probes for the chromosomes most commonly involved in aneuploidy (X, Y, 18, 13, and 21) (94).

PGS is offered at a number of infertility centers in the United States. Misdiagnosis rates for PGS by single-cell blastomere vary with reporting centers. Cytogenetic FISH analysis appears to offer a positive predictive value of 83% and a negative predictive value of 81% when evaluating a single blastomere in conjunction with PGS (95). Laboratory error with FISH and early embryonic mosaicism are primarily responsible for the inaccuracies associated with cytogenetic preimplantation testing (96). It is recommended that a confirmatory diagnosis be performed when PGS indicates a normal karyotype, or normal DNA results in the case of a single-gene disorder by PGD, in the form of an amniocentesis or a CVS, due to the risk of misdiagnosis.

As PGS is becoming readily available to women who seek diagnostic testing before the time of implantation, it will be important to determine risks, benefits, and pregnancy rates using the procedure. This is particularly true for cytogenetic PGS. Although cytogenetic PGS has been shown to lower the rate of spontaneous miscarriage in women who are >35 years of age (96,97), studies demonstrating accuracy in diagnosing

fetal aneuploidy have yielded controversial results. The number of candidate embryos eligible for transfer after PGS for aneuploidy, furthermore, is diminished. Studies examining the loss and nonuse of cytogenetically normal embryos following PGS will need to be conducted.

Fetal Cells, DNA, and RNA in the Maternal Circulation

The same molecular techniques (PCR, FISH) that have allowed the development of PGS have also played a major role in efforts to provide prenatal diagnosis based on the analysis of fetal cells obtained from maternal peripheral blood. If this type of noninvasive testing were developed to the point of cost-effective and accurate diagnostic clinical application, the risks associated with invasive prenatal diagnostic techniques (amniocentesis, CVS) could be avoided. Thus far, its potential has been evaluated more in terms of a screening test.

Earlier studies suggested that fetal cells exist in the maternal circulation (98,99), and work based on PCR technology has confirmed this (100). Requirements to achieve a diagnosis based on fetal cells isolated from maternal blood would include a sensitive and specific method to identify fetal cells, a process to enrich for the infrequent fetal cells, and techniques to analyze the fetal genetic material for prenatal diagnosis. Each of these aspects is currently in the process of development, and a variety of methods have been attempted with varying success.

In order to identify fetal cells, monoclonal antibodies against various fetal cell antigens have been developed. These include antibodies to trophoblasts (101,102), fetal erythrocyte cell surface antigens (103–105), and paternally derived human leukocyte antigens (106,107). To enrich for fetal cells, the monoclonal antibodies have been fluorescently tagged and used in combination with flow cytometry, a method to sort individual cells based on their specific physical and chemical properties. Characteristics selected for include cell size, cell granularity, and the presence of a fluorescent tag (104,105). By report, multiparameter flow cytometry applied to first-trimester maternal peripheral blood enriches from one fetal nucleated erythrocyte in approximately 10^7 to 10^8 maternal cells to approximately 4 fetal cells per 1000 maternal cells (104). The proportion of fetal cells after sorting second-trimester samples was approximately 20 fetal cells per 1000 maternal cells in this study, though other investigators have not described such high yields of fetal cells (108,109). In addition to fluorescence-activated flow cytometry, isolation methods under investigation include immunospecific magnetic-activated cell sorting (110), immunomagnetic beads (111), avidin–biotin columns (112), and discontinuous density-gradient centrifugation (113). The various methods were reviewed and their advantages and disadvantages were analyzed by Holzgreve and colleagues (114).

Techniques for genetic analysis that have been applied to fetal cells isolated from the maternal circulation include PCR and in situ hybridization. PCR has been used to detect Y-chromosome-specific DNA sequences to identify male fetuses in both unsorted (115) and sorted (104,105) maternal peripheral blood. More recently, this technology has been applied to determine fetal rhesus antigen status (116,117). FISH to detect fetal aneuploidies has also been described using fetal cells derived from the maternal circulation (104,118,119).

The ongoing research using fetal nucleated RBC is of critical importance. In a recent publication from Huang et al., a microfluidic device was used to isolate nucleated RBCs from pregnant women and showed a 10- to 20-fold enrichment of nucleated RBCs over other methods described (120).

In recent years, there has been a greater focus on using cell-free fetal DNA or RNA within the maternal plasma or serum for use in prenatal DNA diagnosis. In 1997, Lo et al. demonstrated that cell-free fetal DNA circulates within the plasma and serum of pregnant women (121). Fetal DNA can be detected within the maternal circulation as early as 7 weeks of gestation (122). In 2009, Tobias et al. reported a method of specific magnetic bead-based capture of free fetal DNA from maternal plasma. The team developed a magnetic capture hybridization technique using specific hybridization primers for the extraction of fetal DNA from maternal plasma, which is almost twice as efficient as older methods (123). Both forms of nucleic acids may prove useful for accurate fetal gender determination and fetal RhD blood typing. Their utility for screening and/or detection of fetal aneuploidy remains under active investigation.

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