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Review Article

Fetal Diagnosis

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The fetus could not be considered a patient until ultrasonography stripped the fetus of his/her aura of mystery; until the origin and development of the fetus from embryo to neonate could be explained scientifically. Ultrasonographies enabled accurate delineation of normal and abnormal fetal anatomy with considerable detail and later on live moving pictures. Ultrasonographic imaging appears to have no harmful effect on the mother or on the fetus. The ability of prenatal ultrasonography to diagnose fetal abnormalities, both as a screening tool and for targeted examinations, has also been confirmed. The ability to perform invasive prenatal diagnostic procedures, such as amniocentesis, chorionic villus sampling, and cordocentesis as well as fetoscopy for fetal therapy has been significantly improved by real-time ultrasonography. Three-dimensional ultrasonography and MRI are now also available for fetal imaging. Recently, cell free fetal DNA released into maternal plasma is known as useful tool for non-invasive prenatal diagnosis of genetic diseases. The fetus may become our new patient in the near future.

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Introduction

In 19 century we have almost no idea of the nature of fetal life. There were no methods to obtain accurate information about the fetus and its environment, and even if serious fetal compromise was recognized, there were no safe methods for operative delivery. The risk of neonatal death from prematurity-related complications was high.

By middle of last century the entire perspective of obstetric care had undergone a perceptible shift towards identification, treatment, and prevention of perinatal death and damage. The transition was fueled by the precipitous fall in maternal mortality, and the underpinnings of fetal medicine became established. The flow of information on fetal life has accelerated with each new methodology, such as the introduction of the amniocentesis and the development of obstetrical ultrasound.[1,2] Concurrently, the advances in neonatology have been equally impressive, bringing a tangential perspective to the organization of biophysical and biochemical variables that become apparent in the transition from fetal to neonatal life.

We closed last century with a fully established concept of the fetus as a patient, amenable to observation and examination, and to rational intervention when warranted.

We are starting this century with more dramatic advances such as three-dimensional fetal imaging, noninvasive methods for measurement of fetal biochemical indices, methods to measure fetal organ blood flow with precision, new techniques for in utero surgery, and perhaps even methods for correction of gene therapy.

Prenatal imaging

The development of fetal diagnosis has been dependent on advances in the field of prenatal imaging. Without the ability to accurately visualize the structure and well-being of the fetus, it would not be possible to diagnose or treat the huge range of abnormalities that are now addressed by the special fetal medical care unit.

Ultrasonography

The advent of real-time sonographic scanning during the 1970s was vital for the accurate visualization of the constantly moving fetus. The subsequent development of higher frequency transabdominal and transvaginal transducers resulted in vast improvement in the resolution of fetal images (Figure 1).[3-5] More recent advances include the development of color Doppler, which displays the strength and the direction of a Doppler signal are useful in fetal imaging for low and high flow states and may aid in the detection of fetal movements and assessing placental function (Figure 2).[6,7] Ultrasound imaging is an integral part of obstetric practice today. It is used routinely for

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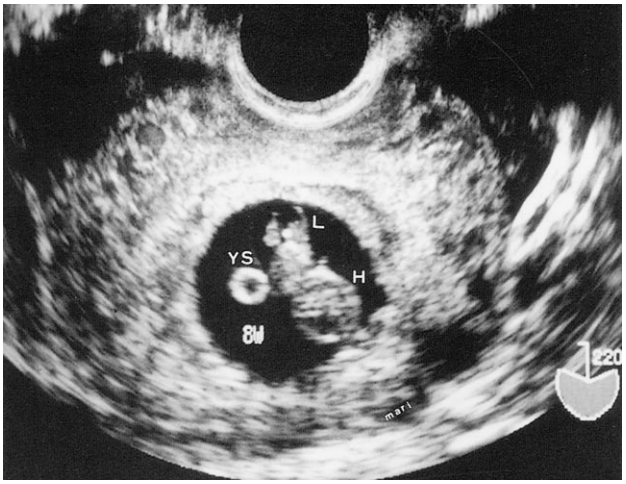

Figure 1. Transvaginal ultrasonography demonstrates the embryo and yolk sac at 8 weeks' gestation. Fetal head (H), legs (L) and yolk sac (YS) are situated within the gestational sac.

