Molecular Diagnostics of Hydatidiform Moles

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Disclosure

- I am not a pathologist......
Topics/Objectives

- Limitations of morphology for diagnosis
- Genetics of hydatidiform moles (HM)
- Ancillary techniques for distinguishing hydatidiform moles from non-molar entities and for subtyping HMs
  - Immunohistochemistry for p57
  - Molecular genotyping
- Examples
Differential Diagnosis

- Types of hydatidiform moles:
  - Complete hydatidiform mole (CHM)
    - Early CHM
  - Partial hydatidiform mole (PHM)
- Non-molar entities capable of simulating hydatidiform moles:
  - Early/hydropic abortus (EA/HA)
  - Abnormal villous morphology (AVM) associated with non-molar type genetic abnormalities:
    - Trisomies
    - Mosaicism (androgenetic/biparental)
Problems We Face In Making The Diagnosis Based on Morphology

1. The subtypes of hydatidiform moles are often misclassified as one another, particularly with specimens from an early gestation.

2. Hydropic abortuses can mimic complete hydatidiform moles (CHM).

3. Villi with abnormal karyotypes (e.g. trisomy) can bear morphologic similarities to hydatidiform moles.


- 50 cases sent to 7 pathologists
- 1 year later the same cases were re-sent
- 35/50 (70%) had agreement between ≥ five participants.
- Agreement between the expert gynaecological pathologists was no better than for the others.
- Intraobserver agreement for each pathologist was good to excellent.
- Neither non-molar pregnancy nor complete mole could be easily differentiated from partial mole.
Morphologic Overlap of Molar and Non-Molar Specimens
...Back To Basics: Conventional Thinking On How Hydatidiform Moles Develop

Complete Mole:

“Empty Ovum”

“Empty Ovum”

This results in androgenetic diploidy (Diandric, 46XX)
Partial Mole:

This results in triploidy (Diandric, biparental, 69 XXY) phenotype.
Why Is It Important To Get A Diagnosis of Hydatidiform Mole “Right?”

<table>
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<tr>
<th>Hydatidiform Mole Subclassification</th>
<th>Percent of Patients with Persistent GTD</th>
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<tr>
<td>Complete Hydatidiform Mole</td>
<td>20%</td>
</tr>
<tr>
<td>Partial Hydatidiform Mole</td>
<td>&lt; 5%</td>
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Follow-up for a diagnosis of hydatidiform mole:
- Weekly bHCG until 3 normal values obtained.
- Monthly bHCG x 6 months.
- NO new pregnancies during the period of follow-up.

...So, the distinction of what is “molar” (including providing subclassification) versus “non-molar” IS important.

_Fukunaga M et al. AJSP  29(7): 942-947, 2005._
Value of “Getting it Right”

- Achieve best diagnosis (morphology is imperfect)
  - “Non-triploid PHM” = misclassified CHM or AVM (trisomy)
- Identify biologically distinct entities with different risks of persistent gestational trophoblastic disease:
  - 15-20% for CHM
  - 0.5-5% for PHM
- Refine clinical management
  - Contraception and serial hCG levels (6-12 months) for molar pregnancy but not for non-molar abortus
  - Implications for patients with infertility
Tools to differentiate CHM, PHM, and NM

- Morphology
- Ancillary testing
  - Protein analysis:
    - P57 Immunohistochemistry (IHC)
  - Genetic analysis:
    - Karyotype
    - FISH
    - Ploidy by Flow Cytometry
    - Genotyping by short tandem repeat (STR) markers
p57 Immunohistochemistry

- p57 is a cell cycle inhibitor.
- p57 is strongly paternally imprinted.
  - Differential DNA methylation of maternal and paternal alleles leads to expression from only one allele.
  - Expression is from the maternal allele.
p57 is a paternally imprinted maternally expressed gene

Immunohistochemical analysis of p57 protein expression can be utilized for diagnosis of hydatidiform moles
Non-molar Specimen: Biparental Diploidy

Positive in villous stromal cells, cytотrophoblast, and intermediate trophoblast (nuclear expression)
Partial Hydatidiform Mole: Diandric (Monogynic) Triploidy

Positive in villous stromal cells, cytotrophoblast, and intermediate trophoblast (nuclear expression)
Complete Hydatidiform Mole: Androgenetic Diploidy

