

**DIAGNOSING CONGENITAL MALFORMATIONS
IN THE FETUS**

*Joseph R. Siebert, PhD
Research Associate Professor*

and

*Raj P. Kapur, MD, PhD
Associate Professor*



Department of Laboratories, Children's Hospital & Regional Medical Center
and Department of Pathology, University of Washington
Seattle, Washington

Society for Pediatric Pathology Workshops (1999 — 2001)

Table of Contents

Historical Perspective	3
Classification of Congenital Anomalies	3
Introduction to the Fetal Autopsy	5
General Approach	5
Challenging Dissections	6
Morphometry	8
Postmortem Imaging	9
Photography	9
Alizarin Staining	10
Artifacts	10
The Fetal Autopsy Service	10
Examination of the Autolyzed Fetus	11
Examination of Fetuses Delivered by Dilatation and Evacuation	15
Examination of the Hydropic Fetus	16
Molecular Testing and Fetal Pathology	17
Additional References	29

HISTORICAL PERSPECTIVE

Fetal abnormalities include stillbirth; perinatal asphyxia; consequences of immaturity; specific infectious or metabolic conditions; and, congenital anomalies (Wigglesworth and Singer, 1998). The following discussion is limited to the anomalies, particularly malformations.

The diagnostic approach to congenital malformations has changed dramatically in recent years. Two or three decades ago, individuals with several anomalies were often classed as “multiple congenital anomalies.” Since that time, our understanding and our classification schemes have evolved to a point where this designation is seldom used, unless to describe a new constellation of findings that defies other diagnosis.

But regardless of diagnostic approaches, the method traditionally used at autopsy has been the same—delineation of morphologic features. In the 1970s-80s, the sophistication of syndrome delineation came into its own (see Cohen 1982). Syndromes were defined anatomically, if somewhat vaguely (e.g., “congenital heart disease;” hypoglossia-hypodactyilia), or by eponym (Potter syndrome; Poland anomaly). Then, with an expanding knowledge base, workers began to define syndromes on the basis of pathogenesis or etiology (e.g., oligohydramnios tetrad; amniotic band disruption sequence; retinoic acid embryopathy). Conditions with a chromosomal, genetic, or molecular abnormality would also be described, and named, more precisely (e.g., trisomy 21, 18q-). At the same time, pathologists used anatomic findings to hypothesize aberrations in maternal or fetal physiology. Now with the advent of Doppler flow, maternal physiology can be ascertained with increased accuracy (e.g., decreased flow in uterine artery).

References

Cohen MM Jr: *The Child with Multiple Birth Defects*. Raven Press, New York, 1982.

Wigglesworth JS and Singer DB: *Textbook of Fetal and Perinatal Pathology* (second edition). Blackwell Scientific Publications, Oxford, 1998.

CLASSIFICATION OF CONGENITAL ANOMALIES

As the understanding of pathogenesis and etiology has increased, congenital abnormalities have come to be classified in a variety of ways. We find the following scheme particularly helpful.

Malformation - morphologic defect resulting from an intrinsically abnormal developmental process (structure is abnormal from its inception)

Deformation - abnormal form, shape, or position of a structure, caused by mechanical factors

Disruption - morphologic defect resulting from extrinsic interference with a normal process

Dysplasia - abnormal organization of tissue

Syndrome: "a recognizable pattern of anomalies which are known or thought to be causally related" (Spranger et al, 1982; Khoury et al, 1994)

Sequence: "pattern of anomalies derived from a known (or presumed) malformation or mechanical factor"

Complex: "those groups of heterogeneous disorders with overlapping characteristics that are difficult to separate into specific conditions" e.g., facio-auriculo-vertebral spectrum, hypoglossia-hypodactylia (Martinez-Frias, 1995)

Association: "derivatives of causally nonspecific disruptive events acting on developmental fields" or "abnormal markers of normal embryologic relationships" (Lubinsky, 1986). In a sense, this is a "wastebasket" category that should constantly change with increasing understanding.

Developmental Field: "basic biological units of individual development and of evolution, and association to represent the idiopathic occurrence of multiple congenital anomalies during blastogenesis" (Opitz, 1985)

A simplified way of viewing these classifications is shown below (see Amer J Med Genet 49:26-28, 1993 for more complete discussion).

	ETIOLOGY	PATHOGENESIS
DISEASE	+	+
SYNDROME	+	--
SEQUENCE	--	+
ASSOCIATION	--	--

(Known + ; Unknown --)

Pathologists must be aware of the subtle distinctions in terminology regarding congenital malformations, and be careful of their usage. For example, "agenesis" and "absence" of a tissue or organ are not synonymous, and have different implications for development as well as counseling.

References

Khoury MJ, Moore CA, Evans JA: On the use of the term "syndrome" in clinical genetics and birth defects epidemiology. Am J Med Genet 49:26-28, 1994.

Lubinsky M: Vater and other associations: historical perspectives and modern interpretations. Am J Med Genet Suppl 2:9-16, 1986.

Martinez-Frias ML et al: Primary midline developmental field. II. Clinical/epidemiological analysis of alteration of laterality (normal body symmetry and asymmetry). *Am J Med Genet* 56:382-385, 1995.

Opitz JM: The developmental field concept. *Am J Med Genet* 21:1-11, 1985.

Spranger J et al: Errors of morphogenesis: concepts and terms. Recommendations of an international working group. *J Pediatr* 100:160-165, 1982.

THE FETAL AUTOPSY

Autopsies are performed to identify a cause of death, of course, but also to further the understanding of the patient's course and disease. They are also performed in order to produce epidemiologic data, identify or clarify quality control issues, and provide information that can be used for grief management and counseling (Pauli et al, 1994; Pauli and Reiser, 1994). The results of a fetal autopsy may have broad impact, in that obstetrical, fetal, maternal, paternal, and familial conditions may be uncovered.

In the same way that children are not just small adults, fetuses are not just small, or very young, babies. The practice of fetal pathology differs from that of pediatric pathology, and hence, the fetal autopsy must be approached with a somewhat different philosophy and different set of skills. The pregnancy and birth processes must be evaluated; gestational age determined; growth and development documented; underlying abnormalities identified; and maternal or fetal therapy evaluated (Ochs et al, 1988).

The anatomic delineation and interpretation of congenital anomalies is quite similar in fetuses and newborns, but as described below, can be complicated by autolysis, distortion secondary to delivery method, or small size. Even the assessment of external features can be complicated—the external genitalia, for example, pass through an “indifferent stage” and sex can be assigned erroneously, particularly in early gestation.

References

Ochs RH et al: Perinatal autopsies: A challenge for the nonpediatric pathologist. *Path Ann* 23:235-255, 1988.

Pauli RM et al: Wisconsin Stillbirth Service Program: I. Establishment and assessment of a community-based program for etiologic investigation of intrauterine deaths. *Am J Med Genet* 50:116-134, 1994.

Pauli RM, Reiser CA: Wisconsin Stillbirth Service Program: II. Analysis of diagnoses and diagnostic categories in the first 1000 referrals. *Am J Med Genet* 50:135-153, 1994.

General Approach

Many of the techniques used in pediatric cases apply to the fetal autopsy. For example, we generally employ careful external (*ex situ*) and internal (*in situ*) examination, followed by Rokitansky *en bloc* evisceration. *In situ* examination includes conducting a search for the

presence of fluids, including gas, and examination of umbilical vessels, diaphragm, and presence and position of viscera. Retroperitoneal structures (e.g., major vessels, duodenum, adrenal glands, kidneys and ureters) are examined *in situ*, although the examination will be performed more completely after evisceration.

Following removal, the organs may be fixed in formalin or dissected fresh. We generally opt to fix smaller or more autolytic specimens for up to 24 hours prior to dissection; larger specimens are dissected fresh. In fetuses younger than 20 weeks, much of this dissection is aided by a high quality dissection microscope. The organ block is dissected in the traditional fashion, except in cases of disruption by dilatation and evacuation (see below). Major vessels and organs are approached from the posterior aspect, their components examined, and separated for weighing and sectioning. Extreme care must be taken when examining the cranial contents, which are especially delicate in early gestation and supremely influenced by autolysis.

Challenging Dissections

Because of its small size and anatomical intricacy, the heart can be difficult to examine in early- and mid-gestational fetuses. In our experience, this part of the autopsy can be facilitated in several ways. Regardless of one's approach, small hearts seem to yield the most information when they are fixed prior to internal examination. It is possible to overlook a VSD in the unfixed heart—myocardium, when soft and pliable, can collapse, filling the defect. For the most optimal examination, fix the heart overnight. Then, if the heart is particularly small, bisect it in the coronal plane and examine each half; in making the cut, direct the blade from the cardiac apex to a point midway between main pulmonary artery and ascending aorta. This will display the entire interventricular septum and reveal a defect if present. When the heart is of sufficient size, or if the case warrants (i.e., strong ultrasonographic or other evidence for cardiac abnormality), it can be opened along the path of blood flow using iris scissors. This dissection may be aided by using a dissecting microscope.



Figure 1: Heart (with endocardial cushion defect) and lungs, with dime

An understanding and ability to thoroughly examine the various aspects of the fetal circulatory system is essential. Most components (i.e., foramen ovale, ductus arteriosus, umbilical arteries and vein) are well understood, and the pathologist will be hampered chiefly by subtle or rare findings. The foramen ovale, for example, should close by three postnatal months; when it is completely closed in the fetus or newborn, the finding is abnormal and associated with hydrops, cardiac failure, or other life-threatening condition.

