Hydatidiform Moles
Ancillary Techniques to Refine Diagnosis
Brigitte M. Ronnett, MD

Context.—Distinction of hydatidiform moles from nonmolar specimens and subclassification of hydatidiform moles as complete hydatidiform mole versus partial hydatidiform mole are important for clinical practice and investigational studies. Risk of persistent gestational trophoblastic disease and clinical management differ for these entities. Diagnosis based on morphology is subject to interobserver variability and remains problematic, even for experienced gynecologic pathologists.

Objectives.—To explain how ancillary techniques target the unique genetic features of hydatidiform moles to establish diagnostic truth, highlight the issue of diagnostic reproducibility and importance of diagnostic accuracy, and illustrate use of p57 immunohistochemistry and polymerase chain reaction–based DNA genotyping for diagnosis.

Data Sources.—Sources are the author’s 10-year experience using ancillary techniques for the evaluation of potentially molar specimens in a large gynecologic pathology practice and the literature.

Conclusions.—The unique genetics of complete hydatidiform moles (purely androgenetic), partial hydatidiform moles (diandric triploid), and nonmolar specimens (biparental, with allelic balance) allow for certain techniques, including immunohistochemical analysis of p57 expression (a paternally imprinted, maternally expressed gene) and genotyping, to refine diagnoses of hydatidiform moles. Although p57 immunostaining alone can identify complete hydatidiform moles, which lack p57 expression because of a lack of maternal DNA, this analysis does not distinguish partial hydatidiform moles from nonmolar specimens because both express p57 because of the presence of maternal DNA. Genotyping, which compares villous and decidual DNA patterns to determine the parental source and ratios of polymorphic alleles, distinguishes purely androgenetic complete hydatidiform moles from diandric triploid partial hydatidiform moles, and both of these from biparental nonmolar specimens. An algorithmic approach to diagnosis using these techniques is advocated.


Hydatidiform moles are abnormal placentas with distinct genetic characteristics/alterations that are responsible for inducing variable trophoblastic proliferation and hydropic change in villous tissue. A comprehensive discussion of the epidemiology and pathogenetic mechanisms of molar pregnancies, which is beyond the scope of the current article, has been provided in a recent review. Distinction of hydatidiform moles from nonmolar specimens and subclassification of hydatidiform moles as complete hydatidiform mole (CHM) versus partial hydatidiform mole (PHM) are important for both clinical practice and investigational studies. The risk of persistent gestational trophoblastic disease (GTD) and clinical management differ for CHMs, PHMs, and nonmolar specimens. However, diagnosis based solely on morphology is subject to diagnostic variability. The unique genetic features of CHMs (purely androgenetic conceptions), PHMs (diandric triploid conceptions), and nonmolar specimens (biparental conceptions with allelic balance) allow for certain molecular techniques, including immunohistochemical analysis of p57 expression (a paternally imprinted, maternally expressed gene) and DNA genotyping, to refine the diagnosis of hydatidiform moles. Although p57 immunostaining alone can identify CHMs, which lack p57 expression because of the lack of maternal DNA, this analysis cannot distinguish PHMs from nonmolar specimens because both express p57 because of the presence of maternal DNA. Polymerase chain reaction (PCR)–based DNA genotyping, which can determine the parental source and ratios of polymorphic alleles, specifically distinguishes purely androgenetic CHMs from diandric triploid PHMs, and both of these from biparental nonmolar specimens. The objectives of this review are:

1. To present the classification and differential diagnosis of hydatidiform moles;
2. To discuss the genetics of molar and nonmolar specimens and explain how ancillary techniques target
these unique genetic features to establish diagnostic truth;
3. To highlight the issue of diagnostic reproducibility for hydatidiform moles and discuss the importance of accurate diagnosis;
4. To illustrate the utility of p57 immunohistochemistry for diagnosis of hydatidiform moles and provide guidance on interpretation;
5. To demonstrate the application of DNA genotyping for diagnosis of hydatidiform moles;
6. To provide an algorithmic approach for diagnosis of hydatidiform moles in the molecular era.

**CLASSIFICATION AND DIFFERENTIAL DIAGNOSIS OF HYDATIDIFORM MOLES**

Table 1 lists the classification and differential diagnosis of hydatidiform moles. Hydatidiform moles include 2 varieties: the complete hydatidiform mole (CHM) and the partial hydatidiform mole (PHM). In addition, an early form of CHM has been recognized. Typical CHMs are comprised of enlarged edematous villi with moderate to marked circumferential trophoblastic hyperplasia, often with cytologic atypia, prominent central cistern formation, and trophoblastic inclusions. 2–4 Early CHMs are characterized by a redundant bulbous villous growth pattern, hypercellular myxoid villous stroma, a labyrinthine network of villous stromal canalicular vascular structures, karyorrhectic debris within stroma, and at least focal trophoblastic hyperplasia on villi and the undersurface of the chorionic plate. 5 Characteristic morphologic features of PHMs include the presence of 2 populations of villi (large, irregular, hydropic villi, and small, immature, fibrotic villi), cisterns in some enlarged villi, markedly irregular villi with scalloped borders and trophoblastic inclusions, and generally mild circumferential trophoblastic hyperplasia. 2,6–9 Invasive hydatidiform moles, which invade the myometrium, are virtually always CHMs. 10 Examples are provided in Figure 1, A through F.