No maternal DNA

Paternal Chr 11 (2 copies)

RNAs

p57 protein

IHC

Negative in villous stromal cells and cytotrophoblast (intermediate trophoblastic cells +)
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Tools to differentiate CHM, PHM, and NM

- Morphology
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* cannot distinguish diandric from digynic triploidy
^ triploidy and trisomy confused with use of limited probes
Molecular Genotyping using Short Tandem Repeat (STR) Loci (AKA microsatellites)

- Identity testing
- Tetranucleotide repeats (4 basepair repeats)
- Dispersed throughout the genome. Non-coding.
- Polymorphic = the number of repeats is variable in the population

Assay:
- Extract DNA from decidua and villi
- PCR amplify 9 STR loci plus the amelogenin locus (XY identification)
- Capillary electrophoresis.
STR PCR

- PCR primers flank the repeat region.
- Amplification of both alleles (maternal and paternal).
- Fluorescent PCR products are analyzed by Capillary Electrophoresis (CE).
Size in bases

Fluorescence intensity
Interpretation of STR Data

- Comparison of loci in the decidua and villi.
- Identify non-maternal (paternal) alleles, and alleles consistent with maternal.
- Calculate ratio of the two alleles.
- Data
  - Number of alleles and allelic ratios
  - Source of alleles (maternal vs paternal)
Non-molar Villous Tissue: Biparental Diploidy
(1 maternal and 1 paternal chromosome complement)

Maternal

Villous

Shared alleles = maternal

Novel alleles = paternal

Informative loci have 2 distinct alleles, with 1:1 ratio
Villous Tissue in CHM: Androgenetic Diploidy
(2 paternal and no maternal chromosome complements)

Maternal

Villous

Heterozygous (~10%)
(dispermy = 2 novel paternal alleles)

Homozygous (~90%)
(monospermy = 1 novel paternal allele)
Villous Tissue in PHM: Diandric Triploidy
(2 paternal and 1 maternal chromosome complements)

Maternal Homozygous (~10%)
(monospermy = 1 novel paternal allele; P:M = 2:1)

Villous

Heterozygous (~90%)
(dispermy = 2 novel paternal alleles)
Loci Establishing Triploidy but Indeterminate for Source (diandric versus digynic)
Non-informative Loci:
Homozygous Loci and Alleles Shared by Maternal and Paternal DNA

Maternal

Villous
Example of diploidy, 1 maternal, 1 paternal chromosome complement (NHM)
Molecular Genotyping: Androgenetic Diploidy = eCHM

- Androgenetic diploidy due to monospermy (multiple loci with paternal alleles only; single peak at each locus = homozygous pattern)
Example of diandric triploidy (PHM)
The genetics of moles are not that cut and dry...
Case 1

- Consultation case:
- 27 yo with “Missed Abortion, Evaluate For Molar Pregnancy”
- Morphology concerning for a partial mole
Molecular Genotyping:
Biparental Diploidy with Trisomy 7 & 13 (paternal)
= Non-molar AVM
Case 2

Consultation Case:
- 31 G1P0, no prior history of GTD
- Patient presented with vaginal bleeding
- Positive urine pregnancy test
- U/S suspicious for a “mole”
- Morphology typical complete mole
- P57 IHC: POS!
Decidua

D3S1358 (3p)

VWA (12p12-pter)

TH01 (11p15.5)

D11S1981 (11pter-p15.1)

Villous

P, P

P, P

P, P

P, P

M

PM, P
Molecular Genotyping:
Androgenetic Diploidy with Trisomy 11 = CHM

- Androgenetic diploidy (multiple loci) due to dispermy (first locus)
- Trisomy 11 (2 alleles, ratio = 2:1, source unknown; additional markers establish extra copy as maternal)
- CHM with retained maternal chromosomes 6 and 11 (trisomies)
- P57 is located on 11p. The presence of a maternal chromosome 11 resulted in aberrant expression of p57 in a CHM
Case 3

- **Consultation Case:**
  - 25 yo G3 P1011 with 6 week pregnancy by LMP
  - Patient presented with profuse vaginal bleeding
  - A yolk sac and fetal pole identified, but no cardiac activity seen, suggesting fetal demise
  - Abnormal hyperechoic soft tissue throughout
  - Endometrial cavity with numerous tiny cystic spaces suspicious for GTD
  - Working diagnosis by contributor was PHM

- JHU pathologist identified two areas of villi that were morphologically distinct. Each was analyzed separately.
Molecular Genotyping Analysis:

DAD

V1
ALL
DAD

V2
MOM + DAD
Diagnosis:

CHM PLUS A NON-MOLAR TWIN!!!
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Possible Hydatidiform Mole

p57 immunohistochemistry

Negative

Morphology appropriate for CHM?