In contrast to other parts of the circulation, the ductus venosus remains elusive to many prosectors. The structure is easily overlooked, partly hidden by the caudate and left lobes of the liver (Figure 2); it is thin-walled when patent, but closes within hours of birth, quickly becoming a tiny fibrous strand. Although it is a subtle anatomic structure, it should be identified in each case, for absence of the ductus venosus carries important clinical consequences that are still understood incompletely.

The ductus arteriosus begins to close at birth, or shortly thereafter. With the contraction of smooth muscle in the ductal wall, the intima becomes wrinkled and can then be differentiated easily from the intimal surfaces of pulmonary arteries and aorta. Rarely, the ductus arteriosus is absent (particularly within the context of right aortic arch) or necrotic.

Umbilical vessels can be altered in several ways. Single umbilical artery is associated with a wide variety of anomalies. Omphalitis, with ascending inflammation of the umbilical vein, is commonly recognized. However, a lesser known change is focal aneurysmal distention, or varix, of the peritoneal umbilical vein. The change must be appreciated, for acute thrombosis or rupture with hemoperitoneum of a varix can lead to fetal death.

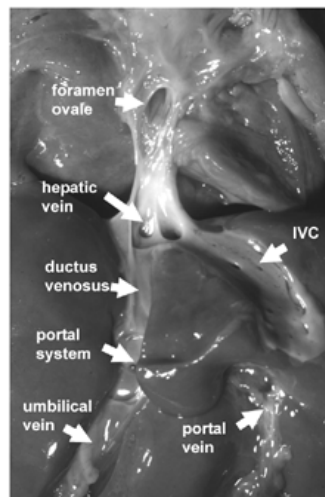


Figure 2: Heart and liver, showing ductus venosus and related structures

Thorough examination of the entire male urethra is also challenging, but necessary in cases of suspected bladder outlet obstruction secondary to posterior urethral valves. Our approach is to continue the abdominal incision inferiorly to the symphysis pubis, then very carefully incise the symphysis pubis in the midline using a scalpel. Valves, when present, lie immediately beneath (posterior to) the symphysis, so great care must be taken to incise only cartilage. The incision is then widened by removing 2 - 3 mm of cartilage on either side of the midline. Following removal of the cartilage, the anterior (and outer) surface of the urethra is exposed. The urethra can be retrieved by removing the entire penis, although we favor a slightly different approach. The muscular portion of the penis can be dissected free of the penile sheath, by careful dissection beneath the skin. This approach has the advantage of leaving the (empty) penile sheath intact (it can be filled with adipose or skeletal muscle prior to suturing the body). The body of the penis,

urethra, and urinary bladder are then removed, generally intact with ureters and kidneys. Finally, the urethra is opened in the midline, carefully, beginning at the urethral orifice.

An alternative approach is to perform gross serial sections of the entire urethra and examine them microscopically. This is helpful in very small specimens, or those with areas of urethral atresia.

References

Goldstein AM et al: Hemoperitoneum due to spontaneous rupture of the umbilical vein. *Am J Gastroenterol* 90:315-317, 1995.

Lev M et al: Premature narrowing or closure of the foramen ovale. *Am Heart J* 65:638-47, 1963.

Machin GA: Re. 'Agenesis of the ductus venosus in a case of monochorionic twins which mimics twin-twin transfusion syndrome' [comment]. *Prenat Diagn* 16:971-972, 1996.

Sepulveda W et al: Fetal prognosis in varix of the intrafetal umbilical vein. *J Ultrasound Med* 17:171-175, 1998.

Shih JC et al: Agenesis of the ductus venosus in a case of monochorionic twins which mimics twin-twin transfusion syndrome. *Prenat Diagn* 16:243-246, 1996.

Sivén M et al: Agenesis of the ductus venosus and its correlation to hydrops fetalis and the fetal hepatic circulation: Case reports and review of the literature. *Ped Pathol Lab Med* 15:39-50, 1995.

Morphometry

Several measures of the fetus are used to estimate gestational age and rate of growth and to corroborate ultrasound findings. A number of relevant tables and graphs are included in this handout (Appendix I). We routinely obtain several measures: crown-heel and crown-rump length, circumferences of the head, chest and abdomen, and foot length. The latter correlates especially well with gestational age.

Although taking these measurements is part of the traditional autopsy, pathologists are encouraged to think critically about the issue of normal measurements. Lengths and circumferences can be misleading within the context of congenital anomaly or postmortem deformation of the body. Crown-heel length is, for example, of limited use in a case of dwarfism.

The routine task of weighing organs must also be performed with care. Because fetal organs weigh so little, one may introduce an unacceptable level of variability according to the method of dissection. Are solid viscera weighed before or after sectioning? Is the heart weighed with or without aorta and other vessels? The prosector should develop a routine, acquiring these measures in a uniform manner. Original references should be consulted, so that the methods by which the data were obtained can be appreciated. Unfortunately, it is unclear how cases were selected, or dissections performed, in some studies. Does a report of normal lung weights

include any cases of pulmonary hypoplasia? Do studies of normal liver weight account for acute congestion?

Choosing an independent variable is particularly important. Oftentimes the independent variable is age, but age is often estimated and inaccurate. Body size, and hence organ weight, varies considerably for a given gestational age. Correlating organ weight to a specific measure of body size, e.g. foot length, is much more accurate.

Postmortem Imaging

Radiographic examination is, of course, especially useful in documenting bony anomalies. It is also an important way to document the presence of all major bony components, as when evaluating a fetus that has been delivered by D&E (see below). Radiography is not particularly helpful in evaluating defects of unossified or cartilaginous tissue, such as myelomeningocele.

Postmortem CT scans and MRI can be helpful, particularly in older gestation fetuses and those with CNS masses or autolysed brains with lesions. Some institutions possess software for producing three-dimensional reconstructions of CT scans.

Infrequently, we have performed postmortem ultrasonography. Tissues are placed in saline first, for support and to mimic amniotic fluid.

Photography

Good quality photographs enhance the autopsy record greatly. To be useful, photos must show the lesion(s) accurately and be technically acceptable (in focus, properly exposed). Dissections should be performed in ways that enhance photography, and the prosector should bear this in mind during preparation. We include case number and ruler, but relegate them to an inconspicuous corner of the photograph.

Photographs of a fetus can be important keepsake for families. We often wrap the fetus in a patterned blanket and photograph the face (possibly with cap) and hand were appropriate. The benefits of this procedure were recently described by Sawyer (1998). The blanket and cap add warmth to the image, but can also hide malformations, as can careful cropping (Figure 3).



*Figure 3: Infant with ectopia cordis and giant omphalocele;
portrait on right is suitable for family keepsake*

Reference

Sawyer DR: Perinatal bereavement: The photographer's role in infant death. *J Biol Photog* 66:35-37, 1998.

Alizarin Staining

For research purposes, staining a fetus with alizarin produces an extraordinary specimen. Counterstaining with alcian blue (to color cartilage) enhances the specimen even more. The procedure is not a trivial one, as skin and most skeletal muscle must be removed and the specimen cleared with potassium hydroxide for optimal viewing.

Artifacts

Many of the artifacts encountered at the fetal autopsy are described below (see "The Autolysed Fetus" and "Examining the D&E").

A rather common artifact is postpartum deformation of the fetus. Obstetrical workers sometimes transport fetuses in containers that are too small; in the process, especially when formalin is added for transport, limbs are fixed in positions that are difficult to assess. Careful communication with physicians, nurses, and other personnel will help avoid this problem.

Subtle artifacts are more difficult to recognize. When a particularly rare finding is encountered multiple times, one should entertain the possibility that the change is an artifact. For example, we made the diagnosis of bifurcated cardiac apices in a fetal heart; however, when we later encountered two additional cases, one with hemorrhage and another with fresh epicardial laceration, we ascribed the change to artifact (trauma secondary to delivery by dilatation and extraction).

The Fetal Autopsy Service

As prenatal imaging has advanced, so has prenatal intervention, which currently takes the form of therapeutic termination of pregnancy. Pediatric pathologists have therefore had to become fetal pathologists. These trends will undoubtedly increase in the future, as fetal surgery becomes more widespread.

The transition from pediatric to fetal pathology involves changes in mindset, knowledge and skill base. Continuing education programs are very important. Recently, Dr. Kapur and I contracted with the Department of Infant and Maternal Health, State of Washington, to visit a number of regional hospitals in an effort to orient pathologists and other health care workers to the field of fetal pathology. The effort was well appreciated, as physicians recognized the need for careful, skilled evaluation of the fetus, and is recommended to pathologists in other areas as well.

We find that collaboration with ultrasonographers, obstetricians, and perinatologists is vital, and schedule monthly conferences to share findings and train each other. The cooperation of nurses, medical genetics counselors, and autopsy assistants is also important.

All of the attributes that contribute to a successful pediatric autopsy service apply to fetal pathology as well. These include prompt reports, possibly including photographs, particularly of special cases; accurate and timely billing of insurance carriers, and so on (see Appendix IV for representative report and billing forms).

EXAMINATION OF THE AUTOLYZED FETUS

Introduction

Autolysis, or maceration, is common in fetal pathology. In many instances, fetal demise for no clinically apparent reason and is not discovered until hours, days, or weeks later. In these situations, family members are often devastated and look to pathological evaluation as the primary means to understand “what went wrong”. With or without such heightened expectations, examination of a macerated fetus can be a challenge.

