Pathologic examination of the products of embryos and fetuses, both from spontaneous abortions (SAbs) and terminations of pregnancy, has become increasingly important. While such examination was once performed primarily for the purpose of furthering scientific understanding of prenatal human development, the practical medical applications of this knowledge have become clear and now form an integral part of the medical assessment and management of fertility issues (1–5). As an understanding of the factors involved in successful pregnancies has developed and as patient demand for information has increased, the role of pathologic examination has grown. Increased use of assisted fertilization techniques has heightened the interest of physicians and patients alike in understanding why pregnancies fail. This chapter will address the examination of disorders encountered in those pregnancies that end spontaneously in the first and second trimesters of gestation; the pathology of fetuses delivered after prenatal ultrasound diagnosis is beyond the scope of this chapter.

It is recognized that many conceptions do not end in live births but, rather, that there is a high rate of spontaneous loss, especially early in gestation. It is estimated that 10% to 20% of recognized pregnancies end as SAbs, with most losses occurring in the first trimester (first 12 to 14 weeks of gestation). With the demonstration of fetal cardiac activity, the miscarriage rate drops to approximately 3% to 12% (6). In a study of women with a normal prenatal visit at 6 to 11 weeks of gestational age (GA), the risk of subsequent SAb was 1.6% or less, considerably lower than for pregnancies overall (7). After the first trimester, approximately 1% to 2% of pregnancies are spontaneously aborted (8). The incidence of stillbirth at term gestation is in the order of 0.1% to 0.5%. These loss rates that persist throughout gestation, together with changing or changed societal approaches and expectations of pregnancy such as delaying childbearing until later in a woman’s reproductive life and increased access to assisted reproduction methods, have led to a tense in understanding the cause of pregnancy loss and the implications for future reproductive success.

GA refers to the number of weeks since the last menstrual period (equivalent to menstrual dates), while developmental age (DA) or conceptual age (CA) refers to the age as determined from the time of fertilization, generally considered as approximately 2 weeks after the last menstrual period. Embryos are assessed by developmental features that correlate with age, usually given as DA. Thus, in a normal gestation, GA is DA plus 2 weeks.

The first trimester of pregnancy is the period of implantation and embryogenesis, with the completion of embryogenesis by 8 weeks of DA (10 weeks of GA). Upon completion of embryogenesis with development of all organ systems, the conceptus is referred to as a fetus. Definitions of fetus and infant vary with locale; in Canada, a fetus is considered an infant once it has reached the GA of 20 weeks or is liveborn at any GA. In the United States, by contrast, the living intrauterine conceptus is referred to as a fetus until the time of live birth. Stillbirth is defined as delivery of a deceased conceptus at or after 20 weeks of GA.

CAUSES OF EARLY SPONTANEOUS ABORTION

It is well recognized that the major causes of early SAb are lethal chromosomal abnormalities, which are usually aneuploid (any karyotype that deviates from two haploid sets of 22 autosomes and one sex chromosome, with each haploid set contributed by one parent) (9). The use of techniques such as comparative genomic hybridization (CGH) and quantitative fluorescence-polymerase chain reaction (QF-PCR) to supplement conventional cytogenetic analysis has increased the detection of chromosome abnormalities because these

*Based upon the prior edition chapter by Deborah E. McFadden
techniques do not require cell culture and can address the issue of free maternal cell contamination. Numerous studies have shown that at least half of early SAbs are chromosomally abnormal when routine cytogenetics is performed. Array CGH may be used in cases in which tissue culture for cytogenetic analysis has failed or in which maternal cell contamination is suspected (10–12). More recent studies of preimplantation blastomeres in in vitro fertilization (IVF) patients have shown that even in young healthy co-uties, fewer than 10% of embryos have a normal karyotype in all blastomeres (13), although it is unclear if this is due to IVF or reflects a typical state of chromosomal instability in early embryonic life.

The distribution of chromosome abnormalities is consistent between various studies, with trisomy accounting for half of all chromosomally abnormal SAbs, polyplioidies and monoplioidies (primarily monosomy X) each accounting for 10% to 15%, and structural rearrangements accounting for approximately 5% (14,15). Trisomy for two chromosomes (double trisomy) is seen in only 3% of cases. The chromosomal abnormalities are a result of a subgroup bcause of unidentified factors that have arisen from a parent who carries a rearrangement predisposing to an unbalanced karyotype in the offspring. Studies of couples who have had recurrent miscarriages show that 5% have balanced rearrangements in the chromosomes involved (16). These in individuals have an increased risk of conceiving chromosomally abnormal pregnancies as well as recurrent miscarriage (17), with the actual risk depending on the type of rearrangement and the chromosomes involved (18). Although the presence of a balanced translocation predicts a risk of increased miscarriage in future pregnancies, the risk of a liveborn infant with a translocation is extremely low, and the proposed benefit of preimplantation genetic diagnosis is to shorten the interval between pregnancies, in order to achieve a successful outcome (19) rather than to improve the overall live birth rate.