No or uncertain

Positive

Molecular genotyping

Androgenetic diploidy

Diandric triploidy

Biparental diploidy

CHM (including early CHM)

PHM

Non-molar
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Diagnosis of Hydatidiform Moles: Morphology and Ancillary Techniques

Keywords: Hydatidiform Moles, molecular, genetics, short tandem repeats, p57, immunohistochemistry

Distinction of hydatidiform moles from non-molar specimens and subclassification of hydatidiform moles as complete hydatidiform mole (CHM), partial hydatidiform mole (PHM), or early CHM are important for both clinical practice and investigational studies. The risk of persistent gestational trophoblastic disease (GTD) and hence, clinical management, differs for CHMs, PHMs, and non-molar specimens. However, diagnosis based solely on morphology suffers from poor interobserver reproducibility and remains problematic even for experienced gynecologic pathologists. The unique genetic features of CHMs (androgenetic diploidy), PHMs (diandric triploidy), and non-molar specimens (biparental diploidy) allow for certain molecular techniques, including immunohistochemical analysis of p57 expression (a paternally imprinted maternally expressed gene) and molecular genotyping, to refine the diagnosis of hydatidiform moles. While p57 immunostaining alone can identify CHMs, which lack p57 expression due to the lack of maternal DNA, this analysis cannot distinguish PHMs from non-molar specimens since both express p57 due the presence of maternal DNA. Short tandem repeat (STR) genotyping, which can determine the parental source of polymorphic alleles, can distinguish among all of these entities by discerning androgenetic diploidy, diandric triploidy, and biparental diploidy to rigorously diagnose CHMs, PHMs, and non-molar specimens, respectively. An algorithmic approach using these techniques to refine morphologic diagnosis has been developed for routine practice. This review discusses current issues in the diagnosis of hydatidiform moles, including the limitations of morphologic diagnosis, the need for refined diagnosis to
assure accurate ascertainment of risk of persistent GTD associated with the different subtypes of hydatidiform moles, and the utility of ancillary immunohistochemical and molecular techniques for providing such refined diagnosis.

**Morphologic diagnosis of hydatidiform moles**

Hydatidiform moles include two 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. Early CHMs are characterized by a redundant bulbous villous growth pattern, hypercellular myxoid villous stroma, a labyrinthine network of villous stromal canaliculi, karyorrhectic debris within stroma, and at least focal trophoblastic hyperplasia on villi and the undersurface of the chorionic plate. Characteristic morphologic features of PHMs include the presence of two populations of villi (large, irregular, hydropic villi and small, immature, fibrotic villi), cisterns in some enlarged villi, markedly irregular villi with scalloped borders and stromal trophoblastic inclusions, and mild circumferential trophoblastic hyperplasia.

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 the diagnosis of hydatidiform moles continues to suffer from poor interobserver reproducibility. Even among experienced pathologists, high interobserver and intraobserver variability exist.
experiences with routine and consultation cases confirm that this diagnostic difficulty persists for both general pathologists and experienced gynecologic pathologists.\textsuperscript{10,11}

The differential diagnosis of hydatidiform moles includes a variety of non-molar entities which can exhibit some features suggestive of a molar pregnancy. These include products of conception specimens with abnormal villous morphology (AVM), early abortuses with prominent trophoblastic hyperplasia, and hydropic abortuses. AVM is a less well defined entity in which villi have some features suggestive of PHM (irregular shapes and sizes with limited trophoblastic proliferation, occasionally with syncytiotrophoblastic "snouts") but lack fully diagnostic morphologic features of PHM; in some cases these changes are associated with genetic abnormalities such as trisomy (but not the diandric triploidy of PHMs).\textsuperscript{10,12} Early non-molar gestations at times have trophoblastic proliferation that is sufficiently prominent to raise concern for a CHM but lack other features of a hydatidiform mole. In the earliest examples, the trophoblastic proliferation can form 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 exhibit only villous edema without trophoblastic hyperplasia. In addition, since 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. Variations in the sizes and shapes of the villi, the extent of hydropic change, and the degree of trophoblastic hyperplasia among CHMs and PHMs are sufficient to result in some
morphologic overlap between a subset of CHMs and PHMs (those at the lower and upper ends, respectively, of their morphologic spectra).