The differential diagnosis of hydatidiform moles includes a variety of nonmolar entities that can exhibit some features suggestive of a molar pregnancy. These include products of conception specimens with abnormal villous morphology, abnormal nonmolar specimens with prominent trophoblastic hyperplasia, hydropic abortuses, and androgenetic/biparental mosaic conceptions. Abnormal villous morphology is a term used to label cases in which villi have some dysmorphic features suggestive of a hydatidiform mole, usually a PHM but sometimes an early CHM, but lack fully developed diagnostic features of either type. In some cases these changes are associated with other (nonmolar) genetic abnormalities, such as trisomy. 11–13 Early nonmolar specimens at times have trophoblastic proliferation that is sufficiently prominent to raise concern for a CHM, usually an early CHM, but lack other features of a hydatidiform mole. In the earliest examples, the trophoblastic proliferation forms a circumferential shell around a very early conceptus, but once some branching of immature villi occurs, the trophoblastic proliferation usually can be recognized as polarized when a radiating pattern from the tips of the villi is appreciated. Hydropic abortuses have edematous villi but lack other features of a hydatidiform mole; any trophoblastic proliferation is generally polarized at one end of the villous structures. Nonmolar androgenetic/biparental mosaic conceptions are unusual specimens which, when encountered at an early gestational age, represent the early form of placental mesenchymal dysplasia. They are characterized by hydropic villi, which can have some cisterns and trophoblastic inclusions, often with some villi having more cellular stroma and notable vascular proliferation, but the villi lack trophoblastic hyperplasia. Some androgenetic/biparental mosaic conceptions also have a molar component in which the villi have trophoblastic hyperplasia and the other features of a CHM; the molar component can be focal and inconspicuous with features of the early form, or it can be more fully developed and readily apparent. Examples of these entities are provided in Figure 1, G through P. In addition, because the individual subtypes of hydatidiform moles can exhibit a spectrum of morphologic features, depending in part on gestational age, CHMs (including the early form) and PHMs are often in the differential diagnosis of one another as well. Parameters assessed to distinguish the subtypes of hydatidiform moles, including variations in the sizes and shapes of the villi, the extent of hydropic change, and the degree of trophoblastic hyperplasia, have sufficiently wide spectra to result in some morphologic overlap between a subset of CHMs and PHMs, namely, those at the lower and upper ends, respectively, of their morphologic spectra.

**GENETICS OF HYDATIDIFORM MOLES AND NONMOLAR SPECIMENS**

The distinct genetics of molar specimens are illustrated in Figure 2. CHMs are purely androgenetic conceptions (only paternal genetic material is present) and usually diploid (2 paternal chromosome complements without a maternal chromosome complement) 3,14; most result from fertilization of an ovum devoid of maternal genetic material by a single sperm that duplicates (monospermy; ~85%), 15–19 but a subset is due to fertilization by 2 sperm (dispermy). 20 Some CHMs are tetraploid rather than diploid, but these are also purely androgenetic. A rare form of CHM, the familial biparental CHM, is not androgenetic but rather is related to mutations in maternal effect genes NLRP7 (NALP7; chromosome 19) and KHCDC3L (C6orf221; chromosome 6), which result in global imprinting alteration leading to preferential expression of paternally imprinted genes in villous trophoblast. 21–23 In contrast, PHMs are characterized by diandric triploidy (2 paternal and 1 maternal chromosome complements), with most arising by fertilization of an

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**Table 1. Classification and Differential Diagnosis of Hydatidiform Moles**

<table>
<thead>
<tr>
<th>Classification and Differential Diagnosis of Hydatidiform Moles</th>
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<tr>
<td><strong>Hydatidiform moles:</strong></td>
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<tr>
<td>Complete hydatidiform mole</td>
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<tr>
<td>Early complete hydatidiform mole</td>
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<td>Partial hydatidiform mole</td>
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<td>Invasive hydatidiform mole</td>
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<td>Nonmolar specimens capable of simulating hydatidiform moles</td>
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<tr>
<td>Abnormal villous morphology, possibly related to other genetic abnormalities (eg, trisomy)</td>
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<td>Early abortus with trophoblastic hyperplasia</td>
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<td>Hydropic abortus</td>
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<tr>
<td>Androgenetic/biparental mosaic conceptions</td>
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<tr>
<td>Nonmolar form (placental mesenchymal dysplasia)</td>
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<tr>
<td>Molar form (usually with complete hydatidiform mole component)</td>
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ovum by 2 sperm (dispermy; ∼99%). Rare examples of PHMs exhibit triandric triploidy (3 paternal and 1 maternal chromosome complements). Nonmolar specimens are usually characterized by biparental diploidy (1 paternal and 1 maternal chromosome complements), but some can be tetraploid. Some nonmolar specimens are digynic triploid conceptions (2 maternal and 1 paternal chromosome complements). In most cases, digynic triploid conceptions do not exhibit molar features, but on occasion they can have some focal dysmorphic features suggesting a PHM, and rare examples occurring in patients with familial recurrent hydatidiform mole associated with mutations in NLRP7 (NALP7) or KHDC3L (C6orf221) can have the morphology and immunophenotype (p57+) of a CHM.

Nonmolar specimens with cytogenetic abnormalities, such as trisomy, can have dysmorphic villi suggesting or simulating PHMs. Androgenetic/biparental mosaic conceptions are genetically distinct from typical hydatidiform moles. The nonmolar forms comprise villi with varying admixtures of both androgenetic (p57−) and biparental (p57+) cell lines within individual villi (often segregated as biparental cytotrophoblast and androgenic stromal cells, but the reverse is possible); these cell lines are most often both diploid but can be a mixture of diploid and triploid or even tetraploid cells. Androgenetic/biparental mosaic conceptions with a molar component, which is most often a CHM/early CHM, have in addition a population of purely androgenetic villi (see the section on ancillary techniques for more details).

**DIAGNOSTIC REPRODUCIBILITY AND IMPORTANCE OF ACCURATE CLASSIFICATION OF MOLAR AND NONMOLAR SPECIMENS**

The diagnosis of hydatidiform moles can often be accomplished on the basis of morphologic assessment alone when characteristic features are well developed. However, a number of studies have demonstrated that there is diagnostic variability (suboptimal interobserver and intraobserver reproducibility) for hydatidiform moles based on routine assessment of hematoxylin-eosin–stained slides, even among experienced pathologists with specialized training. In general, problems in classification can be attributed to several factors, including imperfect histologic criteria for diagnosing hydatidiform moles, variability in how pathologists apply diagnostic criteria, and the known variation in morphologic features dependent on the gestational age of the specimen. With the widespread use of routine first-trimester ultrasonography, the latter factor has become significant because most products of conception specimens, including molar and nonmolar ones, are encountered at much earlier gestational ages when histologic features are less well developed.