With respect to trisomic conception, the most common subset of nonviable aneuploidies, studies of parental origin have dem onstrated that the largest proportion of these is maternally derived. There is a strong positive correlation with maternal age, meaning that the risk of conceiving a trisomic conceptus rises with increasing maternal age (20). Hypotheses to explain this are generally in accordance with a normal chromosomal crossover, altered chromatin cohesion, and altered DNA damage checkpoints. Trisomy affects 3% of pregnancies in 25-year-old women but occurs in 35% of pregnancies in women aged 42 years (21). Most trisomies are the result of errors in meiosis I (22), although this varies for individual chromosomes. For example, trisomy 18 is more typically the result of errors in the second meiotic division, while trisomy 21 is predominantly the result of a trisomy 21 meiotic division errors.

With the p redominant o f SA bs atribute to a neuroployd, it is clear that in order to ensure clinical relevance of SA b e valuations, t he ex amination s hould in clude determination of karyotype, particularly in recurrent pregnancy loss. In the event that the examination proves normal, with normal karyotype and normal villus histology, management of persons having recurrent SAbs shifts and other etiologies for pregnancy loss must be considered (23). The investigation of those who have had chromosomally normal miscarriages with no other pathology is dependent upon the proposed nonchromosomal mechanisms of pregnancy loss. These proposed associations in include exogenous environmental exposures, uterine abnormalities (24), skewed X-inactivation (25,26), disorders of function, immune disorders including conditions with autoantibodies, and thrombophilic conditions (15). The roles of these factors in miscarriage remain under investigation, and the relative significance of each has not been established with certainty. Prospective cohort studies have shown an association between single inherited thrombophilia mutations and miscarriage, a thrombosis m ultiple m utations m ay increases r isk (27–30). Prophylactic anticoagulant treatment has not been shown to improve outcomes in patients with recurrent miscarriages and in hereditary thrombophilias, and screens for these conditions are not currently recommended (31), a finding that p ractice r emains co ntroversial (30). There is less controversy about the role of antiphospholipid antibody in recurrent pregnancy loss. A nti phospholipid antibodies are found more often in women who have recurrent miscarriages (RSAb) than in other women, although the mechanism by which an antiphospholipid antibody causes pregnancy loss is not definitively known. It is speculated that it may be due to interference with trophoblast function and thus the establishment of successful implantation, mediated by early-onset inadequate decidual arteriolar perfusion, and/or to maternal/gestational interface inflammation (32). There are no specific pathologic features iden ti fied in these conditions (33–35). Current guidelines recommend screening in patients with prior venous thromboembolism, with one fetal loss or with multiple early miscarriages. T reatment w ith anticoagulation p rophylactic anticoagulation may improve outcome in these patients (36). With changing reproductive patterns, such as women starting their families later in life and having fewer children, there is enhanced societal desire to diagnose and manage causes of miscarriage. Given that cytogenetic abnormalities account for the majority of第一 trimester abortions, it has been suggested that the evaluation of first trimester SAb with cytogenetic analysis may prove more cost effective than a standard battery of tests such as thyroid function tests, endometrial biopsy, or thrombophilia testing (37). Pathologic examination also serves to identify those conditions not associated with abnormal karyotype that may require additional investigation or treatment to increase the likelihood of successful...
pregnancy and to diagnose conditions in which there is a risk of neoplasia, as with molar gestations and their attendant risk of transformation to gestational trophoblastic neoplasia (GTN) (1).

**FIRST TRIMESTER SPONTANEOUS ABORTION**

**Indication for Cytogenetic Analysis**

Given that chromosome abnormality accounts for the majority of first trimester SAs, an argument could be made for performing cytogenetic analysis on all cases. To assist in the reproductive counseling regarding cause of the SAb and risks for recurrence, karyotype is a vital piece of information. Where embryo-pathology examination was once performed only in cases of recurrent SAs, changes in reproductive patterns and practices have altered, resulting in a broader range of cases referred for embryo-pathologic examination. Those who treat women who have had previous miscarriages are anxious to know whether the pregnancy failure was the result of chromosome abnormality or if there is perhaps another etiology necessitating further investigation. Increasingly, assisted reproductive technologies (ARTs) such as IVF or ICSI are utilized. There are concerns that ARTs are associated with an increased incidence of chromosome abnormalities at prenatal diagnosis and at birth, specifically for sex chromosome abnormalities in pregnancies that are the result of ICSI (38,39). However, many small studies have found a high rate of chromosomal abnormalities in both ART and naturally conceived miscarriages, and a recent meta-analysis of case-controlled studies of chromosomal abnormalities in first trimester miscarriages showed no significant differences between either IVF or ICSI and natural conception, with the risk of abnormalities increased in older women in all groups (40).

Until the natural history of ART pregnancies is delineated, the use of these technologies should be considered as a non-indication for cytogenetic analysis in cases of SAb. In some laboratories, the fact that the majority of first trimester SAs are the result of chromosome abnormality is sufficient indication to perform cytogenetic analysis of all cases examined morphologically. In other laboratories, constraints imposed by funding structures may impose the necessity of specific clinical indication before cytogenetic studies will be funded. These other indications include a history of recent miscarriages (variably defined as two to three or more losses), abnormal villus morphology, abnormal or normal embryo, parental chromosome rearrangement, and maternal age 35 years or greater.