**Importance of accurate classification of molar specimens**

Distinction of hydatidiform moles from non-molar specimens and the subclassification of hydatidiform moles as CHM, PHM, or early CHM are important for both clinical practice and investigational studies. Accurate classification is critical to ascertaining the actual risk of persistent GTD associated with the various subtypes of hydatidiform moles and determining the appropriate nature and duration of clinical follow-up care. Both under-diagnosis and over-diagnosis of hydatidiform moles can result in faulty estimation of the risk of persistent GTD and improper clinical management. The risk of persistent gestational trophoblastic disease (GTD) and hence, clinical management, differs for CHMs, PHMs, and non-molar specimens.\(^2,13-16\)

Although significant geographic variation exits, in the United States the incidence of molar pregnancy is 1 in 1500 live births.\(^15,17\) Based on well defined cases in the modern literature, the risk of persistent GTD following CHM is 15-20\%, while persistent GTD following a PHM is 0.2-4\%.\(^16,18-20\) While most cases of persistent GTD following a hydatidiform mole are invasive moles, 3-5\% present as choriocarcinoma. Until recently, overtly malignant (metastatic) GTD was not thought to occur following a PHM; however, 3 cases of choriocarcinoma and one case of placental site trophoblastic tumor, all arising from antecedent well-documented PHMs, have been described in the literature (in the 3 cases of choriocarcinoma, identical microsatellite polymorphisms were documented in the antecedent PHM and the subsequent GTD).\(^21,22\) 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 receive follow-up with serum human chorionic gonadotropin (hCG) levels in conjunction with contraception until undetectable levels are obtained. In contrast, because persistent GTD following a first trimester non-molar spontaneous abortion is rare (estimated risk of ≤ 0.0002%), spontaneous abortion or termination of a non-molar pregnancy does not inherently warrant follow-up with serum hCG levels or contraception until undetectable levels are obtained. Thus, avoiding misclassification of non-molar spontaneous abortion specimens, particularly those with AVM, as PHMs is of particular importance to patients with infertility for whom mandated contraception is undesirable.

**Ancillary techniques exploit the genetic constitution of molar and non-molar specimens**

In view of the established limitations of morphologic assessment alone, 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 ancillary techniques, including immunohistochemical analysis of p57 expression\(^{19,23-25}\) and molecular genotyping via PCR amplification of STR loci\(^{11,25-27}\), for improving the diagnosis of hydatidiform moles has been demonstrated in a number of recent studies. These techniques exploit the unique genetic features of CHMs, PHMs, and non-molar specimens to improve diagnosis. CHMs are most often diploid with both chromosomal complements being paternal in origin (androgenetic diploidy), whereas PHMs are
triploid with one maternal chromosome complement and two paternal chromosome complements (diandric triploidy). Non-molar specimens are typically diploid with one maternal and one paternal chromosome complement (biparental diploidy); some non-molar specimens can be triploid due to two maternal and one paternal chromosome complement (digynic triploidy) but these specimens do not have the morphologic features of PHMs.\textsuperscript{28,29}

Immunohistochemical analysis of p57 expression in the diagnosis of hydatidiform moles