Examples from a reproducibility study in which diagnoses were established using ancillary techniques are shown in Figures 3 and 4, with details for the cases in these figures provided in Table 2; summary data from the study are provided in Supplemental Figure 1 (find supplemental digital content at www.archivesofpathology.org in the December 2018 table of contents; see the section on ancillary techniques for details of those diagnostic methods).

Distinction of hydatidiform moles from nonmolar specimens and the subclassification of hydatidiform moles as CHM versus PHM are important for both clinical practice and investigational studies. Accurate classification is critical to ascertaining the actual risk of persistent gestational trophoblastic disease (GTD) associated with the various subtypes of hydatidiform moles and determining the appropriate nature and duration of clinical follow-up. Both underdiagnosis and overdiagnosis of hydatidiform moles can result in a faulty estimation of the risk of persistent GTD and improper clinical management. The risk of persistent GTD and clinical management differ for CHMs, PHMs, and nonmolar specimens.

Based on well-defined cases in the modern literature, the risk of persistent GTD following CHM is 9% to 20%, whereas persistent GTD following a PHM is 0% to 4%. Most cases of persistent GTD following a hydatidiform mole are invasive moles, but 3% to 5% present as choriocarcinoma. Despite the lower risk associated with PHMs, metastatic GTD and trophoblastic tumors (choriocarcinoma and placental site trophoblastic tumor) coexist with or subsequent to a diagnosis of a well-documented PHM have been reported. Hence, although localized and metastatic persistent GTD is much more common after a diagnosis of CHM, it can and does occur following a PHM. Consequently, patients with PHMs should receive follow-up with serum β-human chorionic gonadotropin (hCG) levels in conjunction with contraception until undetectable levels are obtained. The risk of persistent GTD for familial biparental CHMs is similar to that of sporadic androgenetic CHMs. In contrast, because persistent GTD following a first-trimester nonmolar spontaneous abortion is rare (estimated risk of ≤0.0002%), spontaneous abortion or termination of a nonmolar pregnancy does not inherently warrant follow-up with serum hCG levels or contraception until undetectable levels are obtained. Thus, avoiding misclassification of nonmolar specimens, particularly those with abnormal villous morphology, as PHMs is of particular importance to patients with infertility for whom mandated contraception during a period of hCG monitoring (even if abbreviated) is undesirable.

**ANCILLARY TECHNIQUES FOR REFINED DIAGNOSIS OF MOLAR AND NONMOLAR SPECIMENS**

In view of the limitations of morphologic assessment and the clinical importance of accurate diagnosis of molar specimens, use of ancillary techniques to refine the diagnosis of hydatidiform moles is recommended. The value of immunohistochemical analysis of p57 expression and DNA genotyping via PCR amplification of short tandem repeat (STR) loci for improving the diagnosis of hydatidiform moles has been demonstrated in a number of studies. These techniques exploit the unique genetic features of molar and nonmolar specimens to improve diagnostic accuracy.

**Immunohistochemical Analysis of p57 Expression**

p57 is the gene product of the paternally imprinted, maternally expressed gene CDKN1C, a cyclin-dependent kinase inhibitor located on chromosome 11p15.5. CHMs, including the early forms, which lack a maternal genetic contribution, have absent (or very limited) p57 expression in villous cytotrophoblast and villous stromal cells (Figure 5, A). In contrast, both PHMs and nonmolar specimens (including those with abnormal villous morphology) contain a maternal chromosome complement and exhibit diffuse p57 expression in these cell types (Figure 5, B and C). This differential p57 expression has been found to be useful in
Figure 1. A and B, Complete hydatidiform mole. Hydropically enlarged villi have trophoblastic hyperplasia (A) as well as cisterns and trophoblastic inclusions (B). C and D, Early complete hydatidiform mole. Cauliflower-like villi have trophoblastic hyperplasia, and cellular myxoid stroma contains canalicular vascular structures and karyorrhectic nuclear debris. E and F, Partial hydatidiform mole. Irregularly shaped villi have scalloped contours, mild trophoblastic hyperplasia, and trophoblastic inclusions. G and H, Nonmolar abnormal villous morphology associated with trisomy. Variably sized, irregularly shaped villi with focal mild trophoblastic hyperplasia simulate a partial hydatidiform mole. I and J, Early nonmolar abortus. Immature chorionic villi have polarized trophoblastic hyperplasia. K and L, Hydropic abortus. Villi are edematous but lack other features of a hydatidiform mole.
the distinction of CHMs (including early forms) from PHMs and nonmolar specimens.\textsuperscript{19,31,32,56–68} In addition, interpretation of p57 immunostains is highly reproducible.\textsuperscript{40,41} However, this marker has the limitation of not being able to discern PHMs from nonmolar specimens (the latter including both biparental diploid nonmolar specimens and digynic triploid nonmolar specimens) because all of these entities maintain p57 expression due to the presence of a maternal chromosome complement. Therefore, other methods, such as genotyping, are required to definitively distinguish PHMs from nonmolar specimens.

To interpret immunohistochemical stains for p57, the presence or absence of nuclear positivity is assessed in villous cytotrophoblast and villous stromal cells, as well as in any intermediate trophoblastic cells and maternal decidua present in the stained section. The p57 immunostain is interpreted as “negative” when villous cytotrophoblast and villous stromal cells are either entirely negative or demonstrate only limited expression (nuclear staining in <10% of these cell types). It is important to also assess the adequacy of the stain by identifying the presence of nuclear p57 expression in intermediate trophoblastic cells and/or maternal decidua cells. Expression in these cellular components serves as an internal positive control in all cases, including CHMs. Expression of p57 in the intermediate trophoblast of CHMs is thought to be related to “epigenetic relaxation” (expression from the parental copy of the gene because a maternal copy is lacking). The p57 immunostain is interpreted as “positive” when the extent of staining is extensive or diffuse in these cell types (expression in >50% of these cells). The interpretation of p57 immunohistochemistry is typically straightforward in that the cellular components in which p57 is differentially expressed (villous cytotrophoblast and villous stromal cells) are almost always uniformly negative or diffusely positive; intermediate/focally positive, discordant, or divergent staining patterns in these cell types are uncommonly encountered. A few studies have described a limited extent of p57 expression (scattered nuclear positivity in villous cytotrophoblast and villous stromal cells) in a minority of cases of both diploid and tetraploid CHMs; this limited extent of expression (present in <10% of these cell types) is still considered compatible with a diagnosis of CHM.\textsuperscript{56,61,62} Typical examples of p57 staining patterns in a CHM, a PHM, and a nonmolar specimen are illustrated in Figure 6. It is also important to ensure that p57 expression in intermediate trophoblastic cells and decidua is at the appropriate level and demonstrates only nuclear expression without excessive nonspecific cytoplasmic staining. When the latter occurs, it is possible to generate some degree of p57 expression in the villous cytotrophoblast and/or stromal cells of a CHM, leading to erroneous interpretation as a positive result that would exclude a diagnosis of a CHM (Figure 7).