**Examination**

Examination of the early pregnancy loss or embryo specimen is quite different from that of a fetal specimen as the latter represents an autopsy examination of a fetus and its placenta. Examination of the products of an early pregnancy loss (spontaneous or missed abortion) is performed to identify pregnancy-related tissue (embryonic and/or placental tissue) to confirm intrauterine pregnancy and to assess their morphology. This examination includes sampling of tissues for additional studies, including for cytogenetic analysis or other means of determining the chromosome complement of the conceptus. Thus, it is imperative that all specimens for embryo-pathology examination are submitted in the fresh rather than fixed state.

An assessment of the products of conception is accomplished by examining the specimen under a dissecting microscope, ideally equipped with a camera. The presence of any embryonic or placental tissue, in cluding implantation site, allows confirmation of intrauterine pregnancy. In the absence of pregnancy-related tissues, intrauterine pregnancy cannot be confirmed, and the report must reflect that. Decidualized endometrium may be seen in a trophoblastic effect, in cluding with an ectopic pregnancy, and is therefore in sufficient for confirmation of intrauterine pregnancy.

The morphology of the chorionic villi is characterized—individual morphology and their distribution over the chorionic sac. Attention to whether the villi appear overly abundant and/or cystic is important because of concerns for complete or partial hydatidiform mole (CHM, PHM). Other features of embryo development, such as a villous, yolk sac, umbilical cord, and nucleated embryonic erythrocytes, should also be assessed.

Tissues are sampled for cytogenetic analysis and can be retained frozen for additional studies as required. In many institutions, tissue is often submitted directly to the cytogenetics laboratory by the clinicians, either from the tissue obtained at dilation and curettage or by chorionic villous sampling. Additional tissue may be retained frozen for array CGH, although this can be increasingly performed on paraffin-embedded tissue (41). Although an argument can be made to utilize array CGH in all cases of SAs to eliminate the labor and risk of culture failure associated with conventional cytogenetic analysis, conversely, CGH will miss some anomalies seen only on karyotype (12,42–44).

The presence of embryonic tissue convirms intrauterine pregnancy and is a feature in favor of a diagnosis other than CHM, a frequent concern as edematous villi are often identical on ultrasound or at gross examination. In determining the developmental stage and thus the age of the embryo, standard developmental criteria can be used (45,46). Embryos may be normal or deformed (Figur e 2-1) according to established criteria, but this does not exclude chromosome abnormality. Most deformed embryos are established criteria, but this does not exclude chromosome abnormality. Most deformed embryos are established criteria, but this does not exclude chromosome abnormality. Most deformed embryos are established criteria, but this does not exclude chromosome abnormality. Most deformed embryos are established criteria, but this does not exclude chromosome abnormality. Most deformed embryos are established criteria, but this does not exclude chromosome abnormality. Most deformed embryos are established criteria, but this does not exclude chromosome abnormality.

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refers to an intact empty sac (Figure 2-2), type II refers to a nodular embryo in which cranial and caudal ends cannot be distinguished (Figure 2-3), type III refers to a cylindrical embryo in which there is some cranial–caudal differentiation with retinal pigment (Figure 2-4), and type IV refers to an embryo in which there is more recognizable embryonic development but delayed growth of limbs and other developmental features (Figure 2-5). While growth disorganization is readily identified and classified, the findings are nonspecific—in all types of growth-disorganized embryos, the incidence of chromosome abnormality is similar to that encountered in SABs in general, with the same types of chromosome abnormalities identified. Ultrasound detection of an embryo does not strictly correlate with the morphologic detection of embryonic tissue, but it has been demonstrated that the rates of abnormal karyotypes are not significantly different between SABs in which an embryonic pole is identified on ultrasound examination and those that appear anembryonic (37,47). Approximately 60% to 70% of both anembryonic and embryonic specimens are chromosomally abnormal, although the spectrum of abnormalities may be different, with fewer monosomy X and viable autosomal trisomies in the anembryonic specimens.

Embryos may show isolated or focal abnormalities such as neural tube defects, facial clefts, or limb abnormalities.
Many of these abnormalities, such as neural tube defects, occur in the setting of chromosome abnormality (48). In the setting of a normal karyotype, the focal defects likely have the same significance as in later gestation, and genetic counseling to discuss the findings and possible recurrence risks is indicated. Chromosomally abnormal embryos may show a number of non-specific abnormalities, such as delay of normal limb development and various types of growth disorganizations. Embryos with trisomies more commonly encountered in later gestation and live births, such as trisomies 13, 18, and 21, may show some features in common with the phenotypes.

**FIGURE 2-5** • Growth-disorganized embryo, GD4—delayed development of head, trunk, and limbs relative to crown-rump length.

**FIGURE 2-6** • Stage 20 embryo with parietal and occipital encephaloceles.

**FIGURE 2-7** • Monosomy X embryo with parietal encephalocele.

**FIGURE 2-8** • Stage 18 embryo with cleft lip, absent digit in the right hand, and coloboma.
observed in the fetal period. Most often, however, embryonic phenotypic manifestations of these trisomies are nonspecific (Figure 2-9).