P57 is the gene product of the paternally imprinted, maternally expressed gene $CDKN1C$, a cyclin-dependent kinase inhibitor located on chromosome 11p15.5. CHMs are characterized by androgenetic diploidy (2 sets of paternal chromosomal complements). Lack of a maternal genetic contribution in CHMs leads to lack of (or very limited) p57 expression in villous stromal cells and cytotrophoblast. In contrast, PHMs are characterized by diandric triploidy (1 set of maternal and 2 sets of paternal chromosome complements) and non-molar specimens are characterized by biparental diploidy (1 set of maternal and 1 set of paternal chromosome complements). Consequently, since both PHMs and non-molar specimens (including those with AVM) contain a maternal chromosomal complement, they are characterized by diffuse expression of p57 in these cell types. This differential p57 immunohistochemical expression has been found to be useful in the distinction of CHMs (including early forms) from PHMs and non-molar specimens; however, the latter two entities cannot be distinguished from one another due to shared (retained) p57 expression patterns.\textsuperscript{19,23-25}
The interpretation of p57 immunohistochemistry is typically straightforward in that the cellular components in which p57 is differentially expressed (villous stromal cells and cytotrophoblast) are almost always uniformly negative or diffusely positive; intermediate, equivocal, or discordant staining patterns in these cell types are uncommonly encountered. In our experience, the kind of equivocal p57 staining seen in case presented here occurs in <5% of cases. A few studies have described a limited extent of p57 expression (scattered nuclear positivity in villous stromal cells and cytotrophoblast) in a minority of cases of both diploid and tetraploid CHMs\textsuperscript{23,30,31}; this limited extent of expression (present in less than 10\% of these cell types) is still considered compatible with a diagnosis of CHM. Thus, to interpret immunohistochemical stains for p57, the presence or absence of nuclear positivity is assessed in villous stromal cells, cytotrophoblast, intermediate trophoblast, and maternal decidua. The p57 immunostain is interpreted as “negative” and satisfactory when maternal decidua and/or intermediate trophoblastic cells exhibit nuclear expression of p57 (serving as internal positive control in all cases, including CHMs) but villous stromal cells and cytotrophoblast are either entirely negative or demonstrate only limited expression (nuclear staining in <10\% of these cell types). This negative result is then interpreted as consistent with a diagnosis of CHM, provided the morphology of the specimen is appropriate. The p57 immunostain is interpreted as “positive” when the extent of staining is extensive or diffuse in these cell types. This pattern of expression is consistent with all forms of non-molar specimens as well as PHMs and cannot distinguish among these entities. Molecular genotyping is required to definitively distinguish a PHM from the other non-molar entities.
Nuclear expression in villous stromal cells and cytotrophoblast in the focally positive range (>10% but <50%) is considered an equivocal result, encompassing the 30% value used in one study as a cut-off for positive and negative results but allowing for a wider range to enable use of quick visual estimation rather than cell counting to determine a result.\textsuperscript{23} Since this amount of staining is uncommonly encountered but of an equivocal extent, cases exhibiting this pattern of expression warrant molecular analysis, particularly when establishing a definitive diagnosis is necessary for clinical management or investigative studies. These equivocal results are different from the aberrant results encountered in some studies. Aberrant retained p57 expression has been reported in mosaic/chimeric (androgenetic plus biparental) conceptions, with discordant expression in different cell types based on the presence or absence of maternal genetic material in those particular cells.\textsuperscript{19,23,32} Two cases of molecularly confirmed androgenetic diploid CHMs with diffusely positive aberrant p57 expression have been reported.\textsuperscript{33,34} Both cases demonstrated a retained maternal chromosome 11 which resulted in the aberrant p57 expression. Relaxation of imprinting (incomplete imprinting) has been suggested as a possible mechanism for the rare cases of weak but diffuse nuclear staining in cytotrophoblast and villous stromal cells in otherwise genetically confirmed CHMs.\textsuperscript{19}

\textit{Molecular techniques in the diagnosis of hydatidiform moles}

A variety of molecular techniques have been used to improve diagnosis of hydatidiform moles. These include formal cytogenetic analysis (karyotyping), determination of ploidy by flow cytometry\textsuperscript{35,36}, fluorescent in situ hybridization
(FISH)\textsuperscript{37,38}, and PCR amplification of STR loci.\textsuperscript{11,25-27} Most of these techniques, including karyotyping and ploidy analyses, have limitations beyond the known technical and interpretive difficulties in that they cannot specifically discern the maternal and paternal chromosomal contributions in a specimen. Thus, while diploid and triploid specimens can be identified to improve both recognition of PHMs and distinction of PHMs from CHMs, CHMs (particularly the morphologically subtle early forms) cannot be distinguished from non-molar specimens (both yield non-specific diploid results), and PHMs cannot be distinguished from digynic triploid non-molar specimens (both yield non-specific triploid results).