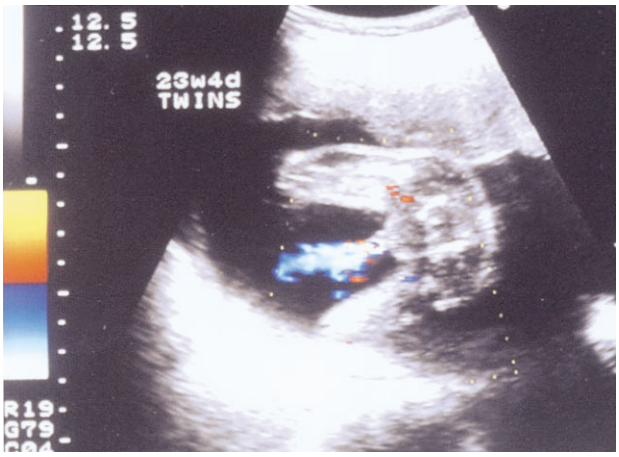

Figure 2. Color Doppler imaging of fetal urination obtained at 23 weeks' gestation. A blue colored jet from the penis was evident.

accurate dating of pregnancy, confirmation of pregnancy location and number of gestations, prenatal diagnosis of congenital malformations, and assessment of fetal well-being. Three-dimensional ultrasonography is now also available for fetal imaging and has the potential to revolutionize the field of prenatal imaging.

3D-ultrasound

Advanced imaging technology has permitted the real-time three-dimensional (3D) reconstruction of data acquired by specially adopted ultrasound machines. This technology permits the increased resolution required for certain fetal malformations such as cleft lip/palate. Routine 2D-ultrasound requires the sonographer to reconstruct the third dimension in a mental image. The real practical value of 3D ultrasound technology is the potential to allow the remote acquisition of ultrasound data by technicians that can be analyzed by appropriate experts (Figure 3).

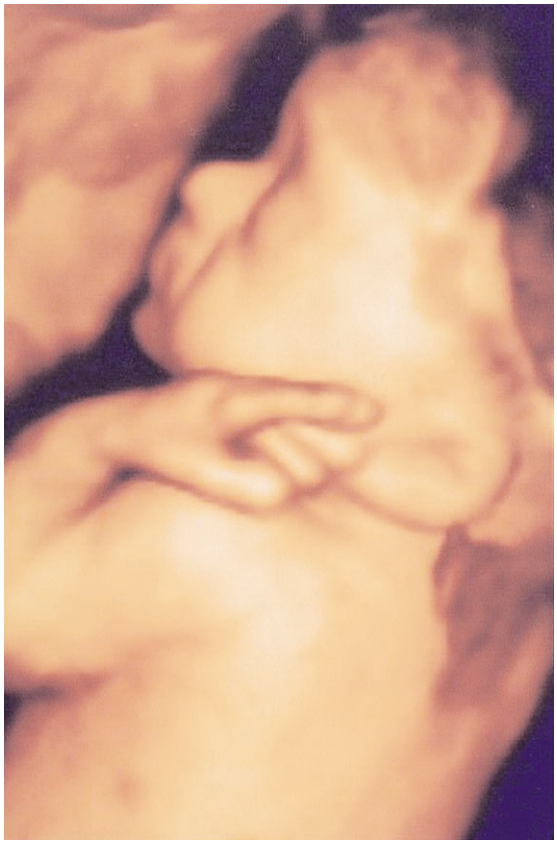

Figure 3. Three dimensional imaging of a normal fetus obtained in the third trimester gestational age.