Triploidy is encountered in approximately 6% of early SAbS; embryonic tissues are often identified. A phenotype thought to be characteristic of embryonic triploidy has been described and includes retarded limb development, facial dysplasia, subectodermal hemorrhage, and cystic chorionic villi (49) (Figure 2-10), but more recent series describe a wide range of phenotypic abnormalities ranging from growth-disorganized to apparently normal embryos (50). These apparently normal embryos are most often seen at approximately stage 16 of development (37 to 42 days), equivalent to approximately 7 to 8 weeks of GA (Figure 2-11). The normal and the growth-disorganized phenotypes have been seen in triploids of both maternal origin and paternal origin.

Histologic examination of placental tissues and decidua should be routinely performed in all cases. Microscopic examination of chorionic villi allows detection of many potential abnormalities including viral infections such as cytomegalovirus (CMV) and bacterial infections such as Listeriosis. In addition, other disorders such as intervillitis or conditions with increased intervillous fibrin are occasionally detected. Villous infarction is distinctly unusual and should raise concerns of maternal vascular/thrombophilic disease. Routine histologic examination of decidua allows for assessment of decidual (maternal) vasculature. In a review of the histopathology of SAbS with known karyotype, 19% of SAbS with a normal karyotype showed evidence of chronic inflammation or perivillous fibrin deposition, in contrast to 8% of those with an abnormal karyotype. The findings were even more frequent (31%) in the subset of SAbS that were chromosomally normal and occurred in a population with recurrent SAbS (51).

Chronic histiocytic intervillitis is a disorder in which there is an abnormal increase in maternal mononuclear cells, predominantly comprised of mature CD68 histiocytes, within the intervillous (maternal) space (Figure 2-12). The lesion is thought to be a maternal immune-mediated disorder; it confers a high recurrence risk in subsequent pregnancies (52–54).

Increased perivillous fibrin is another disorder in which it has been suggested that maternal immune dysregulation may play a role. Perivillous fibrin may also be increased as a degenerative change in response to prolonged intrauterine death, and distinguishing between pathologic and degenerative changes in perivillous fibrin can be difficult. When there is an obvious increase in perivillous fibrin, the lesion may be considered to account for
the loss. Some suggest that an arbitrary threshold of 50% villous involvement be used to make a pathologic diagnosis (55). Although probably etiologically heterogeneous, this entity may also recur and has been associated with recurrent SAbS.

Infection is a clear cause of pregnancy loss, with viruses, spirochetes, and bacteria all playing significant roles. Although first trimester loss may occur with syphilis, it is seen more often in losses occurring in later gestation. Listeriosis, by contrast, causes pregnancy loss throughout gestation (56). Listeriosis may occur as community outbreaks, related to improper food handling, or as a sporadic event related to ingestion of foods known to be at risk of containing Listeria, such as soft or unpasteurized cheeses. Listeriosis is characterized histologically by acute neutrophilic villitis with intervillous abscess formation (Figure 2-13). There is usually also an acute chorioamnionitis. Gram-positive bacilli may be demonstrated on Gram stain, although histology is usually sufficiently characteristic to allow confident diagnosis.

Excluding the small percentage of cases in which infectious, immune, or vascular causes of first trimester SAb are identified, the majority of SAbS are shown to be chromosomally abnormal. Although there are histologic features in chorionic villi that have been suggested as being more commonly observed in aneuploid pregnancies, such as irregular villous outlines, excess trophoblast inclusions, or complex invaginations, in general, these findings are nondiagnostic (57–60). Some trisomies, such as trisomy 22, may be more likely to show these features (61) (Figure 2-14).

Concern for GTN is heightened in the SAb population, as CHM and PHM may present as spontaneous or missed abortions. Historically, it has been shown that fewer than 44% of CHMs or PHMs are detected at routine first trimester ultrasound (62), providing an indication of the necessity for histologic examination of SAbS, even when a gestation appears routine at ultrasound or at the time of evacuation. The risk of GTN requiring chemotherapy has been reported to be between 15% and 28% after diagnosis of CHM (63), underscoring the importance of early diagnosis. The risk of GTN after triploid PHM is less well defined; there are case reports of hchoriocarcinoma occurring after triploid PHM (33, 61, 64, 65), but others have shown that the risk of persistent GTN is rare, occurring in fewer than 5% of cases (39). Given the risks, however, some recommend that these cases be managed as would women who have had a CHM (34).

The diagnosis of early CHM and PHM can be difficult in specimens from early SAbS, perhaps more so than in the past when these pregnancies presented later in gestation. Gross examination of early hydatidiform molar sacs shows cystic change of chorionic villi—grossly, this may be impossible to differentiate from the cystic change in partial moles and the hydropic degeneration occurring in nonmolar SAbS. Histologic diagnosis may not be possible even among experienced pathologists (66): studies have demonstrated considerable interobserver and intraobserver variability in the diagnosis of both CHM and PHM even among placental pathologists (67). A major problem that occurs in routine practice is to distinguish between hydropic abortion (degenerative change) and molar gestation. With recognition that the features in early CHM may be subtle and with the availability of karyotype determination, ploidy determination, and immunohistochemical staining for p57kip2, diagnostic accuracy is increased (68).
CHMs are diploid, with both haploid complements being paternal in origin. Thus, CHMs are androgenetic, with no maternal DNA contribution present. A normal development in this situation is considered to be a reflection of imprinting (see Chapter 3, Table 3-17), since both maternal and paternal genetic contributions are required for normal embryonic and placental development. The classic histopathologic features of CHM are diffuse villus edema (hydropic change), cistern formation, and circumferential syncytiohydatid amnion villotrophoblast hyperplasia. Rudimentary villous vessels may be identified, but intravascular nucleated red blood cells are not seen. Stromal karyorrhexis is a feature of early CHM, thought to be related to increased stromal proliferation and apoptosis (Figure 2-15) (34). Immunohistochemical staining for p57kip2 is useful in the assessment of potential complete molar gestations because of its expression from the maternal allele only. Thus, in a CHM, by definition androgenetic, the normal p57kip2 staining of cytotrophoblast and villous stroma is absent (69,70). p57kip2 staining of triploid PHM is normal because of the maternal haploid contribution and is thus not helpful as a diagnostic aid.