In addition to the typical diffusely positive p57 staining result, variants of positive staining can be encountered occasionally. A p57 immunostain can be considered as “focally positive” when nuclear expression in both villous stromal cells and cytotrophoblast is in the focally positive range (>10% but <50% of the villi in the stained section). In our experience, this degree of staining has only been encountered in PHMs (with some frequency) and nonmolar specimens, but never in molecularly confirmed CHMs, so interpretation as a fundamentally positive result is justified. The p57 immunostain is interpreted as “discordant” when there is any combination/admixture of negative and positive results for villous cytotrophoblast and villous stromal cells within individual villi, including positive staining in cytotrophoblast and negative staining in villous stromal cells (most cases), or vice versa. Discordant p57 expression is characteristic of androgenetic/biparental mosaic conceptions, with discordant expression in different cell types based on the presence or absence of maternal genetic material in those particular cells. In these cases, the p57 cells are androgenetic (usually diploid) and the p57 cells are biparental (usually also diploid, but some can be triploid or tetraploid or a mixture of these).\textsuperscript{31,32,34} The p57 immunostain is interpreted as “divergent” when 2 populations of villi, each with different morphologies, exhibit 2 different staining patterns (eg, a typical “negative” result in one set and typical “positive” result in the other set). A twin gestation comprising a typical androgenetic diploid CHM with a lack of p57 expression and a typical biparental diploid nonmolar abortus with a positive p57 result exemplifies this situation.\textsuperscript{14} Another form of divergent p57 expression (also with discordant expression) is encountered in androgenetic/biparental mosaic conceptions with a molar component (Figure 8). In these cases, the nonmolar androgenetic/biparental mosaic component has discordant p57 expression—usually positive staining in cytotrophoblastic cells and negative staining in villous stromal cells (Figure 8, B through D)—and the molar component, which has features of a monozygous.

Figure 2. Genetics of hydatidiform moles. A, Complete hydatidiform mole. Most commonly, 1 sperm enters an ovum devoid of maternal nuclear genetic material and duplicates, giving rise to a purely androgenetic conception (paternal genome only), which is usually diploid (~85% monospermic/homozygous). B, Partial hydatidiform mole. Most commonly, 2 sperm fertilize an ovum, giving rise to a diandric monogynic triploid conception (~99% dispermic/heterozygous).
CHM, is negative for p57 (Figure 8, E through H); thus, the 2 components have divergent patterns relative to each other (1 discordant and 1 negative).

In addition to these variants of positive p57 results, both aberrant retention and loss of p57 expression are rarely encountered in specific situations. Two molecularly confirmed androgenetic CHMs with diffuse p57 expression attributable to a retained maternal chromosome 11 (location of the p57 gene) have been described (Figure 9, A and B). Two molecularly confirmed PHMs—one diandric triploid and the other triandric tetraploid—with loss of p57 expression attributable to loss of the maternal copy of chromosome 11 have been reported (Figure 9, C and D).

**DNA Genotyping**

Molecular genetic analysis of the type provided by PCR-based DNA (STR) genotyping offers greater diagnostic discriminatory capability than other genetic techniques in that CHMs, PHMs, and nonmolar specimens can be distinguished from one another by specifically discerning the purely androgenetic nature of CHMs from the diandric triploidy of PHMs, and both of these from the biparental allelic balance of nonmolar specimens. Thus, this technique can establish diagnostic truth. Other ancillary techniques, including conventional cytogenetics (karyotype), DNA ploidy analysis (flow cytometry, image analysis), and fluorescence in situ hybridization, have the limitation of not being able to establish maternal/parental contributions of chromosome complements and cannot absolutely determine the true diagnosis. Thus, although diploid and triploid results obtained with these latter techniques can improve recognition of CHMs and PHMs in the context of sufficiently developed morphologic alterations, CHMs (particularly some early forms) cannot be distinguished from...
and PHMs cannot be distinguished from diploid triploid specimens on the basis of these results alone and with uterine biopsy. Using DNA genotyping, PHMs and digynic triploidy cannot be distinguished from nonmolar specimens because of shared p57 expression patterns. This technique, used in conjunction with morphology, is the best one suited in fact for the diagnosis and classification of early CHMs, nonmolar specimens with abnormal villous morphology, and even digynic triploid specimens as PHMs. Digynic triploid specimens usually do not exhibit the morphologic features of PHMs, but on occasion, the villous morphology features of PHMs can have some focal dysmorphic features to suggest a PHM, which can lead to potential overdiagnosis as such if ploidy analysis, rather than DNA genotyping, is used.

STRs are repetitive DNA sequences that are highly polymorphic in the population. These genetic markers have been developed for identity, forensic, criminal, and relationship (paternity) testing. DNA genotyping using STR analysis generally involves PCR amplification of multiple STR loci using fluorescently labeled PCR primers, followed by capillary electrophoresis (Figure 10, A). The material used for analysis is formalin-fixed, paraffin-embedded tissue sections. Areas of pure villous and decidual tissue are identified on a stained section, which is used to guide macrodissection of these tissues from serial unstained sections (Figure 10, B through D). The analysis of hydatidiform moles, which is a complex and challenging task, involves the use of DNA genotyping and STRs to confirm the diagnosis of PHMs and to rule out other entities such as nonmolar pregnancies.