After demise, the fetus remains in a 37°C liquid environment, which is conducive to rapid tissue degeneration. If bacteria are present (e.g. demise associated with chorioamnionitis), the process may be accelerated. Further autolysis may continue after delivery, particularly if the fetus is not refrigerated. The cumulative changes may obscure underlying pathology and prevent some types of analysis, but should not dissuade the pathologist from his/her efforts to perform a comprehensive study.

Estimating the interval from demise to delivery

An estimate of the time of death relative to delivery should be attempted in every case. It should be based on a combination of clinical and pathological data, and acknowledge the limitations that exist for a particular case. A useful series of papers that examined the ontogeny of anatomic changes that follow fetal demise was written by Genest and his colleagues (citations below). These workers analyzed a series of gross and microscopic changes in the fetus and placenta and determined which correlated well with the interval since birth. In an individual case, application of all of the “good-predictor” indices provides a reasonable basis for estimating intrauterine retention time. The estimate should be compared with clinical information that is available in the case and any discrepancies should be noted.

Personal note: Although the indices listed in these papers are generally useful, they are based on data from relatively a relatively small sample size (71 - 150 stillbirths), particularly when one considers potential affects of gestational age/fetal size on autolysis rates. In many instances, we have found the correlation of these “good-predictors” to be inconsistent.

The mode of demise can influence the pattern of autolytic changes. Fetal death due to intracardiac or umbilical vein injection with potassium chloride as part of an elective termination of pregnancy causes far more rapid and profound autolysis of the heart, liver, and brain than

expected based on intrauterine retention alone. It is possible that prolonged agonal hypoxia (e.g., prolonged severe fetal bradycardia) or metabolic disorders may similarly affect some organs more severely than others.

References

Genest DR, Singer DB: Estimating the time of death in stillborn fetuses: III. External fetal examination; a study of 86 stillborns. *Obstet Gynecol* 80:593-600, 1992.

Genest DR, Williams MA, Greene MF: Estimating the time of death in stillborn fetuses: I. Histologic evaluation of fetal organs; an autopsy study of 150 stillborns. *Obstet Gynecol* 80:575-84, 1992.

Genest DR: Estimating the time of death in stillborn fetuses: II. Histologic evaluation of the placenta; a study of 71 stillborns. *Obstet Gynecol* 80:585-92, 1992.

Artifacts Associated with Fetal Autolysis

Significant autolysis introduces potential artifacts that the pathologist and clinician must appreciate.

1. Dehydration usually follows fetal demise and by 2 weeks leads to mummification. Loss of fluid can obscure antemortem edema/hydrops, particularly in mild-to-moderately hydropic fetuses. As such, nuchal edema (“thickening”), which is often noted by prenatal ultrasonography, can be difficult to confirm after fetal demise. Recognition of redundant nuchal skin may be possible in this context.
2. Laxity of joints occurs in the macerated fetus are often lax. As a result, arthrogryposes that are clearly evident in non-autolyzed specimen may defy resolution. Pterygia (skin folds across joints that restrict complete movement) may provide a clue to antemortem restriction of joint mobility. However, even in non-autolyzed fetuses, pterygia do not form across certain arthrogryptic joints (e.g. talipes) or in association with all clinically-significant arthrogryposes.
3. Herniation of CNS tissue through intervertebral foramina and into the retroperitoneum, scrotum, or vascular system is a well-documented phenomenon that can lead to embarrassing erroneous diagnoses (e.g. neuroblastoma). The brain and spinal cord rapidly liquefy after fetal demise. As the fetus is expelled through the cervix, compression of the head can drive CNS tissue into the retroperitoneum to form a mass. Histologically this mass contains autolyzed primitive neural tissue that can be mistaken for a peripheral neural tumor.

Reference

Kalousek DK, Pantzar T, Craver R: So-called primitive neuroectodermal tumor in abortive previable fetuses. *Pediatr Pathol* 8:503-11, 1988.

4. Degenerating epithelial cells in the small airways of autolyzed fetal lungs can be easily mistaken for neutrophils and interpreted as evidence for ascending bacterial infection. For some reason, pyknosis of sloughed endothelial cells often assumes a multilobular character that

resembles degenerating polymorphonuclear white blood cells. When dealing with a macerated fetus, it is prudent to demand “classic” histological features for neutrophils in the lungs.

5. Hemorrhages into fetal tissues are often found following demise and sometimes invoked as the cause of death. In many sites (adrenals, periventricular germinal matrix, subarachnoid space), hemorrhage probably represents an agonal event in which vulnerable vasculature beds bleed due to intermittent perfusion. We have seen a number of examples of scalp in fetuses, which died prior to delivery. I suspect that these result from external compression of the fetus during labor, even though the fetal heart is inactive.

6. Umbilical cord “narrowing” is a controversial subject. Narrowing of the umbilical cord diameter, particularly in the 1-2 cm segment immediately adjacent to the abdominal wall is a common finding in autolyzed fetuses. It is usually associated with histological loss of Wharton’s jelly (focal dehydration?) and less commonly with obvious antemortem vascular obstruction (thrombosis, hemorrhage, severe congestion). Sometimes excessive spiraling of the cord with apparent torsion may be present at the site of narrowing. In cases with corroborative anatomic evidence for obstruction, most pathologists are comfortable ascribing clinical significance to the lesion. However, in the larger percentage of cases in which no associated obstructive sequelae are found, I am reluctant to invoke “cord stricture” as the cause of fetal demise. The literature regarding the subject is inconclusive.

References

Benirschke K: Obstetrically important lesions of the umbilical cord. *J Reprod Med* 39:262-72, 1994.

Glanfield PA, Watson R: Intrauterine fetal death due to umbilical cord torsion. *Arch Pathol Lab Med* 110:357-8, 1986.

Hallak M, Pryde PG, Qureshi F, Johnson MP, Jacques SM, Evans MI: Constriction of the umbilical cord leading to fetal death. A report of 3 cases. *J Reprod Med* 39:561-5, 1994.

Kiley KC, Perkins CS, Penney LL: Umbilical cord stricture associated with intrauterine fetal demise. A report of two cases. *J Reprod Med* 31:154-6, 1986.

Sun Y, Arbuckle S, Hocking G, Billson V: Umbilical cord stricture and intrauterine fetal death. *Pediatr Pathol Lab Med* 15:723-32, 1995.

Limitations Imposed by Autolysis

The macerated fetus imparts some limitations to comprehensive evaluation that can not be overcome.

1. Liquifaction of the brain may be so severe that anatomic studies are simply not possible. At a minimum, the base of the skull should be examined since malformations of the brain may be evident from anomalies in the cranial base (e.g. absent crista galli in holoprosencephaly). For soft, but not liquefied brains, dissection under water or fixation prior to complete exposure and removal can benefit the examiner, but neither technique provides the anatomic resolution one expects from non-autolyzed tissue. Prior fixation of other organs (e.g. heart) also improves

consistency and facilitates dissection. The value of histology for macerated fetuses is often questioned, particularly when nuclear staining is all but absent. However, architectural details of most organs are usually maintained and provide some insight into their antemortem status. Although viral changes, metabolic cytopathy or more subtle diagnostic findings may be lost, architectural distortion due to necrosis, calcification, or large inflammatory infiltrates may be apparent without cytological detail. Surprisingly, in some cases (e.g., nuclear inclusions in parvovirus B19), specific histological changes can be found despite moderate-to-severe autolysis.

2. Cytogenetic studies and many biochemical tests are not possible with devitalized fetal tissue. It is important to keep in mind that embryo-derived cells in the fetus remain despite fetal demise and that a placental biopsy can substitute for a fetal tissue specimen for many tests. Placenta tissue should be taken as a shallow sample from the fetal surface to avoid contamination by maternal tissues.

The indications for post-mortem cytogenetic studies following fetal demise have not been established clearly, particularly if the fetus is non-dysmorphic. In the past, a karyotype was only sought for dysmorphic fetuses. However, the situation was complicated by discovery that spontaneous fetal demise and / or intrauterine growth retardation are associated with confined placental mosaicism (see below). In these pregnancies, aneuploidy is confined to the placenta such that the fetus is diploid and generally shows no anatomic malformations, but may be small for gestational age. Diagnosis requires demonstration of aneuploidy in the placenta by either traditional cytogenetic studies of cultured cells from placental villi or molecular diagnostic techniques (e.g. comparative genomic hybridization) that can be performed with placental DNA (frozen tissue). A recent informal survey of perinatal pathologists in the USA and elsewhere indicated a wide-range of practices, but few groups that pursue placental studies for confined placental mosaicism for every unexplained stillbirth.

References

Kalousek DK, Barrett I: Confined placental mosaicism and stillbirth. *Pediatr Pathol* 14:151-9, 1994.

Johnson A, Wapner RJ: Mosaicism: implications for postnatal outcome. *Curr Opin Obstet Gynecol* 9:126-35, 1997.

Kalousek DK, Vekemans M: Confined placental mosaicism. *J Med Genet* 33:529-33, 1996.

Moore GE, Ali Z, Khan RU, et al: The incidence of uniparental disomy associated with intrauterine growth retardation in a cohort of thirty-five severely affected babies. *Am J Obstet Gynecol* 176:294-9, 1997.