Triploidy may be either paternal (diandric) or maternal (digynic) in origin. Older studies demonstrated that diandry was the predominant origin of triploidy, while more recent studies have shown that the distribution of diandric triploidy and digynic triploidy is somewhat more complex than that. In early pregnancy, the incidence of diandric triploidy is the norm. Triploidy can be confirmed by conventional cytogenetics, flow cytometry, or molecular testing.

Unlike CHM, the presence of intravillous nucleated blood cells is the norm. Triploidy can be confirmed by conventional cytogenetics, flow cytometry, or molecular testing.

SECOND TRIMESTER PREGNANCY LOSS

With completion of fetal embryonic period, organogenesis is complete. The forces that lead to fetal loss during a second trimester pregnancy (a approximately 5% to 10%), a second trimester loss are more heterogeneous, with a concomitant broader range of implications for counseling and management of future pregnancies. Examination of fetuses second trimester fetal loss is similar to the investigation of intrauterine death in later gestation, requiring complete postmortem examination, including placental evaluation, with the goals of 1) identifying the cause of intrauterine demise, 2) assessing recurrence risk in future pregnancies, and 3) determining management options.

The extent of fetal postmortem examination varies between situations, with external examination of a fresh fetus sufficient in some laboratories, while others provide complete autopsy examination. The latter is the only way of adequately assessing such specimens, and examination of a fresh fetus is the only way of adequately assessing such specimens, and examination of a fresh fetus with external examination other than complete autopsy with examination of the placenta should be considered in complete. As many of the causes examined in the fetal pathology service come after diagnosis of intrauterine fetal death, maceration of tissues is a problem that must be addressed. The characteristic features of various disorders are present but may be altered or obscured by the effects of maceration. Maceration is noted in agnosis and should be considered a limitation to the examination of affected fetuses. Second trimester specimens may also come from elective terminations due to fetal structural, chromosomal, or other genetic anomalies. In these cases, both the fetus and placenta are often markedly disrupted, with or without a normal diagnosis of PHM. In the fetal and infant population, digyny clearly predominates.

PHM is characterized, classically, by two populations of villi, some with hydropic change and cistern formation and others that are small and not hydropic. The external villous contours are irregular and have been described as foordlike. Invaginations or claustrations of trophoblast are ree seen—so-called trophoblast psuedoinclusions—that represent complex infoldings of chorionic villi. These may be acutely deformed or aca, appearing to be syncytiothrophoblast, and circumferential trophoblast hyperplasia may be focal.

FIGURE 2-15 • Early CHM with stromal karyorrhexis.
performed, skin ederal survey is do ne a s in dicated, a nd in ternal examination w ith di section o f a ll o rgan sys tems, in cluding cen tral nerv ous system (CNS) to the ex tent that the methodology of ter mination permits, is conducted. Ex ter nal examination includes an assessment of all growth parameters and comparison to estab lished nor mative va lues for det ermination of GA. Par tic ular at tention should be given to the detection of in tr auter ine growth restriction (IUGR), with consider a tion given to the demise-to-delivery interval of spontaneous pregnancy losses that might alter estimation of GA at demise. Sections from all iden ti fi able organ systems are re s igned for histologic exam ination. Cyto genetic and/or other molec ular studies s hould be ini ti ated if necessary where maternal and fetal cir cum stances warrant investigation. U nixed and nonma cere ted fetal and placen tal tissues can be frozen in the event that the tissue is required for CGH or other genetic studies. When in dic ated, tissues can be submitted for specific molecular analysis, for disorders such as hemoglobinopathies and inborn errors of metabolism. Spec ies-specific infectious disease cultures, including viral cultures, can be submitted as clinically appropriate. In cases of suspected skeletal dysplasia of unknown genetic origin, fibroblast cultures can be submitted and initiated; the resultant cell line can be frozen and retained for potential future genetic testing.

It is not possible to outline all of the disorders encountered in the pathology of second trimester losses. Suffice it to say that all of the pathology encountered in later gestation, intrauterine deaths, and pregnancy losses also occur in the second trimester, a nd o ccur a s proportion o f c hromosomal anormalities responsible for second trimester SABs (see Chapter 4).