Figure 4. Challenging examples of hydatidiform moles from a reproducibility study. A through C, Complete hydatidiform mole, p57 genotyping-proven androgenetic. This example was recognized as molar, but the consensus assessment was a partial hydatidiform mole with a focal polyplody-proven androgenetic interpretation. There was no consensus diagnosis in either of 2 rounds assessing morphology. When p57 was used, the consensus interpretation was a complete hydatidiform mole (see Table 2 for details).

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each locus are identified for both the maternal (decidua) and villous tissues, and the patterns are compared. Alleles in the villous tissue are identified as paternal (nonmaternal) or likely maternal (also possibly paternal because of shared alleles). The copy number/dosage of each allele relative to the other can be determined by calculating an allelic ratio, which compares either the peak height or peak area of the 2 alleles. In general, when 2 alleles are present in equal dosage, the ratio will be approximately 1:1. When 1 allele is in double dosage compared with the other (eg, trisomy/triploidy), the ratio will be approximately 2:1. Specific details for the interpretation of STR data can be found elsewhere. 

Diagrams illustrating the cellular components of molar, nonmolar, and mosaic entities and corresponding genotyping data are provided in Figures 11 and 12. Complete hydatidiform moles are composed of p57 villous cytotrophoblastic cells and stromal cells. By genotyping, they are diagnosed by the finding of paternal to maternal allele ratios of 2:1 at informative loci. This is manifested as either 3 unique alleles, 2 paternal and 1 maternal, with equal ratios, or as 2 alleles with a 2:1 ratio and with the allele in double dosage being paternal (Figure 11, C and D). Rare triandric tetraploid examples (4 sets of chromosome complements, with 1 maternal in origin and 3 paternal in origin) have paternal to maternal allele ratios of 3:1 at informative loci. Nonmolar specimens are most often composed of p57 biparental diploid cells. They are diagnosed as such when the genotyping demonstrates balanced biparental allele ratios (ratios of 1:1) at informative loci (Figure 12, A and B). Those with abnormal villous morphology related to other nonmolar genetic alterations, such as trisomy, can have an altered ratio at one or even a few loci if the affected chromosomes are covered by the markers used in the genotyping kit. The number of loci with imbalanced ratios and the allele pattern will depend on the number of chromosomes involved and the parental source, but most loci will have balanced biparental allele ratios. Nonmolar specimens with digynic triploidy will have 2:1 allele ratios at informative loci, but none of these will demonstrate evidence of 2 novel/obligate paternal alleles. Nonmolar mosaic conceptions most commonly are com-

| Table 2. Diagnostic Reproducibility Data for Examples in Figures 3 and 4 |
| Example | Round 1: H&E | Round 2: H&E | Round 3: H&Ep57 | Genotyping Diagnosis |
| Figure 3, A through C | Path 1: CHM | Path 1: CHM | Path 1: CHM | Path 1: Negative | Androgenetic CHM |
| Path 2: CHM | Path 2: CHM | Path 2: CHM | Path 2: CHM | Path 2: Negative |
| Figure 3, D through F | Path 1: PHM | Path 1: PHM | Path 1: PHM | Path 1: Positive | Diandric triploid PHM |
| Path 2: PHM | Path 2: PHM | Path 2: PHM | Path 2: PHM | Path 2: Positive |
| Figure 4, A through C | Path 1: CHM | Path 1: PHM | Path 1: CHM | Path 1: Negative | Androgenetic CHM |
| Path 2: PHM | Path 2: PHM | Path 2: CHM | Path 2: CHM | Path 2: Negative |
| Figure 4, D through F | Path 1: PHM | Path 1: PHM | Path 1: PHM | Path 1: Negative | Androgenetic CHM |
| Path 2: CHM | Path 2: CHM | Path 2: CHM | Path 2: CHM | Path 2: Negative |
| Path 3: NM | Path 3: NM | Path 3: NM | Path 3: NM | Path 3: Negative |

Abbreviations: CHM, complete hydatidiform mole; H&E, hematoxylin-eosin; NM, nonmolar specimen; PHM, partial hydatidiform mole.

a Three gynecologic pathologists (Path 1, Path 2, and Path 3) reviewed an H&E-stained slide in rounds 1 and 2 and rendered diagnoses; they then reviewed the H&E slide with a p57 immunostain in round 3 and rendered diagnoses in conjunction with interpretation of the p57 immunostain.

b Cannot distinguish diandric triploidy from digynic triploidy.

c For digynic triploid nonmolar specimens and examples with trisomy for the chromosome(s) probed; distinction of triploidy and trisomy requires use of appropriate probes.

d Cannot distinguish androgenetic diploidy/tetraploidy from biparental diploidy/tetraploidy.

e Cannot specifically distinguish diploid/tetraploid from tetraploid.

Table 3. Methods for Distinction of Molar and Nonmolar Specimens

<table>
<thead>
<tr>
<th>Diagnostic Technique</th>
<th>Complete Hydatidiform Mole</th>
<th>Partial Hydatidiform Mole</th>
<th>Nonmolar Abortus</th>
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<tbody>
<tr>
<td>Ploidy/DNA Content</td>
<td>Good for examples with adequately developed characteristic features, but imperfect diagnostic reproducibility limit performance</td>
<td>Triploidb (some tetraploid)</td>
<td>Diploidb (rare tetraploid)</td>
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<tr>
<td>Hybridization</td>
<td>2 signalsa (4 signals)</td>
<td>3 signalsb (4 signals)</td>
<td>2 signalsb (4 signals)</td>
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<td>FISH</td>
<td>Negative</td>
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<td>Positive</td>
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<tr>
<td>DNA Genotyping</td>
<td>Androgenetic conception</td>
<td>Diandric triploid</td>
<td>Bi parental conception with allele balance (except for trisomic or monosomic loci)</td>
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a Cannot distinguish androgenetic diploidy/tetraploidy from biparental diploidy/tetraploidy.

b Cannot distinguish diandric triploidy from digynic triploidy.

c For digynic triploid nonmolar specimens and examples with trisomy for the chromosome(s) probed; distinction of triploidy and trisomy requires use of appropriate probes.
posed of villi with a mixture of p57+ biparental villous cytotrophoblastic cells and p57- androgenetic villous stromal cells (p57-discordant villi). By genotyping, they demonstrate an excess of androgenetic alleles with variable paternal to maternal allele ratios typically greater than 2:1 at informative loci, reflecting the admixture of androgenetic and biparental cell lines within individual villi (enrichment for paternal alleles attributable to the mixture of some cells with 2 paternal copies [no maternal copy] and other cells with 1 paternal and 1 maternal copy of a given allele; Figure 12, C and D). Androgenetic/biparental mosaic conceptions with a nonmolar mosaic component and a molar component showing features of a CHM comprise p57-discordant and p57-C0 villi, respectively, with each component being similar to the pure forms of these entities. When each component can be individually genotyped, the results generated will also be the same as for the pure forms of these entities (Figure 12, C and D).