Robinson WP, Barrett IJ, Bernard L, et al: Meiotic origin of trisomy in confined placental mosaicism is correlated with presence of fetal uniparental disomy, high levels of trisomy in trophoblast, and increase risk of fetal intrauterine growth restriction. *Am J Hum Genet* 60:917-27, 1997.

EXAMINATION OF FETUSES DELIVERED BY DILATATION AND EVACUATION

Introduction

Cervical dilatation and extraction of the fetus in parts is a method of pregnancy termination that is less expensive and potentially less painful than labor induction. The fetus is almost invariably damaged during the delivery procedure; however, the outcome is quite variable. In some instances, the fetus is relatively undisturbed and the pathological exam is almost identical to an intact fetus. At another extreme, the fetus is received as numerous parts admixed with placental tissue. Since this procedure is often used following prenatal diagnosis of fetal anomalies, part of the pathologist's role is to confirm/exclude specific anomalies. It is critical that he/she is aware of the prenatal diagnoses when the examination is performed. An effort should be made to locate and evaluate as many fetal tissues as possible, note any large missing tissues, and convey a clear narrative that indicates which organs, if any could not be adequately evaluated.

General Approach

Our approach to fragmented fetuses is to collect the fragments with a sieve, arrange them anatomically, photograph and radiograph the entire group, and examine each piece individually. Certain organs often partition together because of their proximity in the fetus and vulnerability of adjacent sites to dissociation. These relationships are helpful, since identification of a specific organ (e.g. thymus) may be facilitated by recognition of adjacent organs (e.g., heart, ribs). If any major skeletal structures are missing, the clinician is notified immediately, as it may represent retained parts that could be responsible for prolonged maternal hemorrhage. Otherwise, the statement "all major fetal parts including head, trunk, pelvis and four extremities were identified" appears in the report. Although fragments are evaluated individually, an effort should be made to integrate the findings and summarize conclusions about organ systems, as though the fetus were intact. Organs or organ relationships that can not be evaluated should be noted, along with the reason (traumatic damage vs. missing).

Limitations Imposed by D&E Delivery

Fragmented fetal specimens impose an inherent group of limitations that need to be recognized by the pathologist, clinician, and family. Information that can be obtained from fragmented fetal tissues depends on the nature of the fetal anomalies and the location/severity of the trauma associated with delivery. In general certain sites are more vulnerable than others. In our experience, the brain, body wall and in situ relationships between organs are frequently so disrupted that anomalies can not be excluded. In contrast, cardiac, gastrointestinal, or genitourinary defects are frequently preserved. Isolated neural tube defects, skeletal dysplasias, and cytogenetic disorders are relatively easy to confirm. In contrast, agenesis of an organ (e.g. renal agenesis) can be difficult to diagnosis confidently since it may have been destroyed in the delivery process. Whenever possible, corroborative findings (e.g., flattened adrenal in renal agenesis) should be sought.

It is important to educate clinicians about these limitations. The likelihood that a brain malformation will be preserved after D&E is so small that patients may elect an alternative

method of delivery, particularly if the diagnostic findings have prognostic significance (e.g. aqueductal stenosis).

EXAMINATION OF THE HYDROPIC FETUS

Introduction

Hydrops, or fetal edema, is a non-specific sign that complicates many pregnancies and may be the only prenatal diagnostic finding. Ultrasonographic approaches to detect fetal edema are relatively sensitive and focus, in part, on fluid accumulation in the posterior neck. A relatively subtle change in “nuchal thickness” is one indication that a fetus is at risk for more severe edema and a long list of associated disorders. The list of primary disorders that can lead to hydrops includes structural, metabolic, physiologic, infectious, chromosomal and idiopathic etiologies. A practical approach to hydropic fetuses is to collect the following necessary data for the reasons indicated.

References

Machin GA: Hydrops revisited: literature review of 1,414 cases published in the 1980s. *Am J Med Genet* 34:366-90, 1989.

Van Maldergem L, Jauniaux E, Fourneau C, et al: Genetic causes of hydrops fetalis. *Pediatrics* 89:81-6, 1992.

Cytogenetic studies

In general, cytogenetic studies should be performed on all fetuses with idiopathic hydrops, particularly if malformations are present. The common trisomies (18, 13, 21) can each present with hydrops and monosomy X (Turner syndrome) accounts for a sizeable fraction of severely hydropic female fetuses. In fetal Turner syndrome, edema is often very severe with formation of a massive nuchal cystic hygroma. Aortic coarctation, hypoplastic left heart malformation, and renal anomalies are also common with monosomy X.

The “Plumber’s” Approach to Fetal Hydrops

Most of the primary etiologies associated with fetal edema can be understood because of their direct or indirect effects on hydrostatic pressure in capillary beds. Much rarer are conditions that reduce oncotic pressure (hypoproteinemia, congenital nephrotic syndrome) or disrupt vascular/lymphatic integrity. Increased venous pressure can result from cardiac failure or venous obstruction. Cardiac failure may be primary (cardiomyopathy, heart malformation, myocarditis, arrhythmia) or secondary (anemia, hypervolemia, arteriovenous shunt). Many, but not all, of these etiologies have grossly recognizable findings. Tumors that compress great veins or are the substrate for arteriovenous shunts can lead to hydrops through more than one mechanism. Anastomoses between circulatory systems of monochorionic twins can lead to transfusion and volume overload of one twin fusion with secondary failure. Approaching the autopsy with causes of cardiac failure in mind is often helpful.

Freeze liver and placenta

I recommend routinely freezing liver and placenta from every fetal autopsy (see below). However, it is particularly important for fetal hydrops. Once more common causes of hydrops are excluded, metabolic disorders and hemoglobinopathies must be considered in the differential diagnosis. Hepatomegaly, splenomegaly, or cytopathological changes may suggest a metabolic disease (e.g. storage disease). If so, confirmation by biochemical or DNA analysis of frozen tissue can be performed. A fetus with profound extramedullary hematopoiesis and hydrops may have thalassemia, which could be confirmed by electrophoretic or DNA studies.

Cultures

Bacterial infections, excluding syphilis, are not generally associated with hydrops. Viral cultures, particularly for CMV, Herpes, or parvovirus, might be useful but are general not necessary since specific inclusions, immunohistochemistry, and serology are all available to establish the diagnosis. The incidence of viral myocarditis due to other agents is not well enough understood to know the value of cardiac viral cultures in this setting.

Maternal history and serology

Maternal history is important when dealing with any fetal case. However, it is particularly useful for hydropic fetuses since it may provide valuable clues to the specific etiology. Risk factors for infectious causes should be sought (e.g., exposure to children with erythema infectiosum, consumption of unprocessed undercooked meat). If a TORCH panel has not been obtained, it should be requested as soon as possible.

MOLECULAR TESTING AND FETAL PATHOLOGY

(Adapted from handout prepared originally for the Molecular Diagnostics and Pediatric Pathology Symposium, Spring 1998)

Advances in molecular genetics have had a growing impact on the field of fetal pathology. In some respects, rapid developments in molecular diagnostics and related disciplines have been a "two-edged sword" - providing objective methods to confirm specific diagnoses, but adding a layer of complexity to the practice of fetal pathology. Because the list of mutations responsible for recognizable patterns of human malformation grows at an exponential rate, it is impossible for even the most devoted practitioner to keep abreast of the candidate genes for specific conditions and / or perform comprehensive molecular testing for every candidate case and gene. Furthermore, the resources and expertise necessary to perform mutational analysis on specific genes often resides in one or a small number of research laboratories which are generally not established, nor necessarily interested, in performing such analysis as a diagnostic service. For these reasons, establishment and use of molecular diagnostic testing by most pathologist must be done prudently with the greatest attention given to preservation of tissue samples in a state where analysis can be performed in an outside laboratory at a later date, if desired.

The emphasis of the following sections is practical measures that may be utilized in the routine evaluation of fetal pathology specimens. Recommendations are given for specific diagnostic tests that should be accessible to all pathologists, and resources are described for more esoteric

analyses that which may be sought in specific instances. In the interest of space, medicolegal and ethical issues are not discussed, but some appropriate references are given as these topics may influence decisions the practice of fetal pathology.

What should be done routinely?

Molecular diagnostic tests include a variety of modalities, which each impose different requirements for tissue handling (Table 1).