In general, losses occurring in the second trimester can be either intrinsic to the conceptus or a consequence of abnormalities in the intrauterine environment. In the latter setting, the fetuses are normally developed, usually well preserved, and the findings raise concerns for uterine anomalies, cervical incompetence, and/or ascending infection (76).

Uterine abnormalities are present in approximately 15% of women who have recurrent miscarriage (77). Pathologic examination of the aborted fetus and placenta cannot make this diagnosis, but the finding of a nonmacerated, anatomic, and chromosomally normal fetus with no evidence of ascending infection may be suggestive of a clinical and/or anatomic abnormality in second trimester losses/deliveries.

Ascending infection is a common cause of second trimester preivable or viable preterm deliveries (3,78). Particularly in a primigravida, there may be an n eces sary hi story to the diagnosis in the absence of maternal abnormalities or a nonspecific infection. Pathologic examination of the abortus may show features characteristic of Listeriosis, including fibrous, fibroptic placenta, and an inflammatory response to intrauterine infection (63,79).

Although a broad range of organisms is responsible for chorioamnionitis, routine culturing of the products of SABs is not performed. When there is clinical concern for specific notable diseases, such as Listeriosis or syphilis, aerobic and anaerobic cultures may be indicated. Listeriosis can occur as outbreaks, and thus, knowledge of its role in gestational infection is important from an epidemiologic perspective as well as from a clinical one. Listeriosis is caused by Listeria monocytogenes. Infection may be caused by exposure to foods such as unpasteurized or soft cheeses as well as incompletely processed meats; outbreaks have been related to contaminated production techniques. Listeriosis may be subclinical or may be associated with gastrointestinal diseases, such as vomiting and diarrhea, or generalized malaise that includes fever and myalgia. Infection in pregnant women is associated with an increased risk of intrauterine death and SABs, including both first and second trimester SABs.

Pathologically, Listeriosis may present as an early abortus with small white nodules/plaques on the skin of the fetus (70). Histologic examination shows necrosis with hemorrhage, a characteristic lesion of Listeriosis. Lysis of fetal organs may be present in the form of neutrophils in the fetal chorionic vessels and/or in the vessel of the umbilical cord (chorionic and/or umbilical vasculitis, respectively). Ascending infection has been associated with uterine trauma in mid trimester gestations and has been reported to be more likely when there is a fetal inflammatory response to intrauterine infection (63,79).

**FIGURE 2-16** Listeriosis. A: Gross examination shows small white nodules/plaques on the skin of the second trimester fetus (arrows). B: Histologic examination shows necrosis with abundant bacteria, shown to be Gram-positive bacilli, culture positive for Listeria monocytogenes.
Other infections, including viral infections, can account for intrauterine death in the second trimester, with CMV being the viral infection most commonly encountered in fetal death occurring in developed countries (80). Fetuses affected by CMV may appear grossly normal, aside from effects of retention after fetal death, but may also show hepatic calcifications and CNS abnormalities. Histologic examination of fetal organs may show variably severe tissue-destructive mononuclear inflammation, and CMV inclusions may be readily identified by routine histology or aided by immunohistochemical staining or in situ hybridization. Classically, the placenta shows a “dirty” lymphoplasmacytic villitis, with villous vascular and stromal kerorrhexis and hemosiderosis. CMV inclusions are often readily identifiable on routine H&E stains (Figure 2-18), although immunohistochemistry or in situ hybridization can also be utilized for confirmation, as necessary. There may be a discrepancy between the severity of placental manifestations and those of the fetal organs; and in fact, it often appears that fetal organs are more likely to show inclusions and inflammation relative to the extent of placental inflammation.

Syphilis is encountered more commonly in the obstetric population of developing countries, including being responsible for fetal deaths. Spirochetes may be readily identified in fetal organs, and the placenta shows the features described elsewhere (see Chapter 9), including villitis, villus edema, and obliterator vascular changes.

Chromosomal abnormalities are encountered less often in second trimester losses than in those occurring in the first trimester, and the type of aneuploidy encountered is less varied, bearing a closer resemblance to the range observed nearer term (see Chapter 3). The trisomies encountered during second trimester losses are 21, 13, and 18, as well as monosomy X and triploidy—are the most commonly identified abnormalities in the midgestational period. A minority of chromosomally abnormal fetuses survives to term; in fact, it is estimated that only 20% of trisomy 21 conceptions, 5% of trisomy 18 conceptions, and 1% of monosomy X conceptions survive to be liveborn. The mechanism allowing some of the chromosome abnormalities to survive into later gestation has been suggested as due to placental mosaicism for both aneuploidy and normal cell line (81).