DNA genotyping can greatly improve the diagnosis of hydatidiform mole, but interpretive challenges and unusual scenarios exist that require the careful correlation of morphology, p57 results, and genotyping data. These include the following:

1. Specimens in which only villous tissue is available for analysis. Lack of maternal decidual tissue precludes determination of the parental source of polymorphic alleles and their ratios. In this situation, analysis of the villous tissue can yield results for which allelic balance and triploidy can be discerned, but the parental contributions cannot be determined without comparison of the patterns in the villous and decidual tissues. Thus, a nonmolar biparental pattern with allelic balance cannot be distinguished from the heterozygous form of purely androgenetic molar conception, and diandric versus digynic triploidy cannot be distinguished. The information obtained from the analysis would be essentially identical to that obtained with genotyping for chromosome number. However, even without the maternal tissue control, the presence of homozgyosity at all STR loci in villous tissue establishes a diagnosis of a homozygous androgenetic CHM. In this situation, androgenicity is inferred (because alleles cannot be confirmed as nonmaternal) and supported because this pattern is only obtained in purely androgenetic conceptions (when sufficient loci are tested, non-molar conceptions never have purely homozgyous loci).

2. Mosaic conceptions, particularly those with a molar component (CHM). The presence of 2 morphologically distinct populations of villi (mosaic villi without trophoblastic hyperplasia and molar villi with trophoblastic hyperplasia) can lead to misclassification as a PHM. In addition, failure to recognize the discordant p57 expression pattern in some villi (often the majority) and the p57- molar component (which can be focal) contributes to misinterpretation as a p57+ PHM. These can generate complicated genotyping results, which can be challenging to interpret, particularly when the different villous components of the molar form are admixed in tested

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**Figure 5.** Mechanism of p57 expression in molar and nonmolar specimens. **A.** An androgenetic complete hydatidiform mole has aberrant loss of p57 expression because the maternal copy is absent (no expression from methylated paternal copies). Immunohistochemical analysis of p57 expression demonstrates that the nuclei of villous cytotrophoblast and stromal cells are negative (intermediate trophoblastic cells are always positive and serve as internal positive control). **B.** A partial hydatidiform mole has normal expression of p57 from the maternal copy. Immunohistochemical analysis of p57 expression demonstrates that the nuclei of villous cytotrophoblast and stromal cells are positive (intermediate trophoblastic cells are also positive). **C.** A biparental nonmolar conception has normal expression of p57 from the maternal copy. Immunohistochemical analysis of p57 expression demonstrates that the nuclei of villous cytotrophoblast and stromal cells are positive (aggregates of intermediate trophoblastic cells are also positive).
Figure 6. Examples of p57 expression in typical molar and nonmolar specimens. A and B, Early complete hydatidiform mole, with negative p57 immunostain. Villous cytotrophoblast and stromal cells are negative; intermediate trophoblastic cells are positive, serving as an internal positive control. Genotyping confirmed this as a purely androgenetic conception (see Figure 11, B). C and D, Partial hydatidiform mole, with positive p57 immunostain. Villous cytotrophoblast and stromal cells are positive. Genotyping confirmed this as a diandric triploid conception (see Figure 11, D). E and F, Nonmolar abnormal villous morphology related to trisomy, with positive p57 immunostain. Villous cytotrophoblast and stromal cells are positive. The features simulate a partial hydatidiform mole, and the p57 result does not distinguish these entities. Genotyping confirmed this as a biparental conception with allelic balance and a trisomy (see Figure 12, B).
Figure 7. Complete hydatidiform mole with different p57 results obtained in different laboratories related to technical factors. A and B. Villi show typical features of a complete hydatidiform mole. C, The p57 immunostain performed in the originating laboratory demonstrates positivity in villous cytotrophoblast and stromal cells, which led to doubt regarding the morphologic diagnostic impression and a request for consultation. D, Decidual tissue on the p57 immunostain has higher than desirable nonspecific cytoplasmic staining, indicating that the positive result may be unreliable. E, The p57 immunostain performed in the consulting laboratory is negative (with adequate internal positive control in intermediate trophoblastic cells), confirming the morphologic impression. F, Decidual tissue on the p57 immunostain from the consulting laboratory demonstrates the appropriate nuclear expression without any nonspecific cytoplasmic staining. Genotyping confirmed this as a purely androgenetic conception.
Recognition of the different p57 staining patterns in mosaic molar conceptions is necessary for specific macrodissection of the distinct villous components to ensure accurate genotyping and correct interpretation of these complex specimens.

3. Individual, double, and rare multiple trisomies. These have the potential to be interpreted erroneously as triploidy if a sufficient number of informative loci are not evaluated, resulting in misclassification as a diandric triploid PHM if the extra chromosomes are paternal in origin.12

4. Rare cases of biparental CHMs. These share morphology and lack of p57 expression with typical androgenetic CHMs, but the DNA genotyping result of biparental diploidy could be misinterpreted as a nonmolar conception in the absence of correlation with morphologic features and p57 results.

5. Rare CHMs with aberrant retained p57 expression due to a retained maternal chromosome 11 (maternally derived trisomy of chromosome 11 in the setting of an otherwise purely androgenetic conception).7,76

6. Rare PHMs with aberrant loss of p57 expression due to loss of the maternal copy of chromosome 11.19,77

7. Complete hydatidiform moles occurring in the setting of a twin/multiple gestation. Failure to recognize the morphologically and immunohistochemically distinct villous populations can lead to incorrect interpretation as a PHM (“2” populations of villi, with some being p57+) and false assurance that a CHM has been excluded because of p57 positivity in at least some villi. Similar to mosaic specimens, recognition of 2 populations of villi with different p57 staining patterns is critical for accurate genotyping and interpretation of results.