Table 1: Handling Tissues for Molecular Diagnosis

Molecule	Type of Test	Example	Requirements
PROTEIN	Immunohistochemistry	Infections (CMV, parvovirus) Myopathy (typing, dystrophin) Neoplasms	Fixed; Frozen in OCT
	Electrophoresis	Thalassemia	Frozen; refridg. blood
	Western blot	HIV testing	Frozen; refridg. blood
	ELISA/RIA	TORCH serology	Frozen; refridg. blood
	HPLC	Amino acid metabolic defect	Frozen; refridg. blood
	GC-MS	Smith-Lemli Opitz syndrome	Frozen; refridg. blood
	Enzyme assay	Glycogen storage disease	Frozen; refridg. blood
	Protein processing	Osteogenesis imperfecta	Culture
DNA	Southern blot	Specific genes	Frozen; culture
	PCR	Specific mutations (CF, MEN 2B)	Frozen; culture > fixed
	FISH	Aneuploidy Deletion (VCFS)	Culture, touch prep > fixed
	Cytogenetics	Aneuploidy (Confined placental mosaicism)	Culture
	+ breakage study	Fanconi	
	+ puffing assay	Roberts	
	Flow cytometry	Triploidy / tetraploidy	Fresh; fixed
Comp. Genome Hyb.	Deletions, amplifications	Frozen	
RNA	RT-PCR +/- sequence	Specific genes (Achondroplasia)	Frozen (<12h) >> fixed
	Northern blot	Expression level, mutat. screen	Frozen (<12h)

Awareness of these requirements will influence the manner in which tissue samples are collected and stored. As a general rule, some unfixed tissue should be frozen in every case such that many of the analysis described above can be performed later, if desired. In practice, this can be accomplished by using 1.5 cc plastic vials which can be labeled directly with appropriate identifiers and stored in 5" x 5" x 2" freezer boxes which hold up to 100 tubes. Ideally, the tissue chosen should be as non-autolyzed as

possible. We routinely freeze a portion of liver from non-macerated fetuses, as it includes both DNA and many of the enzymes that might be assessed for storage diseases. In macerated cases, we freeze a shallow biopsy of placenta including villi which is procured from the fetal surface to minimize maternal contamination. Additional tissues are frozen only when indicated, i.e. RNA from a particular site of gene expression is required (i.e., congenital neoplasm). The optimal specimen is obtained immediately after delivery, snap-frozen, and stored in liquid nitrogen. However, the majority of specimens are obtained several hours to days after delivery and stored frozen at -70°C. Although RNA will degrade more rapidly, DNA from most organs is suitable for Southern blotting even 3-5 days after delivery (Larsen et al., 1992).

Reference

Larsen S, Rygaard K, Asnaes S, Spang-Thomsen M: Northern and Southern blot analysis of human RNA and DNA in autopsy material. *APMIS 100:498-02*, 1992.

What special tests need to be initiated when the fetus is examined?

Storage of frozen tissue will insure that an adequate sample is available for many types of molecular analyses that might not be considered or available until after the autopsy is done. However, some forms of molecular testing must be initiated at fetal examination, particularly assays that require cell culture (cytogenetics, FISH), histochemistry, or fresh tissue (flow cytometry). Each pathology group (often in conjunction with other specialists) needs to establish criteria which will be used to identify appropriate cases for these type of analyses, and procedures which facilitate timely procurement of specimens by trained personnel. Some specific issues to be considered are the following.

When should tissue be submitted for cytogenetic analysis?

Strict guidelines for the application of cytogenetic studies are not as readily available as one might think, given that the technique has been in existence for several decades. Never-the-less, general agreement seems to exist for karyotype analysis in two situations, recurrent abortion and multiple malformations. However, inconsistency exists regarding definition of these terms. Most clinicians and textbooks agree that cytogenetic studies are indicated with the third unexplained pregnancy loss. However, some sources suggest submission of tissue for karyotyping from the second such loss, particularly during the first trimester (Colwell et al, 1991). In practice, it is often prudent to encourage clinicians to make judgements on individual cases based on their knowledge of the patient's pregnancy history, anxiety levels, etc.. Similar confusion exists regarding how many and what type of malformations need be present to prompt cytogenetic analysis. Many geneticists and prenatal diagnosticians believe that cytogenetics are indicated if two or more "major" malformations exist. However, we observe inconsistencies in our own practice and those of our colleagues such that no standard has been established. While the "2-or-more" rule is a good starting point, flexibility is required in some cases.

Reference

Colwell KA, Wilson, RD: Recurrent pregnancy loss: genetic aspects. *BC Med J 33:593-5*, 1991. [The same issue contains multiple articles on the subject of "recurrent pregnancy loss."]

How do I obtain a cytogenetic sample from a macerated fetus?

If you are concerned that a fetal tissue sample will not yield viable cells for culture, a sample of placenta should be submitted for cytogenetic analysis. Even long after fetal demise, a significant fraction of embryo-derived cells in the placenta are kept alive by maternal perfusion. Sampling should be done just under the chorionic plate to avoid maternal contamination.

Velocardiofacial syndrome (VCFS) / DiGeorge Syndrome

1. Definition

VCFS is an autosomal dominant condition characterized by variable penetrance and expressivity. Anatomic findings include cleft palate (usually not lip), cardiac malformations (particularly interrupted aortic arch, truncus arteriosus, and other outflow tract anomalies), renal anomalies (hydronephrosis, unilateral agenesis), dysmorphic face (“long”, malar “flatness”, narrow orbital fissures, “square” nasal root, small mouth, retrognathia), neural tube defects, minor ear anomalies, slender hands/digits, short stature, microcephaly, umbilical hernia, scoliosis, cryptorchidism, and hypospadias. In addition, many patients have learning disabilities and major psychiatric morbidity (i.e. schizophrenia) is more common than in the normal population. Many individuals with VCFS also have hypocalcemia, T-cell dysfunction, and thymic hypoplasia indicative of phenotypic overlap with DiGeorge Syndrome.

2. Molecular Diagnosis

Both VCFS and DiGeorge Syndrome are very frequently associated with macro-, or more frequently, microdeletion at 22q11.2. These deletions can be demonstrated by fluorescence in situ hybridization (FISH) using commercially available probes specific for part of the deleted DNA. FISH can be performed on metaphase chromosomal preparations from cell cultures, or interphase nuclei from cell cultures, air-dried touch preparations, or nuclei extracted from paraffin-embedded tissue blocks. Metaphase preparations are easiest to interpret and offer the added information found in routine karyotypes, but are more costly. Interphase preparations can be performed more quickly, since cell culture is not required, but specifically address the issue of 22q11.2 deletions and provide no additional cytogenetic information. Recently, a strong candidate gene (designated “UFD1L”) for VCFS was identified. However, the genetics of VCFS/DiGeorge syndrome are seemingly complex and phenotypic variability may reflect contiguous gene mutations, rather than a single gene disorder (Scambler et al, 1999). The entire gene is deleted in patients with large deletions detectable by FISH with a 22q11.2 probe (see below), and a patient with the VCFS phenotype carried a small deletion of part of this gene that was not detectable by FISH.

3. What fetuses should have FISH analysis for 22q11.2 deletion?

The answer to this question is somewhat controversial and may change as more is learned about the spectrum of phenotypic presentations associated with this deletion. A number of studies have been published or are on-going to clarify the minimal phenotypic abnormalities which may present in 22q11.2 deletion patients and to determine the diagnostic yield for testing all patients with these anomalies. At present, the general consensus is that most patients will have at least 2 clinical findings (i.e. cardiac malformation and cleft palate). Unfortunately, one of the two may

be “unusual” facial features, hypocalcemia, or learning disabilities which are difficult or impossible to recognize in a fetus. However, any fetus with 2 or more findings suggestive of VCFS, or a single finding and a family history of such anomalies, should be tested.

What about individuals with apparently isolated cardiac malformations? No standard currently exists for this issue. Conflicting data exists regarding the association between isolated conotruncal malformations, and may reflect differences in ascertainment or clinical evaluation (Table 2). Given that other phenotypic features of VCFS may not be apparent in fetuses, and patients with conotruncal cardiac malformations are at a particularly high risk for a 22q11.2 deletion (see Table 3), testing all such cases for 22q11.2 deletions could be justified, but may be impractical. At a minimum, patients with interrupted aortic arches appear to be at exceedingly high risk and probably should all be evaluated.

Table 2: Frequency of 22q11.2 Deletions in “Non-syndromic” Conotruncal Malformations

Study	22q11.2 deletion
Goldmuntz et al, 1993	5/17 cases
Amati et al, 1995 (Tetralogy of Fallot)	0/107
Takahashi et al, 1995	0/59
Debrus et al, 1996	0/36
Fokstuen et al, 1998	0/59
Marino et al, 1999	1/304

Table 3: Frequency of 22q11.2 Deletions in Specific Cardiac Malformations^a

Cardiac Defect	22q11.2 deletion	Reference
“Conotruncal” malformations	7.8% (n=64)	Takahashi et al, 1995
Table 3, continued		
Transposition of Great Arteries	12.5% (n=32)	Melchionda et al, 1995
Tetralogy of Fallot	21% (n=33)	Trainer et al, 1996
Interrupted Aortic Arch	68% (n=161)	Van Mierop et al, 1986

^a Both isolated and cases with other anomalies.
n = total number of cases in study.

see also Frohn-Mulder et al, 1999

What about isolated cleft palate? Isolated cleft palate probably does not warrant FISH analysis for 22q11.2 deletion. A study of 33 postnatal individuals with isolated posterior cleft palate found no deletions, in contrast to 10 deletions detected in 12 patients with clinical VCFS, including cleft palate (Mingarelli et al, 1996).

4. How should samples be obtained?

Nuclei from any fetal tissue, including placenta, are the appropriate substrate for FISH analysis. If cytogenetic studies are indicated, then an additional request to perform 22q11.2 FISH should suffice. Non-placental tissue is preferable, particularly for female fetuses, to exclude the possibility of maternal contamination. But placental tissue is preferable if the fetus is macerated. If cytogenetic studies are not indicated, then FISH analysis can be performed on interphase nuclei from touch preps of any non-autolyzed fetal tissue. Thymus and spleen work well. The turn-around-time for such samples is usually short (less than 48 hours). FISH can also be performed retrospectively on interphase nuclei extracted from paraffin blocks. However, this is more labor intensive.

References

Amati F, Mari A, Digilio MC, et al: 22q11 deletions in isolated and syndromic patients with tetralogy of Fallot. *Hum Genet* 95:479-82, 1995.