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. STR analysis generally involves PCR amplification of multiple STR loci using fluorescently labeled PCR primers, followed by sizing of the PCR products by capillary electrophoresis. For the analysis of hydatidiform moles, the alleles at each locus are identified for the both the maternal (decidua) and villous tissues, and the patterns are compared. Alleles in the villous tissue are identified as paternal (non-maternal) or likely maternal (also possibly paternal due to 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 two alleles. In general, when two alleles are present in equal dosage, the ratio will be approximately to 1:1. When one allele is in double dosage compared to the other (e.g. trisomy/triploidy), the ratio will be approximately 2:1. Specific details for interpretation of STR data can be found in Murphy \textit{et al.}\textsuperscript{39} Molecular genetic analysis of the type
provided by STR genotyping offers greater diagnostic discriminatory capability than other genetic techniques in that CHMs, PHMs, and non-molar specimens can be specifically distinguished from one another based on identification of the parental source of polymorphic alleles and their ratios. In particular, this analysis can discern androgenetic diploidy, diandric triploidy, and biparental diploidy to rigorously diagnose and distinguish CHMs, PHMs, and non-molar specimens, respectively.

STR genetic analysis is particularly important for the diagnosis of PHMs, which continue to pose diagnostic difficulty and cannot be distinguished from non-molar specimens, especially those exhibiting AVM of the type associated with other (non-molar type) genetic abnormalities, due to shared p57 expression patterns. This technique, used in conjunction with morphology, is the best suited for assuring that specimens interpreted as PHMs are in fact diandric triploid gestations, thus preventing misclassification of early CHMs, non-molar specimens with AVM, and even digynic triploid specimens as PHMs. Digynic triploid specimens do not exhibit the morphologic features of PHMs but can cause confusion with PHMs if simple ploidy analysis or FISH, rather than molecular genotyping, is used without morphologic evaluation.

Genotyping can also resolve the discrepancy between morphology and p57 results in the rare cases of androgenetic diploid p57-positive CHMs with retained maternal genetic material.

Although molecular genotyping can greatly improve/facilitate the diagnosis of CHMs PHMs, and non-molar specimens, it is not without its own interpretive challenges. One limitation encountered with this technique concerns 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 would yield a result of either diploidy or triploidy, but the parental contributions (biparental versus androgenetic diploidy, or digynic versus diandric triploidy) cannot be determined without comparison of the patterns in the villous and decidual tissues. The information obtained from the analysis would be essentially identical to what would be obtained with karyotyping, ploidy, or FISH analysis.

Infrequent yet well documented cases of mosaicism and chimerism found in hydatidiform moles can lead to genetic results that are difficult to interpret.\textsuperscript{19} Individual, double, and rare multiple trisomies 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. The rare cases of biparental CHM also pose an interpretive challenge. This disorder, which appears to result from an autosomal recessive inheritance pattern mapped to 19q13.4, presents clinically, morphologically, and immunohistochemically (negative p57 result) as multiple CHMs which appear to have a risk of persistent GTD similar to that of conventional CHM (uniparental androgenetic diploidy).\textsuperscript{40-42} Molecular genotyping in such a case would result in biparental diploidy that could be misinterpreted as a non-molar gestation in the absence of correlation with morphologic features and p57 results.

\textbf{Summary}

\textit{Algorithmic approach to diagnosis of hydatidiform moles}
Our experiences with p57 immunohistochemistry and molecular genotyping for diagnosis of hydatidiform moles led to the development of a diagnostic algorithm for evaluation of products of conception specimens having any features suggesting the possibility of a hydatidiform mole.\textsuperscript{11,39} This algorithm is applicable to formalin-fixed paraffin-embedded tissues in routine practice. Evaluation of hematoxylin and eosin-stained slides is followed by immunohistochemical analysis of p57 expression. If the morphologic features suggest a CHM and the p57 result is negative (with satisfactory internal positive control), a diagnosis of CHM can be confidently established. Molecular genotyping can confirm a diagnosis of CHM for a p57-negative specimen by demonstrating androgenetic diploidy but is not necessary for routine diagnosis, provided the p57 result is satisfactory. Cases with features suggesting the possibility of a hydatidiform mole, but lacking fully developed diagnostic morphologic features, and having a positive or equivocal p57 result are subjected to molecular genotyping. A diagnosis of PHM is established by demonstrating diandric triploidy. Triploidy of uncertain source (extra chromosomal complement equivocal for paternal versus maternal origin due to shared alleles between villous and decidual [maternal] tissue) is suggestive of a PHM when morphologic abnormalities are appreciated in the specimen; in the absence of morphologic alterations, this could be a digynic triploid non-molar specimen. Biparental diploidy established a specimen as non-molar (with the rare exception of biparental CHM).