Fetal MRI

Magnetic resonance imaging has been available for clinical use for over 20 years, but until recently it has rarely been applied in prenatal diagnosis. The potential for MRI in prenatal diagnosis was recognized early on, with reports of imaging of fetal abnormalities. However, a serious limitation of these early attempts at prenatal MRI was the long acquisition times of standard spin-echo images. The amount of fetal movement that would occur during image acquisition degraded the images obtained. The quality of the images that were possible prompted the use of paralyzing agents administered either by intramuscular injection into the fetus or umbilical cord injection to obtain motion-free spin echo images. The invasive nature of this approach dampened the initial enthusiasm for prenatal MRI. The development of ultrafast MRI sequences and fast scanners to overcome fetal movement artifact has resulted in significant improvement in the quality and usefulness of the image. The ultrafast scanning technique eliminates the artifacts caused by fetal movement, making fetal sedation or paralysis unnecessary for prenatal MRI. These ultrafast sequences are now being successfully employed in imaging the human fetus. Fetal anomaly has been well described by MRI scanning in fetuses over 18 weeks of gestation (Figure 4). MRI has the potential to become a powerful adjunct to the evaluation of the abnormal fetus discovered on ultrasound and

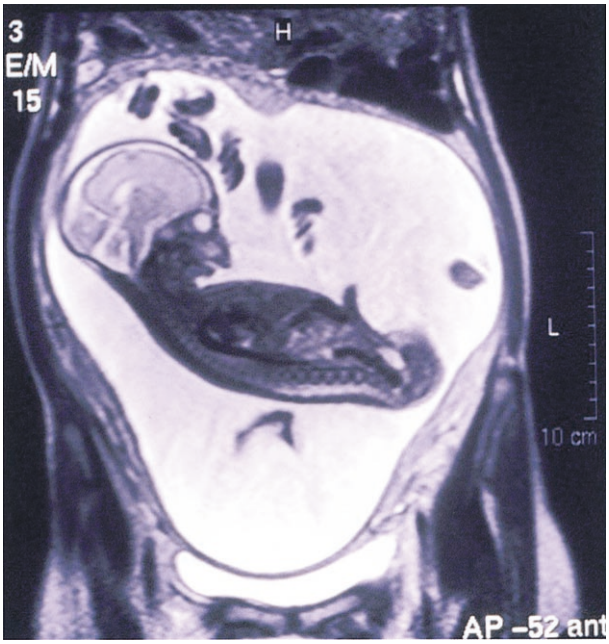

Figure 4. Magnetic resonance imaging demonstrates the fetus with micrognathia and severe polyhydramnios at 30 weeks' gestation.

is increasingly being used to facilitate appropriate prenatal counseling for parents.

Classification of congenital abnormalities

The term congenital abnormality refers to fetal malformations or disorders conferred by birth, rather than inherited as is typically assumed. Although, there are numerous congenital abnormalities, the overall prevalence of disorders is approximately 2 per 100 of pregnancies. The classification of common congenital abnormalities is structural, chromosomal, genetic, and miscellaneous (Table 1).

Structural abnormalities

Structural abnormalities constitute the majority of congenital abnormalities encountered in clinical practice. Fetal central nervous system anomaly, congenital heart disease, intestinal obstruction, abdominal wall defect and obstructive uropathy have established ultrasonographic screening program. The remainder of fetal structural malformations occurs less commonly and is sporadic in nature.

Chromosomal abnormalities

The most common chromosomal abnormalities can be classified as either aneuploidies (usually trisomies) or sex chromosome abnormalities. Although any chromosome may be affected, the majority of trisomies result in first trimester miscarriage except for trisomies 13 (Patau's), 18 (Edward's) and 21 (Down's). Screening programs are geared mainly towards the antenatal detection of Down's syndrome, which is the commonest chromosomal abnormality at birth.[8]

The cumulative prevalence of monosomy X (Turner's syndrome), 47, XXY (Klinefelter's syndrome) and other sex chromosome abnormalities is greater than Down's syndrome. Routine screening for these conditions is not available and the diagnosis is often made incidentally.