Mingarelli R, Digilio MC, Mari A, et al: The search for hemizygoty at 22q11 in patients with isolated cleft palate. *J Craniofac Genet Dev Biol* 16:118-21, 1996.

Debrus S, Berger G, deMeeus A, et al: Familial non-syndromic conotruncal defects are not associated with a 22q11 microdeletion. *Hum Genet* 97:138-44, 1996.

Fokstuen S, Bottani A, medeiros PF, et al: 22q11.2 deletions in a series of patients with non-selective congenital heart defects: incidence, type of defects and parental origin. *Clin Genet* 53:63-9, 1998.

Frohn-Mulder IM, Wesby Swaay E, Bouwhuis C, et al: Chromosome 22q11 deletions in patients with selected outflow tract malformations. *Genet Counsel* 10:35-41,1999

Goldmuntz E, Driscoll D, Budarf ML, et al: Microdeletions of chromosomal region 22q11 in patients with congenital conotruncal cardiac defects. *J Med Genet* 30:807-12, 1993.

Johnson MC, Watson MS, Strauss AW: Chromosome 22q11 monosomy and the genetic basis of congenital heart disease. *J Pediatr* 129:1-3, 1996.

Lindsay EA, Greenberg F, Shaffer LG, et al: Submicroscopic deletions at 22q11.2: variability of the clinical picture and delineation of a commonly deleted region. *Am J Med Genet* 56:191-97, 1995.

- Marino B, Digilio MC, Toscano A, et al: Congenital heart defects in patients with DiGeorge/Velocardiofacial syndrome and del22q11. *Genet Counsel* 10:24-33, 1999.
- Mingarelli R, Digilio MC, Mari A, et al: The search for hemizyosity at 22q11 in patients with isolated cleft palate. *J Craniofac Genet Dev Biol* 16:118-121, 1996.
- Melchionda S, Digilio MC, Mingarelli R, et al: Transposition of the great arteries associated with deletion of chromosome 22q11. *Am J Cardiol* 75:95-98, 1995.
- Nickel RE, Magenis RE: Neural tube defects and deletions of 22q11. *Am J Med Genet* 66:25-7, 1996.
- Scambler PJ: Engineering a broken heart. *Nature* 401:335-6, 1999.
- Takahashi K, Kido S, Hoshino K, et al: Frequency of a 22q11 deletion in patients with conotruncal cardiac malformations: a prospective study. *Eur J Pediatr* 154:878-81, 1995.
- Trainer AH, Morrison N, Dunlop A, et al: Chromosome 22q11 microdeletions in tetralogy of Fallot. *Arch Dis Child* 74:2-63, 1996.
- Van Mierop LH, Kutsche LM: Cardiovascular anomalies in DiGeorge syndrome and importance of neural crest as a possible pathogenetic factor. *Am J Cardiol* 58:133-137, 1986.
- Yamagishi H, Garg V, Matsuoka R, Thomas T, Srivastava D: A molecular pathway revealing a genetic basis for human cardiac and craniofacial defects. *Science* 283:1158-61, 1999.

Fanconi Anemia

1. Definition

Fanconi anemia (FA) is a clinically and genetically heterogeneous disorder. The traditional features include pancytopenia, hyperpigmentation (generalized or cafe-au-lait spots), skeletal malformations, small stature, urogenital anomalies, and a high incidence of malignancies, primarily leukemia. However, penetrance for each of these findings is highly variable and over time other anomalies have been added to this list (Table 4). Those who practice fetal pathology must be acutely aware of this condition since two-thirds of FA patients exhibit one or more congenital malformation and the bone marrow disorder will not be evident in the fetus. Many of the clinical features of FA overlap with VACTERL association, a sporadic disorder. The distinction is critical since FA is an autosomal recessive. Evidence exists for mutations in eight different genetic loci which can lead to FA. Accurate timely diagnosis has important consequences for living patients and their siblings.

Table 4: Congenital Anomalies in Fanconi Anemia

Anomaly	Frequency in FA population ^a
---------	---

Radial ray defects	49 % (found in 74% of those with malformations; 27% unilateral)
Renal	49 %
Heart	13 %
Other skeletal anomalies	
Hip dislocation	6 %
Vertebral	3 %
Scoliosis	3 %
Many others	< 1%
Anorectal	5 %
Duodenal atresia	5 %
Hydrocephalus	5 %
Tracheoesophageal fistula	3 %

^a Based on Giampietro et al., 1993

2. Molecular Diagnosis

A well-established sensitive and relatively specific method to confirm the diagnosis of FA is demonstration of hypersensitivity a patient's cells to clastogenic agents in vitro. Clastogenic agents (diepoxybutane, mitomycin C) cause DNA cross-linking and lead to chromosomal breaks which can be resolved cytogenetically. More breaks occur in cells from FA patients than in normal cells. Most cytogenetic laboratories are competent at this analysis. In order to perform this assay, cells must be cultured for cytogenetic analysis and a subset treated with the clastogenic drug. Therefore, a fresh fetal tissue sample is required.

Eight "complementation groups" (probably distinct genetic loci) have been identified for FA (Joenje et al, 1997). The genes corresponding to three of these, FACA, FACC, and FACG, have been cloned and mutational analysis has been reported for some patients. A small number of research laboratories have the ability to analyze DNA from FA patients for mutations in one or both of these genes. In principle, such analysis could be conducted retrospectively from frozen tissue, stored DNA, or even from parents. However, at this time, such analysis is not commercially available and would have to be negotiated with the scientists involved. Such analysis is fairly labor intensive since many different types of mutations need to be excluded. Therefore, it is best to perform clastogenic hypersensitivity assays when FA is considered.

3. When should molecular tests be performed for FA?

No standard of practice has been established. Most geneticists would agree that any fetus with a radial ray reduction defect (i.e. radial agenesis, absent thumb) should be evaluated for FA, regardless of other anomalies, unless another clear cause of the limb anomaly exists (i.e. amniotic band). The results of clastogenic hypersensitivity assays on fetuses with other isolated anomalies from Table 3 have not been reported, but the yield would probably be low. Testing should be strongly considered in a fetus without a radial ray defect, but with multiple other anomalies in Table 3 (i.e. VACTERL association).

4. How should samples be obtained?

Any non-autolyzed fetal tissue, including placental fibroblasts, can be used to grow cells for clastogenic hypersensitivity studies. Tissues should be taken as soon as possible, under as sterile conditions as possible, and transported to the cytogenetics laboratory in appropriate medium. As clastogenic hypersensitivity studies are not routinely done on all cytogenetic samples, they need to be specifically requested. Clastogenic studies are quantitative assays that require significant experience to perform and interpret. Workers in a given laboratory may not be comfortable using fetal fibroblasts or other cell types in these assays, particularly if appropriate normative data have not been collected from similar cell types.

References

D'Andrea AD, Grompe M: Molecular biology of Fanconi anemia: implications for diagnosis and therapy. *Blood* 90:1725-36, 1997.

Giampietro et al: The need for more accurate and timely diagnosis in Fanconi anemia: a report from the International Fanconi Anemia Registry. *Pediatrics* 91:1116-20, 1993.

Glanz A, Fraser FC: Spectrum of anomalies in Fanconi anemia. *J Med Genet* 19:412-6, 1982.

Joenje H, Oostra AB, Wijker M, et al: Evidence for at least eight Fanconi anemia genes. *Am J Hum Genet* 61:940-4, 1997.

Confined Placental Mosaicism

1. Definition

Dagmar Kalousek (British Columbia Children's Hospital, Vancouver) provided valuable input for this section of the handout.

Placental mosaicism refers a mixture of cytogenetically normal and abnormal cells in the same placenta. The abnormal genotype may also extend to some or all cells in the fetus or be confined to the placenta (confined placental mosaicism, CPM). Frequently, CPM is due to the presence of a trisomic cell population (see Kalousek, 1994 for an excellent review). CPM is common, detectable in 1-2% of chorionic villus samples, and poses an increased risk for pregnancy loss (22%), intrauterine growth retardation, and preterm labor. Trisomies for almost every autosome have been described in CPM, although the individual rates for specific chromosomes vary and IUGR may be associated with a finite subset.

2. Molecular Diagnosis

Placental mosaicism can be evaluated by cytogenetic studies, fluorescence in situ hybridization, or comparative genomic hybridization. FISH techniques have been developed to evaluate all autosomes simultaneously in metaphase preparations or interphase nuclei. In addition, comparative genomic hybridization (CGH), using DNA which can be extracted from fresh or frozen tissue, will reveal most unbalanced cytogenetic abnormalities. These approaches are less expensive than conventional cytogenetics and may be commonplace in coming years, but at

present, most laboratories do not perform CGH and are only set up to evaluate a small number of autosomes by FISH on a given sample. Therefore, traditional cytogenetic studies are the most common diagnostic modality at present. Cytogenetic studies can either be performed on “direct preparations” of chorionic villi or cell cultures. The former allow analysis of both trophoblast and stromal cells, whereas the latter reflect only cells derived from villous stroma. Although the information obtained from both direct and culture preparations furthers our understanding of CPM, its origins, and its impact on gestation, karyotypes from cell cultures appear to be a sensitive indicator of clinically relevant aneuploidy at term. In their cell culture analysis of 34 term placentae from pregnancies complicated with CPM on chorionic villus sampling in the first trimester, Kalousek and colleagues demonstrated identical mosaicism in 17, a subset which included all 6 cases of intrauterine growth retardation (Kalousek et al., 1991).