8. Donor egg conceptions. Lack of clinical history and failure to correlate morphology and p57 results with genotyping results can lead to misinterpretation of a nonmolar specimen as a heterozygous/dispermic CHM on the basis of genotyping data alone.78

**ALGORITHMIC APPROACH TO DIAGNOSIS OF HYDATIDIFORM MOLES**

Algorithmic approaches using ancillary techniques applicable to formalin-fixed, paraffin-embedded tissues have been proposed for refining the diagnosis of hydatidiform moles.34,19,44,72,79 Reproducibility studies demonstrate that routine microscopic evaluation without use of ancillary techniques, even in the hands of gynecologic pathologists...
and even when a consensus diagnosis is used, leads to incorrect classification of at least 20% of cases.\textsuperscript{40,41} This suggests that there are inherent limitations in the ability of morphologic assessment to provide an accurate diagnosis of all cases, which is likely related to the known morphologic overlap of the entities and the lack of fully developed morphologic features in early examples. Given that experienced gynecologic pathologists already have subspecialty training and focused practice in gynecologic pathology, it is unlikely that there is any way to improve diagnosis using traditional morphologic (hematoxylin-eosin) assessment alone. Thus, use of an algorithmic approach that combines p57 immunohistochemistry and DNA genotyping for improving the diagnosis of hydatidiform moles is recommended (Figure 13).

The proposed approaches use standard histology for morphologic evaluation to select tissue for analysis and advocate genotyping as the preferred molecular technique (use of less specific techniques, such as flow cytometric DNA ploidy analysis or fluorescence in situ hybridization, is discouraged). One approach uses universal assessment of p57 expression by immunohistochemistry for all potentially molar specimens, with triage to genotyping based on the p57 result. If the morphologic features suggest a CHM and the p57 immunostain is negative (with satisfactory internal positive control), a diagnosis of CHM can be confidently established (with rare exceptions\textsuperscript{19,77}). Analysis of p57 expression by immunohistochemistry is highly reproducible and is a technique that can be performed in most immunohistochemistry laboratories without the need for highly specialized equipment and expertise, such as that required for genotyping. DNA genotyping can confirm a diagnosis of CHM for a p57\textsuperscript{−} specimen by demonstrating a purely androgenetic DNA pattern but is not necessary for routine diagnosis, provided the p57 result is satisfactory. If the clinical scenario suggests a recurrent familial case, then genotyping would be indicated to establish a diagnosis of a biparental rather than androgenetic CHM.

Figure 9. Examples of aberrant p57 expression. A and B, Complete hydatidiform mole with aberrant p57 expression attributable to a retained maternalcopy of chromosome 11 (location of p57). Villi demonstrate typical features of a complete hydatidiform mole, but diffuse p57 expression in villous cytotrophoblast and stromal cells is not expected for that diagnosis. Genotyping demonstrated a purely androgenetic conception with the exception of the locus on chromosome 11, where there was evidence of trisomy (2 paternal copies and 1 maternal copy). C and D, Partial hydatidiform mole with aberrant loss of p57 expression attributable to loss of the maternal copy of chromosome 11. Villi demonstrate features suggesting an early complete hydatidiform mole, and the loss of p57 expression would support that interpretation. However, genotyping demonstrated a diandric triploid conception with the exception of the locus on chromosome 11, where there was evidence of uniparental disomy (2 paternal copies and no maternal copies).
Figure 10. Methods for genotyping. A, Polymerase chain reaction amplification of short tandem repeat loci. Fluorescently labeled primers are used to amplify each locus to generate copies of the maternal and paternal alleles. When these are of different sizes (heterozygous, locus 1), this will generate 2 peaks on capillary electrophoresis (1, pink and blue peaks). When these are the same (homozygous/shared, locus 2), this will generate only 1 peak (2, purple peak). B through D, Method for obtaining samples from formalin-fixed, paraffin embedded tissue sections. Areas of pure villous (marked V) and pure decidual (marked D) tissue are circled with a marking pen on a stained section (B). On a sequential unstained section, these loci are covered with a solution to facilitate removal from the slide (C, blue solution applied). These areas are carefully macro-dissected from the tissue section (D, tissue removed in circled areas).

Figure 11. Diagrams of molar entities with genotyping examples. A, Diagram of a villous structure from a complete hydatidiform mole demonstrates that the villus is composed of p57\(^-\) androgenetic diploid cytotrophoblastic cells and p57\(^-\) androgenetic diploid stromal cells. B, Per genotyping, all informative loci demonstrate that villous tissue contains only novel/paternal alleles (blue arrows) without maternal alleles (pink arrows), indicating a purely androgenetic conception. The presence of a single allele at all loci indicates a monospermic (homozygous) complete hydatidiform mole. C, Diagram of a villous structure from a partial hydatidiform mole demonstrates that the villus is composed of p57\(^+\) diandric triploid cytotrophoblastic cells and p57\(^+\) diandric triploid stromal cells. D, Per genotyping, 1 fully informative locus (CSF1PO) demonstrates that villous tissue contains a maternal allele (pink arrows) and a novel/paternal allele (blue arrow), with a paternal to maternal allele ratio of 2:1. All other loci are consistent with triploidy but are not fully informative because they do not establish the parental origins as a result of allele sharing (purple arrows). The combined findings indicate a diandric triploid conception, and the presence of 3 distinct alleles at 1 locus (VWA) in the setting of at least 1 locus establishing diandry (CSF1PO) indicates a dispermic (heterozygous) partial hydatidiform mole.
immunostain is positive, then a CHM has been excluded (with rare exceptions\textsuperscript{75,76}). For p57\textsuperscript{+} cases, DNA genotyping is required to definitively distinguish a PHM from a nonmolar specimen. When the molecular analysis reveals a diandric triploid result, a diagnosis of a PHM is established. When genotyping yields a biparental conception with allelic balance (paternal to maternal allele ratio of 1:1), indicating a biparental nonmolar conception, another approach uses universal genotyping based on morphologic assessment suggesting any kind of hydatidiform mole, with selective application of p57 immunohistochemistry to address any discordance between morphology and genotyping. In this approach, p57 immunohistochemistry is used only when there is a discrepancy between the morphology and the genotyping result (eg, rare cases of familial biparental CHMs, mosaicism, or CHM in a twin gestation).