Genetic disorders

Numerous genetic syndromes exist, the majority of which are sporadic but some with established patterns of inheritance. The latter are relatively uncommon and are screened for only after the family has undergone genetic counseling regarding the disease, chance of recurrence, diagnostic tests and possible therapeutic interventions.

Congenital viral infections

Fetal infection with rubella, cytomegalovirus, toxoplasmosis and parvovirus are known to have potentially serious deleterious effects.

Table 1. Classification and prevalence of common congenital abnormalities

Congenital abnormality	Examples	Incidence per 1,000 births
Structural	Congenital heart disease	4-6
	Neural tube defects	2-6
	Cleft lip/palate	1-2
	Talipes equinovarus	1
Chromosomal	Trisomy 21 (Down's syndrome)	1.5
	Monosomy X (Turner's syndrome)	0.3
	Other trisomies (13 and 18)	0.3
Genetic	Muscular dystrophy	0.5
	Sickle cell disease	Depends on ethnicity
Miscellaneous	Viral infection	0.2

The risk of a congenitally infected fetus being affected is inversely proportional to the gestational age. Hence, although the chance of fetal infection is low in early pregnancy, if infected the fetus is likely to be seriously affected and the pregnancy is doomed to miscarriage. Therefore, the most susceptible pregnancies are those infected at 12-18 weeks gestation, when infected fetuses are likely to be seriously affected yet survive.

Screening and diagnostic tests

The distinction between screening and diagnosis is often blurred in common usage (Table 2). Screening tests are performed on all women in order to identify a subset of patients who are at high-risk of the disorder. They do not confer any risk to the pregnancy and are performed for disorders with a relatively high prevalence and for which there are accurate prenatal diagnostic tests. Screening tests include maternal biochemistry, maternal virology, and ultrasound. Diagnostic tests on the other hand are carried out on pregnancies that have been identified as high-risk by a prior screening test. They are usually invasive and carry a small risk of miscarriage. Inevitably the risks of being affected by the condition are severe enough to warrant consideration for a diagnostic test. Diagnostic tests include amniocentesis, cordocentesis, and chorionic villus sampling. Cells obtained from invasive prenatal diagnostic tests are cultured until enough cells in mitosis are available to make a cytogenetic diagnosis. The more rapidly dividing the tissue used, the quicker the results are available. Hence the time for diagnosis for amniocentesis, chorionic villus sampling (CVS) and cordocentesis is 2-3 weeks, 1-2 weeks and 24-48 hours. With CVS, the sampled chorionic villi have so many cells already in mitosis, that a direct result may be available in 24-48 hours. Fetal DNA obtained from invasive tests can be used for DNA probe, PCR or linkage analysis.

Invasive diagnostic tests

A number of different tests exist to enable sampling material of fetal origin (Table 3). The sample obtained can be used for cytogenetic, biochemical, enzymatic or DNA analysis to give a prenatal diagnosis. Generally these tests are invasive in nature and carry a small risk of miscarriage.

Amniocentesis

A thin needle is passes transabdominally under ultrasound guidance into the amniotic cavity. A small amount of amniotic fluid is removed, which is contains fetal fibroblasts. This test is usually performed at or after 15 weeks gestation. The procedure-related miscarriage rate for this test is 0.5%. Although, it is technically possible to

Table 2. Difference between prenatal screening and diagnostic tests

Item	Screening	Diagnosis
Population tested	All women	Women at high risk
Purpose of test	Select a high risk group	Diagnose abnormality
Usual methods of testing	・Maternal history ・Maternal biochemistry ・Maternal virology ・Ultrasound	・Ultrasound ・Amniocentesis ・Chorion villus sampling ・Cordocentesis
Pre-requisite to test	Diagnostic test available	Patient aware of potential risks
Risk of test	Anxiety about positive results of the tests	Small risk of miscarriage