3. When should placental tissue be submitted for cytogenetic analysis for placental mosaicism?

It is clear that CPM poses a markedly increased risk (22%) for either IUGR or spontaneous second and third trimester fetal demise. On this basis, placental cytogenetics could be advocated in all such cases. In practice, most groups reserved these studies for cases of IUGR, because the yield in losses without IUGR has not been established but is probably low. The presence of placental lesions which might produce IUGR (i.e., infarcts) should not discourage studies for CPM since placental insufficiency seems to be part of the pathogenesis in at least some cases.

4. How should samples be obtained?

Placental villi should be sampled from at least 2 sites. These samples should be taken as soon as possible after delivery with as sterile technique as possible. Tissue should be dissected from just beneath the chorionic plate at a shallow depth to minimize the risk of maternal contamination, and placed in appropriate transport medium for routine cytogenetic studies. Cytogenetic studies requires fresh tissue. Although it is possible to evaluate specific chromosomes by FISH in interphase nuclei extracted from paraffin, it is impractical to use this approach to analyze all possible trisomies. In the near future, methodology will probably exist to perform such analysis routinely.

References

Hahnemann JM, Vejerslev LO: European collaborative research on mosaicism in CVS (EUCROMIC) - Fetal and extrafetal cell lineages in 192 gestations with CVS mosaicism involving single autosomal trisomy. *70:179-87, 1997.*

Kalousek DK et al: Confirmation of CVS mosaicism in term placentae and high frequency of intrauterine growth retardation association with confined placental mosaicism. *Prenatal Diagn 11:743-50, 1991.*

Kalousek DK, Barrett I: Confined placental mosaicism and stillbirth. *Pediatr Pathol 14:151-9, 1994.*

Fetal Akinesia Syndromes

Decreased fetal movement in utero is associated with a pattern of anomalies that vary in severity, but usually include multiple arthrogryposes. A variety of terms, e.g. "multiple arthrogryposes complex", "lethal multiple pterygia", and "Pena-Shokeir phenotype", have been applied to fetuses with variations of these findings. The underlying etiologies in each case are diverse, but include musculoskeletal and neuropathic disorders. As such, a comprehensive evaluation of skeletal muscle has been recommended for fetuses with signs of severe fetal akinesia. As part of the diagnostic evaluation, frozen sections of skeletal muscle should be prepared and studied histochemically, in the same manner as post-natal muscle biopsies are evaluated. As the histochemical studies rely on endogenous enzyme activities, prompt sampling and freezing is important to prevent false negative results. Electron microscopy, which may be of value in some cases, also requires prompt sampling and fixation for optimal results.

Triploidy

Triploidy may be suggested by specific fetal and/or placental anomalies. In particular, partial molar change in the placenta is a strong, but not specific, indication of triploidy. The diagnosis can be confirmed by a number of tests, but flow cytometric analysis affords a relatively inexpensive and rapid method. Placental tissue works well, particularly if the fetus is autolyzed. The presence of maternal contamination provides an internal control for diploid DNA content, but will obscure true diploid/triploid mosaicism.

Resources

1. How can I locate a laboratory which may offer molecular testing for a specific genetic disorder?

At present, we are not aware of a single reference source for molecular testing sites. However, two resources, GeneTests™ and GeneClinics™ are available at the University of Washington and Children’s Hospital and Regional Medical Center, Seattle, WA. "GeneTests is a genetic testing resource that includes a genetics laboratory directory, a resource list of genetics clinics, and an introduction to genetics counseling and testing concepts." "GeneClinics is a medical knowledge base relating genetic testing to the diagnosis, management, and genetic counseling of individuals and families with specific inherited disorders." Both services are free to health care providers, but require a one-time registration. They can be accessed as indicated in Table 5.

Table 5: Selected Internet Resources

	GeneTests™	GeneClinics™
Internet	http://www.genetests.org	http://www.geneclinics.org
Phone	(206) 527-5742	(206) 221-4674
FAX	(206) 527-5743	(206) 221-4679
e-mail	genetests@genetests.org	geneclinics@geneclinics.org

Services of the University of Washington (GeneTests™, GeneClinics™) and

Children's Hospital and Regional Medical Center (GeneTests™), Seattle, WA.

In addition to these programs, the Internet offers many Websites sponsored by groups or individuals who are interested in specific diseases. Some of these refer to laboratories which offer testing for specific conditions. A catalog of genetic syndromes with updated references is available at no cost through On-line Mendelian Inheritance in Man (OMIM, <http://www3.ncbi.nlm.nih.gov/omim/>). In general, OMIM does not provide specific testing sites, but often discusses the potential for molecular testing and gives references that can be used to contact experts in the field.

Finally, the E-mail list server (PEDPATH@u.washington.edu) initiated by the member of the Society for Pediatric Pathology provides an opportunity to present cases to pediatric pathologists around the world.

Ethical Issues and Molecular Diagnosis

This handout does not address the difficult ethical issues raised by some molecular diagnostic tests and the manner in which they are applied. The implications of some forms of testing, possible need for informed consent, and differences between “diagnostic” and “research” utilization of fetal tissues have been discussed at length in the academic literature and lay press. Although universal standards have not been established to deal with these issues, pathologists need to be aware of these concerns and their medical-legal implications.

References

Anonymous: ACMG Statement. Statement on storage and use of genetic materials. *Am J Hum Genet* 57:1499-1500, 1995.

Clayton EW, Steinberg KK, Khoury MJ, et al: Informed consent for genetic research on stored tissue samples. *JAMA* 274:1786-92, 1995.

Durfy SJ: Ethics and the human genome project. *Arch Pathol Lab Med* 117:466-9, 1993.

Geller G, Botkin JR, Green MJ, et al: Genetic testing for susceptibility to adult-onset cancer: the process and content of informed consent. *JAMA* 277:1467-74, 1997.

ADDITIONAL REFERENCES

Addis and Gray: Body size and organ weight. *Growth* 14:49-80, 1950.

Addis and Gray.: Body size and gonadal weight. *Growth* 14:81-92, 1950.

Benjamin DR, Juul S, Siebert JR: Congenital posterolateral diaphragmatic hernia: Associated malformations. *J Pediatr Surg* 23:899-903, 1988.

Benirschke K and Kaufmann P: *Pathology of the Human Placenta* (third edition). Springer-Verlag, New York, 1997.

Bergsma D: *Birth Defects Atlas and Compendium*. The Williams and Wilkins Co., Baltimore, 1973.

Bernstein J: A classification of renal cysts. In, *The Cystic Kidney*, eds KD Gardner and J Bernstein, Kluwer Academic Publishers, Dordrecht, 1990, pp. 147-170.

Bosma JF: *Anatomy of the Infant Head*. The Johns Hopkins University Press, Baltimore, 1986.

Boyd: Weight of the thymus. *Am J Dis Child* 43:1162-1214, 1932.

Chan A et al: Prevalence of neural tube defects in South Australia, 1966-91: effectiveness and impact of prenatal diagnosis. *BMJ* 307:703-706, 1993.

Cohen MM Jr: *The Child with Multiple Birth Defects*. Raven Press, New York, 1982.

Cohen MM Jr: *Craniosynostosis: Diagnosis, Evaluation, and Management*. Raven Press, New York, 1986.

Coppoletta JM and Wolbach SB: Body length and organ weights of infants and children: Study of body lengths and normal weights of more important vital organs of body between birth and 12 years of age. *Am J Pathol* 9:55-70, 1933.

Craft H and Brazy JE: Autopsy: High yield in neonatal population. *AJDC* 140:1260-1262, 1986.

Crelin ES: *Functional Anatomy of the Newborn*. Yale University Press, New Haven, 1973.

Currarino G, Williams B, and Dana K: Kidney length correlated with age: Normal values in children. *Radiology* 150:703-704, 1984.

de la Cruz MV, et al: A qualitative and quantitative study of the ventricles and great vessels of normal children. *Am Heart J* 60:675-690, 1960.

DeSa DJ: Intimal cushions in foetal placental veins. *J Path* 110:347-352, 1973.

- Dimmick JE and Kalousek DK: *Developmental Pathology of the Embryo & Fetus*. J.B. Lippincott Company, Philadelphia, 1992.
- Emery JH and Mithall A: The number of alveoli in the terminal respiratory unit of man during late intrauterine life and childhood. *Arch Dis Child* 35:544-547, 1960.
- Fitzsimmons J et al: Long-bone growth in fetuses with Down syndrome. *Am J Obstet Gynecol* 161:1174-1177, 1989.
- Fox H: *Pathology of the Placenta* (second edition). Vol. 7, Major Problems in Pathology, W.B. Saunders Company Limited, London, 1997.
- Genest DR et al: Estimating the time of death in stillborn fetuses: I. Histologic evaluation of fetal organs; an autopsy study of 150 stillborns. *Obstet Gynecol* 80:575-584, 1992.
- Genest DR: Estimating the time of death in stillborn fetuses: II. Histologic evaluation of the placenta: a study of 71 stillborns. *Obstet Gynecol* 80:585-592, 1992.
- Genest DR and Singer DR: Estimating the time of death in stillborn fetuses: III. External fetal examination: a study of 86 stillborns. *Obstet Gynecol* 80:593-600, 1992.
- Gilbert SF: *Developmental Biology* (fifth edition). Sinauer Associates, Inc., Sunderland, Mass., 1997.
- Golbus MS and Berry LC Jr: Human fetal development between 90 and 170 days postmenses. *Teratology* 15:103-108, 1977.
- Goodman RM and Gorlin RJ: *The Malformed Infant and Child*. Oxford University Press, New York, 1983.
- Gorlin RJ, Cohen MM Jr, and Levin LS: *Syndromes of the Head and Neck* (third edition). Oxford University Press, New York, 1990.
- Gruenwald P and Minh HN: Evaluation of body and organ weights in perinatal pathology. *Amer J Clin Path* 34:247-253, 1960.
- Hall JG, Froster-Iskenius UG, and Allanson JE: *Handbook of Normal Physical Measurements*. Oxford University Press, Oxford, 1989.
- Hoda RS and Kahn EL: Diagnostic yield in autopsies of stillborns: A ten year retrospective study in a university hospital [abstract]. *Am J Clin Path* 95:279, 1991.
- Iffy et al: The rate of growth in young human embryos of Streeter's horizons XIII to XXIII. *Acta Anat* 66:178-186, 1967.
- Jauniaux E et al: Embryonic remnants of the umbilical cord: Morphologic and clinical aspects. *Human Path* 20:458-462, 1989.