In view of the known imperfection of morphologic diagnosis and the limitations of other ancillary techniques noted above, we advocate this approach in routine clinical practice to provide the most refined diagnosis of hydatidiform moles possible and
thereby assure accurate ascertainment of risk of persistent GTD and appropriate clinical management. In routine practice, laboratories lacking access to these techniques can seek consultation from reference laboratories offering these assays. In the setting of limited resources, when ancillary testing is not feasible, cases demonstrating any features suggestive of a hydatidiform mole can be diagnosed descriptively, indicating a concern for a molar gestation, with a recommendation that serum hCG levels be followed to guide subsequent management. However, in investigational pursuits, all molar specimens should be evaluated with ancillary techniques to assure rigorous classification of cases, particularly when designed to ascertain risk of persistent GTD associated with the various subtypes of hydatidiform moles.
Complete hydatidiform mole (typical example). Villi are enlarged and hydropic with some circumferential trophoblastic hyperplasia; some have cisterns and trophoblastic inclusions. P57 immunostain demonstrates that villous stromal cells and cytotrophoblast are negative (intermediate trophoblastic cells serve as internal positive control). Molecular genotyping demonstrated androgenetic diploidy (data not shown).
Early complete hydatidiform mole (typical example). Villi have bulbous contours, mild hydropic change, and circumferential trophoblastic hyperplasia. Villous stroma is hypercellular, with prominent karyorrhectic debris and small canalicular vessels. P57 immunostain demonstrates that villous stromal cells and cytotrophoblast are negative (intermediate trophoblastic cells serve as internal positive control). Molecular genotyping: STR analysis of the villous tissue demonstrates only paternal (P) alleles (lack of maternal alleles) at each STR locus (3 loci are shown). These results are most consistent with androgenetic diploidy, and confirm a diagnosis of complete hydatidiform mole.
Partial hydatidiform mole (typical example). Villi are variably sized and irregularly shaped due to prominent villous scalloping, with focal mild circumferential trophoblastic hyperplasia. P57 immunostain demonstrates that villous stromal cells and cytotrophoblast are positive. Molecular genotyping: STR analysis of the villous tissue demonstrates one maternal (M) and two paternal (P) alleles at each STR locus (3 loci are shown). These results are most consistent with diandric triploidy, and establish a diagnosis of partial hydatidiform mole.
Abnormal villous morphology. Villi are variably sized and somewhat irregularly shaped, with foci of mild trophoblastic hyperplasia manifested as syncytiotrophoblastic tufts. P57 immunostain demonstrates that villous stromal cells and cytotrophoblast are positive. Molecular genotyping: STR analysis of the villous tissue demonstrates one maternal (M) and one paternal (P) allele at each STR locus (3 loci are shown). These results are most consistent with biparental diploidy, and exclude a diagnosis of hydatidiform mole (of note, analysis of additional STR makers identified trisomies of chromosomes 16 and 21 in this specimen).
Partial hydatidiform mole (problematic example). Villi are hydropic and variably sized rather than scalloped, with some circumferential trophoblastic hyperplasia; some villi have slightly cellular stroma. These features suggest a differential diagnosis of hydropic early abortus versus early complete hydatidiform mole. P57 immunostain is equivocal in that some villi have negative stromal cells and cytotrophoblast whereas others have positive stromal cells with a few positive cytotrophoblastic cells. Molecular genotyping: STR analysis of the villous tissue demonstrates one maternal (M) and two paternal (P) alleles at each STR locus (3 loci are shown). These results are most consistent with diandric triploidy, and establish a diagnosis of partial hydatidiform mole.
References