**Trisomy 21**

Trisomy 21 syndrome in the fetus shows the same range of developmental anomalies observed in liveborns (Chapter 3, Table 3-8), although the external facial and somatic phenotype may be less well developed. It is frequently associated with abnormalities of maternal serum markers (Chapter 3, Table 3-11). In addition, it is not unusual for midtrimester trisomy 21 to present as hydrops fetalis, with generalized subcutaneous edema and nuchal thickening that can be mistaken for a nuchal hygroma (Figure 2-19). The only
feature observed more commonly in those trisomy 21 cases presenting as intrauterine demise as opposed to those terminated after prenatal cytogenetic diagnosis is hydrops fetalis; the other anomalies do not appear to be different between the two groups and thus do not provide an explanation to account for the survival of only some fetuses until later gestation. Although the presence of features such as atrioventricular canal-type cardiac defects, or transverse palmar creases, may suggest trisomy 21, cytogenetic analysis is required for confirmation. While cell sorting reveals an increased ratio of megakaryocytic and erythroid precursors relative to myeloid precursors in the fetal liver of second trimester trisomy 21 fetuses (82), transient myeloproliferative disorder, which predominates in the third trimester, diagnosed postnatally or postmortem (83). There is an empiric risk of recurrence of trisomy 21 on the order of 1% after an affected pregnancy (second trimester or later), whereas other chromosome abnormalities, such as monosomy X, are not associated with the increased risk of recurrence. If the trisomy 21 is the result of a Robertsonian translocation, carried in a balanced form by one parent, the risk for recurrent trisomy 21 is substantially higher.

Trisomy 18
Fetuses with trisomy 18 may present as intrauterine demise without external anomalies and may be associated with abnormal maternal serum screening, including very low estradiol levels. However, these fetuses typically demonstrate IUGR, which can be difficult in a case where there is maceration, as retention after fetal death may account for some of the discrepancy in fetal growth parameters. Of all the trisomic conceptions, these fetuses display the widest range in external and internal phenotype. Hydramnios is a hallmark, and trisomy 18 fetuses universally exhibit short stature, with the head mid-way between the internipple line and the umbilicus. In addition, they often exhibit some degree of ascites. The hands show flexion of the fingers, with the second and fifth fingers characteristically clasped over the third and fourth, respectively. Feet may show prominent heels and may be rounded, so-called rocker bottom feet, though these features are subjective and can be overinterpreted. Internal examination may show normal or abnormal renal abnormalities, including hydronephrosis, while other abnormalities such as gastrointestinal malformations are common, including CNS defects (e.g., hydrocephalus) as well as other anomalies in phenotype (e.g., gastroschisis). Although more female than male infants are liveborn with trisomy 18, among elective terminations, the sex ratio is equal, and it has been suggested that male infants are more likely to die in utero (84–86).
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Trisomy 13

Trisomy 13 may also present as unanticipated fetal death, with only 5% of all trisomy 13 conceptions surviving to be liveborn. The facial anomalies are explainable by paucity of midfacial tissue, which are also reflective of brain anomalies, the most dramatic of which is holoprosencephaly. Facial anomalies include cleft lip and palate, proboscis, hypotelorism, and synophthalmia (Figure 2-21). Postaxial polydactyly of the hands and/or feet is common. There may be an omphalocele as well as internal anomalies affecting a variety of systems including the kidneys, which may be enlarged and show cystic change, and the heart, which characteristically shows tetralogy of Fallot or truncus arteriosus (see Chapter 3, Table 3–10). At gross examination, the differential diagnosis includes Meckel–Gruber and pseudotrisomy 13 syndromes.

Monosomy X

Monosomy X is also known as Turner syndrome. In the fetal period, this most commonly presents as hydrops fetalis, often with a very large cystic hygroma. Accentuation of the subcutaneous edema on the dorsal aspects of the hands and feet is characteristic but nonspecific. These fetuses are female and show normal female genitalia, internally and externally. Characteristic anomalies in clude left-sided cardiac anomalies such as hypoplasia of the aortic arch and/or left ventricular hypoplasia. Renal anomalies in clude horseshoe kidney (Figure 2-22).

Triploidy

Triploidy is the presence of an entire extra haploid set of chromosomes, which may be of maternal (digynic) or paternal (diandric) origin. Diandric triploidy has been reported in anywhere from 20% to 85% of triploid pregnancies, likely depending on the developmental stage of the pregnancy at the time of ascertainment, with diandric triploidy cases predominating in earlier abortions (50,72,87). Most but not all cases of diandric triploidy are associated with PHM (46). In the fetal period, digynic triploidy predominates (72). In general, triploidy is characterized by anomalies that affect almost every organ system and can be present in both digynic and diandric triploidies. Syndactyly of the third and fourth fingers and second and third toes is a characteristic feature of triploidy, in dependent of parental origin. Despite the fact that the chromosome abnormality is numerically the same, it has been proposed that imprinting leads to a fetal/placental phenotype that correlates with the parental origin of triploidy (88), although not all series have found differences based on parental origin (50). Digynic triploidy gives rise to marked asymmetric IUGR, with the head size being relatively well preserved compared to the trunk and extremities, which are markedly growth restricted (Figure 2–23). There is adrenal hypoplasia, as has been observed in other cases of severe IUGR, consistent with the concept that placental function can be operative in intrauterine adrenal growth and development. Other anomalies are varied and affect all organ systems. The placenta is abnormally small and shows no villus edema or trophoblastic hyperplasia. In diandric triploidy, growth is better preserved,
but there may be symmetric IUGR (Figure 2-24). The placenta shows changes of PHM with villus edema and cistern formation; focal circumferential syncytiotrophoblast hyperplasia, which can have a lacy appearance; and trophoblast invaginations into the villus stromal core (so-called trophoblast pseudoinclusions). Fetal growth and placental differences are reflected in abnormalities observed in maternal serum screening, with digynic triploids showing markedly decreased estriol and human chorionic gonadotropin (hCG), while the diandric triploids have markedly increased levels of alpha-fetoprotein (AFP) and hCG.