In laboratories with experience and excellent technical support, these approaches yield a definitive diagnosis in a very high proportion of cases.\textsuperscript{19} These approaches are advocated when there is any suspicion for a hydatidiform mole, which includes either a clinical concern for a hydatidiform mole (eg, abnormally elevated $\beta$-hCG level, abnormal ultrasound findings, clinical diagnosis of “rule out molar pregnancy,” etc) or pathologic concern because of

Figure 12. Diagrams of nonmolar and mosaic entities with genotyping examples. A, Diagram of a villous structure from a nonmolar specimen demonstrates that the villus is composed of p57\textsuperscript{+} biparental diploid cytotrophoblastic cells and p57\textsuperscript{+} biparental diploid stromal cells. B, Per genotyping, fully informative loci demonstrate that villous tissue contains 1 maternal allele (pink arrows) and 1 novel/paternal allele (blue arrows) with allelic balance (paternal to maternal allele ratio of 1:1), indicating a biparental nonmolar conception. C, Diagram of a nonmolar androgenetic/biparental villous structure demonstrates that the villus is composed of p57\textsuperscript{+} biparental diploid cytotrophoblastic cells and p57\textsuperscript{+} androgenetic diploid stromal cells. Villi of this type can represent an entire specimen (nonmolar form of androgenetic/biparental conception) or can be accompanied by a purely androgenetic villous component in which some villi are composed of p57\textsuperscript{+} androgenetic diploid cytotrophoblastic cells and p57\textsuperscript{+} androgenetic diploid stromal cells (androgenetic/biparental mosaic conception with a molar component; mixture of villous types shown in Figures 11, A, and 12, C). D, When genotyping is performed on the mixture of cell lines in the nonmolar mosaic villi (D, middle), several informative loci show that villous tissue contains both maternal alleles (pink arrows) and novel/paternal alleles (blue arrows), with a paternal to maternal allele ratios of >2:1. In conjunction with the p57 staining pattern indicating a mixture of p57\textsuperscript{+} (purely androgenetic) villous stromal cells and p57\textsuperscript{+} (biparental) cytotrophoblastic cells (see Figure 8, B through D, for histology), and fluorescence in situ hybridization (FISH) results indicating that all villous stromal cells and cytotrophoblastic cells are diploid (data not shown), these ratios reflect an enrichment for paternal alleles attributable to the mixture of purely androgenetic cells with 2 paternal alleles and biparental cells with 1 paternal and 1 maternal allele. For the molar component (D, lower) which is uniformly p57\textsuperscript{+} (see Figure 8, E through H, for histology) and also has uniformly diploid cells per FISH analysis (data not shown), several informative loci demonstrate that villous tissue contains only paternal alleles, consistent with a purely androgenetic molar (complete hydatidiform mole) component.

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some morphologic abnormality of chorionic villi. Use of the p57 triage method represents a compromise between traditional morphologic assessment alone, which has limitations, and genotyping of all cases, which is clearly costlier. The p57 component of the algorithm should capture essentially all CHMs, the most important group to readily identify for clinical management purposes. Because PHMs have a low but real risk of persistent GTD and are managed as molar pregnancies per current guidelines (sometimes with abbreviated follow-up relative to management of CHMs), and because overdiagnosis of nonmolar specimens as molar pregnancies is associated with infertility patients, genotyping of all p57-positively molar specimens per the algorithm provides for a definitive distinction of PHMs and nonmolar specimens, allowing for refined management of these entities. In the setting of limited resources, use of ancillary techniques can be focused on identifying the entity with the greatest risk for persistent GTD, namely, CHMs, by selectively applying only p57 immunohistochemistry to assist in diagnosing CHMs and foregoing genotyping for distinction of PHMs from nonmolar specimens. For the latter situation, an equivocal diagnosis, such as “abnormal villous morphology. PHM cannot be excluded.” might need to be rendered. Because the risk of persistent GTD for PHMs is much closer to that of nonmolar specimens than that of CHMs, this may well be acceptable for routine practice, with the understanding that an equivocal diagnosis will potentially lead to clinical management as a PHM at least for some abbreviated time frame, and that this approach does have accompanying costs (clinic visit, multiple serum β-hCG levels, contraception) which might well rival the cost of genotyping. With such a limited approach, it also needs to be understood that an apparently unequivocal diagnosis of either PHM or a nonmolar specimen established on the basis of morphologic assessment alone is not guaranteed to be accurate, even when rendered by an experienced gynecologic pathologist. Therefore, given the established suboptimal performance of morphologic assessment alone, even for experienced gynecologic pathologists, the most ideal method of correctly classifying all hydatidiform moles and nonmolar specimens is a combined approach including correlation of morphologic features, p57 immunohistochemistry, and DNA genotyping. In investigational pursuits, all molar specimens should be evaluated with ancillary techniques to ensure the rigorous classification of cases, particularly when designed to ascertain risk of persistent GTD associated with the various subtypes of hydatidiform moles.

**SUMMARY**

Studies using genotyping to establish true gold standard diagnoses have confirmed that morphologic diagnosis of hydatidiform moles continues to be negatively impacted by interobserver diagnostic variability both in routine and specialized gynecologic pathology practice. Diagnostic variability compromises investigations of the epidemiology, pathogenesis, and behavior of hydatidiform moles by using inaccurately classified cases. The modern approach to diagnosis of hydatidiform moles should require integration of ancillary techniques, particularly p57 immunohistochemistry and DNA genotyping, into routine practice as much as possible, with the goals of providing refined diagnosis, accurate assessment of the risk of persistent GTD associated with different subtypes of hydatidiform moles, and the appropriate guidance of clinical management.

**References**