Jones KL: *Smith's Recognizable Patterns of Human Malformation* (fifth edition). WB Saunders Co., Philadelphia, 1997.

Kalousek DK et al: *Pathology of the Human Embryo and Previabile Embryo: An Atlas*. Springer-Verlag, New York, 1990.

Keeling JW: *Fetal Pathology*. Churchill Livingstone, Edinburgh, 1994.

Keeling JW (ed): *Fetal and Neonatal Pathology* (second edition). Springer-Verlag, London, 1993.

Khang-Cheng H et al: Analysis of brain weight (Part I and II). *Arch Pathol Lab Med* 104:635-645, 1980.

Khoury MJ, Moore CA, Evans JA: On the use of the term "syndrome" in clinical genetics and birth defects epidemiology. *Am J Med Genet* 49:26-28, 1994.

Kissane JM: *Pathology of Infancy and Childhood* (second edition), The CV Mosby Co., St. Louis, 1975.

Lassau JP, Bastian D, Cabanis EA, Pourcelot L: *Atlas of Neonatal Anatomy: Correlation of gross anatomy, computed tomography and ultrasonography*. Masson, Paris, 1982.

Lemire RJ et al: *Normal and Abnormal Development of the Human Nervous System*. Harper and Row, Hagerstown, 1975.

Lemire RJ, Beckwith, and Warkany J: *Anencephaly*. Raven Press, New York, 1978.

Linn S et al: No association between coffee consumption and adverse outcomes of pregnancy. *N Engl J Med* 306: 141-145, 1982.

Lubinsky M: Vater and other associations: historical perspectives and modern interpretations. *Am J Med Genet Suppl* 2:9-16, 1986.

Macpherson TA et al: Perinatal mortality and morbidity: The role of the anatomical pathologist. *Seminars in Perinatology* 10:179-186, 1986.

Macpherson TA and Valdes-Dapena M: The Perinatal Autopsy, in *Textbook of Fetal and Perinatal Pathology* (second edition). eds JS Wigglesworth and DB Singer, Blackwell Science, Malden, Mass, 1998, pp. 87-110.

Martinez-Frias ML et al: Primary midline developmental field. II. Clinical/epidemiological analysis of alteration of laterality (normal body symmetry and asymmetry). *Am J Med Genet* 56:382-385, 1995.

McKusick VA: *Mendelian Inheritance in Man – Catalogs of Autosomal Dominant, Autosomal Recessive, and X-linked Phenotypes*. Tenth edition. The Johns Hopkins University Press, Baltimore, 1992.

McKusick VM: Polycystic Kidneys (Adult Polycystic Kidney Disease), On-line version of *Mendelian Inheritance in Man* updated by Welsh Medical Library, Johns Hopkins University, MD, MIM # 173900.

McKusick VA: *Mendelian Inheritance in Man: A Catalog of Human Genes and Genetic Disorders*. The Johns Hopkins University Press, Baltimore, 1998.

Moore KL and Persaud TVN: *Before We Are Born: Essentials of Embryology and Birth Defects*. W.B. Saunders Company, Philadelphia, 1998.

Mueller RF et al: Evaluation of a protocol for postmortem examination of stillbirths. *N Engl J Med* 309:586-590, 1983.

Myers J and Segal RJ: Weight of the spleen. I. Range of normal in a nonhospital population. *Arch Path* 98: 33-35, 1974.

Naeye RL: Do placental weights have clinical significance? *Hum Pathol* 18:387-391, 1987.

Naeye RL: *Disorders of the Placenta, Fetus, and Neonate: Diagnosis and Clinical Significance*. Mosby Year Book, St. Louis, 1992.

Nishimura H et al: Normal and abnormal development of human embryos: First report of the analysis of 1,213 intact embryos. *Teratology* 1: 281-290, 1968.

Ochs RH et al: Perinatal autopsies: A challenge for the nonpediatric pathologist. *Path Ann* 23:235-255, 1988.

Opitz JM: The developmental field concept. *Am J Med Genet* 21:1-11, 1985.

O'Rahilly R and Müller F: *Developmental Stages in Human Embryos*. Carnegie Institution of Washington, Washington, D.C., 1987.

O'Rahilly R and Müller F: *Human Embryology and Teratology*. Wiley-Liss, New York, 1992.

Pauli RM et al: Wisconsin Stillbirth Service Program: I. Establishment and assessment of a community-based program for etiologic investigation of intrauterine deaths. *Am J Med Genet* 50:116-134, 1994.

Pauli RM, Reiser CA: Wisconsin Stillbirth Service Program: II. Analysis of diagnoses and diagnostic categories in the first 1000 referrals. *Am J Med Genet* 50:135-153, 1994.

Persaud TVN, Chidley AE, Skalko RG: *Basic Concepts in Teratology*. Alan R. Liss, Inc., New York, 1985.

Reed GB, Claireaux AE, and Bain AD (eds): *Diseases of the Fetus and Newborn: Pathology, radiology, and genetics*. The C.V. Mosby Company, St. Louis, 1989.

- Sadler TW: *Langman's Medical Embryology*. (sixth edition) The Williams and Wilkins Company, Baltimore, 1990.
- Sawyer DR: Perinatal bereavement: The photographer's role in infant death. *J Biolog Photog* 66:35-37, 1998.
- Schulz DM and Giordano DA: Hearts of infants and children. *Arch Path* 74:464-471, 1962.
- Schulz DM, Giordano DA, and Schulz DH: Weights of organs of fetuses and infants. *Arch Pathol* 74:244-250, 1962.
- Shankle WR, Landing BH, and Gregg J: Normal organ weights of infants and children: Graphs of values by age, with confidence intervals. *Pediatr Pathol* 1:399-408, 1983.
- Siebert JR: Small-intestinal length in infants and children. *Am J Dis Child* 134:593-595, 1980.
- Siebert JR et al: *Holoprosencephaly: An Overview and Atlas of Cases*. Wiley-Liss, New York, 1990.
- Siebert JR, Benjamin DR, Juul S, Glick PL: Urinary tract anomalies associated with congenital diaphragmatic defects. *Am J Med Genet* 37:1-5, 1990.
- Sperber GH: *Craniofacial Embryology*. Third edition. Wright PSG, Bristol, 1981.
- Spranger J et al: Errors of morphogenesis: concepts and terms. Recommendations of an international working group. *J Pediatr* 100:160-165, 1982.
- Stalsberg H: The thymus in infants dead from acute disorders. *Acta Path Microbiol Scand* 79:37-42, 1971.
- Stevenson RE, Hall JG, and Goodman RM: *Human Malformations and Related Anomalies* (2 vols). Oxford University Press, New York, 1993.
- Tähkä H: The weight of the thymus in children of 0-2 years of age. *Acta Pediatr* 40:469-485, 1951.
- Tähkä H: Weight of adrenal gland. *Acta Pediatr Scand* 40:1 (Suppl 81), 1951.
- Tanimura T et al: Weight standards for organs from early human fetuses. *Anat Rec* 171:227-236, 1971.
- Taussig HB: *Congenital Malformations of the Heart* (two volumes). Harvard University Press, Cambridge, 1960.
- Taylor JR: Growth of human intervertebral discs and vertebral bodies. *J Anat* 120:49, 195_.
- Usher R and McLean F: Intrauterine growth grids. *J Pediatr* 74:901, 1969.

Valdes-Dapena M and Huff DS: *Perinatal Autopsy Manual*. Armed Forces Institute of Pathology, Washington, 1983.

Warkany J: *Congenital Malformations*. Year Book Medical Publishers, New York, 1971.

Warkany J, Lemire RJ, Cohen MM Jr: *Mental Retardation and Malformations of the Central Nervous System*. Year Book Medical Publishers, Inc., Chicago, 1981.

Wigglesworth JS: *Perinatal Pathology*. W.B. Saunders Co., Philadelphia, 1984.

Wigglesworth JS and Singer DB: *Textbook of Fetal and Perinatal Pathology* (second edition). Blackwell Scientific Publications, Oxford, 1998.

Winter RM, Knowles SAS, Bieber FR, Baraitser M: *The Malformed Fetus and Stillbirth: A Diagnostic Approach*. John Wiley & Sons, Chichester, 1988.

Zak R: *Growth of the Heart in Health and Disease*. Raven Press, New York, 1984.