**Hydrops Fetalis**

Hydrops fetalis can present with second trimester fetal demise and warrants complete evaluation for diagnosis, as in those cases diagnosed as later gestational stillbirths (Figure 2-25). Although historically hydrops fetalis was often associated with Rh isoimmunization, with prophylactic anti-D treatment, isoimmune hydrops fetalis now accounts for less than 10% of cases. Nonimmune hydrops fetalis is much more common. The increase in extravascular fluid in these cases may be due to abnormal pathophysiology involving the heart, kidney, liver, or vessels leading to obstructed lymphatic flow, congestive heart failure with increased central venous pressure, or low plasma oncotic pressure. The differential diagnosis is extensive and includes chromosome abnormalities, infection such as CMV and parvovirus B19, hemoglobinopathies such as thalassemia, fetal arrhythmias, congenital pulmonary airway malformations, tumors (e.g., sacrococcygeal teratoma, placental chorangioma(tosis), obstructive cardiac masses), and metabolic disorders (see Chapter 4) (89,90).

Accordingly, the approach to hydrops fetalis includes complete autopsy examination (91) with maternal reserved for cytogenetic analysis, viral cultures, PCR for parvovirus, initiation of fibroblast cultures, and retention of a variety of tissues for freezing at −70 °C in the event that additional studies such as alpha-thalassemia gene studies are required. With the exclusion of these entities, one is left with the diagnosis of hydrops fetalis of undetermined etiology. Because of the possibility of an undetected metabolic condition leading to hydrops, genetic counseling considers the risk of autosomal recessive conditions; thus, the risk of recurrence may be as high as 25% for each subsequent pregnancy.

**Twinning**

Monozygous twinning is associated with an increased risk of in traterine fetal demise, a nd m ay o ccur o n t he b asis of p lacental vascular anastomoses, leading to twin–twin...
transfusion syndrome (Figure 2-26) or twin-reversed arterial perfusion (TRAP) sequence (92,93) in monochorionic twins. Monochorionic monoamniotic twins (MCMA) are at risk for cord entanglement, which may lead to compromise of umbilical cord blood flow and fetal death; however, cord entanglement in this setting surprisingly does not usually affect fetal survival (94,95). Instead, MCMA twins have a higher rate of mortality than monochorionic diamniotic (MCDA) twins, primarily due to chromosomal or congenital anomalies or TRAP sequence (96). Twin–twin transfusion syndrome can be suggested in monochorionic twins if there are growth and/or perfusion discrepancies between the two fetuses (92). TRAP is a condition in which the umbilical cords of MCMA twins are implanted very close to one another, establishing large bore vascular anastomoses involving chorionic vessels. It is hypothesized that some event leads to an imbalance in the shunting of blood, resulting in reversed perfusion such that one twin receives deoxygenated blood from the other via retrograde flow through its umbilical artery. This results in hypoxia in the recipient twin with resultant bony deformities and organogenesis that is most severe cranially. Thus, the characteristic phenotype is an acardiac, acephalic twin (Figure 2-27). This perfusion abnormality may result in the intrauterine death of both twins, or the still-perfused acardiac twin may be delivered at term with the coexisting twin.

**Umbilical Cord Compromise**

Umbilical cord compromise can occur in a variety of settings and can result in fetal death (97–100). In some cases, there may be no gross fetal features to suggest the cause of death, whereas other cases show an absence of cord entanglement that could have occurred postmortem and that thus suggest the diagnosis (Figure 2-28). Of note, there has been considerable controversy as to whether the twist at the junction of the umbilical cord with the abdominal wall, often observed in macerated fetuses, is a cause of cord blood flow compromise or a postmortem artifact. Some authors have identified placental histologic features that indicate or at least support a diagnosis of fatal umbilical cord blood flow restriction (see Chapter 9) (101,102). This underscores the importance of placental evaluation as a critical component in investigating the cause of midtrimester intrauterine demise.

**Limb-Body Wall Complex**

Limb–body wall complex (LB WC), a disorder within the spectrum of short cord or placental adhesion sequence, is characterized by limb anomalies, including limb aplasia or hypoplasia, associated with a large anterior body wall defect and an abnormally short umbilical cord, usually in chromosomally normal fetuses (Figure 2-29) (103,104). With routine use of detailed antenatal ultrasound examination, these
cases now result most commonly in pregnancy termination but may also present as early intrauterine demise, presumably due to fatally compromised umbilical cord blood flow.

**Postprocedure Pregnancy Loss**

Loss of pregnancy can occur after invasive prenatal procedures such as chorionic villus sampling and amniocentesis. The rate varies with the situation, usually being 0.5% to 3% of first trimester spontaneous abortion. Intrauterine death occurs more commonly in first trimester spontaneous abortion. Am J Obstet Gynecol 2008;53(7):622–628.


17. Ozawa N, T agashima T, et al. Prenatal examination should be undertaken as with any other pregnancy loss, as appropriate for the GA but to include both embryofetal and placental evaluation.

**References**