Table 3. Details of prenatal diagnostic procedures

Item	Amniocentesis	Chorion villus sampling	Cordocentesis
Gestation	15-40 weeks	10-40 weeks	20-40 weeks
Route	Transabdominal	Transabdominal or transcervical	Transabdominal
Cells sampled	Fetal fibroblasts	Trophoblast cells	Fetal white blood cells
Procedural risk of miscarriage	0.5%	1%	1%
Direct karyotype result	None	24-48 hrs	Not needed
Culture karyotype result	2-3 weeks	1-2 weeks	24-48 hrs
Mosaicism rate on karyotype	None	1%	None

do amniocentesis at earlier gestations, this is generally avoided, as it is associated with a higher rate of miscarriage, neonatal talipes and respiratory difficulties.

Chorionic villus sampling (CVS)

A thin needle is passes transabdominally or transcervically under ultrasound guidance into the placenta. Chorionic villi, which are feto-placental in origin, are aspirated or biopsied through this needle. This test is usually performed at or after 10 weeks gestation. Although the miscarriage rate after CVS is thought to be higher (2-3%), this is because the background spontaneous miscarriage rate of pregnancy is higher at 10 weeks. The procedure-related miscarriage rate of CVS is 1%, as with amniocentesis. Although, it is technically possible to do CVS at earlier gestations, this is generally avoided, as it is associated with a higher rate of cleft lip/palate and digital amputation abnormalities.

Cordocentesis

A thin needle is passes transabdominally under ultrasound guidance into the umbilical cord to sample fetal blood. This test is usually performed at or after 20 weeks gestation. The procedure-related miscarriage rate for this test is 1%. Although, it is technically possible to do this test at earlier gestations, this is generally avoided, as it is associated with a higher rate of miscarriage.

Counseling for parents

The early prenatal detection of congenital abnormality allows both parents and medical cares to plan the management for the pregnancy. Alternatively, the time, mode and place of delivery may be planned in order to ensure the optimal prognosis for the neonate. Parental decisions in prenatal diagnosis are dependent on their prior beliefs and expectations. Accurate provision of information regarding the incidence, likely outcome, screening and diagnosis of congenital abnormalities is an essential part of pregnancy care.

Future development

Fetal cells in the maternal circulation

The isolation and analysis of fetal cells in maternal blood for prenatal diagnosis has not been achieved, but may become sensitive enough to be competitive with other non-invasive techniques. Attention is now being directed on choice of the best fetal cell type, selection of optimal methods of cell enrichment, consistency and diagnosis reproducibility of cell recovery and analysis. Most investigators are focused on the isolation of fetal nucleated red blood or trophoblastic cells. Approximately 1.2 nucleated fetal cell/mL of whole blood from women carrying a male fetus were detectable. Although relatively high proportions of fetal nucleated cells to maternal nucleated cells were reported, not all individuals carrying male fetuses showed male metaphases. This was explained on the basis of fetal cells being rare events in maternal blood. In the late 1970s, flow sorting technology was applied to enrich for fetal cells. In pregnancies where the fetus was male, Y-chromatin was used as an independent marker to verify that the cells were of fetal origin. The validation of a reliable technique for the safe, non-invasive acquisition of fetal cells will revolutionize prenatal diagnosis.[9]

Fetal DNA in the maternal circulation

Using nested primer polymerase chain reaction (PCR) to amplify for Y sequences, relatively high proportions of fetal DNA to maternal DNA were reported.[10,11] (Figure 5) The amount of fetal DNA in the maternal blood increases with progression of pregnancy, and 3.4-6.2% of the total maternal plasma DNA during pregnancy was of fetal origin. Therefore cell free fetal DNA in pregnant women's plasma is useful for non-invasive prenatal diagnosis especially for detection of fetal sex, RhD blood type and gene mutations of paternal origin. Previous studies indicated that pregnant women with pre-eclampsia, placenta previa and fetal chromosome abnormalities tend to have elevated levels of fetal DNA in their plasma. Since functional or structural abnormalities of the placenta and destruction of the trophoblast may be associated with these disease, it is suggested that cell free fetal DNA is of placental origin. This implies that quantitative analysis of fetal DNA may be valuable to screen for placental dysfunction.[12-14]

On the other hand, both maternal cells and maternal DNA are often present in the umbilical cord blood. When umbilical cord blood is used for bone marrow transplantation, the presence of maternal cells in umbilical cord blood plasma is a theoretical risk factor for graft-vs-host diseases and may lead to vertical transfer of infectious agents to a fetus. We showed that the different rates of

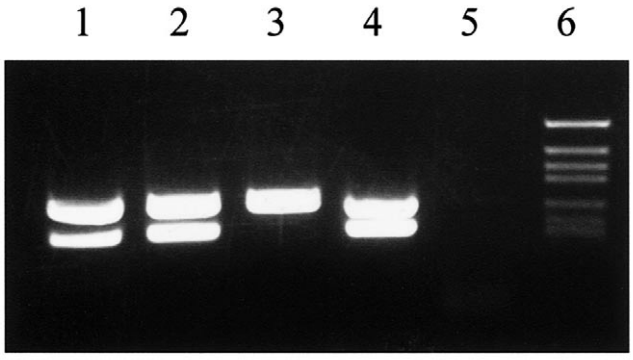

Figure 5. Polymerase chain reaction (PCR) analysis of maternal plasma demonstrates fetal sex. **Lane 1.** Cell-free plasma DNA from 9 weeks pregnant women was detectable by PCR assay and its result showed that fetus was male. **Lane 2.** Cell-free plasma DNA from 11 weeks pregnant women was detectable and its result showed that fetus was male. **Lane 3.** Only 301-bp allele by X chromosome specific primer set was amplified from cell-free plasma DNA from women with female pregnancy. **Lane 4.** Both 301-bp allele by X chromosome specific primer set and 239-bp allele by Y chromosome specific primer set were amplified from cell-free plasma DNA from women with male pregnancy. **Lane 5.** Water as a negative control. **Lane 6.** Size marker.

detection of maternal DNA in umbilical cord blood plasma between the labor and non-labor groups.[15] Although the actual source of maternal DNA in umbilical cord blood plasma remains unknown, it is plausible that some maternal DNA in plasma passes through the placenta, and this may be increased by a mechanical force produced by uterine contraction, which could force placental blood into the umbilical cord. Our results suggest the possibility of trafficking of maternal cells into umbilical cord blood during labor. Because such maternal DNA contamination may suggest the presence of microchimerism in umbilical cord blood, the results may give an insight into the mechanism of fetomaternal circulation and vertical transmission of infectious agents.

Preimplantation genetic diagnosis

Couples at high risk of having pregnancies with inherited diseases may benefit from preimplantation genetic diagnosis (PGD) the early stages of human zygote/embryo development. The development of PGD allows parents to avoid the decision to terminate a pregnancy. PGD has evolved from the development of safe and effective techniques for embryo biopsy and the appropriate methods of genetic diagnosis by FISH or PCR.

Fluorescent in-situ hybridization (FISH)

Rapid FISH analysis of interphase cells has become an essential part of routine cytogenetics in prenatal diagnosis. A variety of DNA probes are available for use, ranging from chromosome-specific to single-gene copy probes. The use of FISH analysis has significantly speeded up the time from sampling to a reliable cytogenetic diagnosis. Amniocentesis, which is a technically simpler procedure than CVS, now can produce results to exclude specific genetic diagnosis in a matter of a few days. The accuracy of these results is similar to that of direct results after CVS.

Conclusions

The fetus has come from the biblical seed to an individual patient with medical problems that can be diagnosed and treated. Although the fetus cannot make an appointment or even cry for help, this patient occasionally needs a physician. The possibility of fetal therapy raises new questions about the pathophysiology of fetal organ development and the technical feasibility of intervention before birth. It also raises complex ethical questions about risks and benefits and about the rights of the mother and fetus as patients. We are only beginning to address these difficult questions.

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