Hydatidiform Moles: Morphology and Ancillary Techniques to Refine Diagnosis

Brigitte M. Ronnett, M.D.
Department of Pathology
The Johns Hopkins University School of Medicine
Baltimore, MD USA

Topics/Objectives

• Morphology, genetics, and differential diagnosis of hydatidiform moles
• Limitations of morphology for diagnosis
• Ancillary techniques for distinguishing hydatidiform moles from non-molar entities and for subtyping hydatidiform moles
  – Immunohistochemistry for p57
  – Molecular genotyping
• Examples illustrating application of these techniques
Cultural Linguistic Care

The Latina population, similar to other ethnic groups with a high birth rate and possibly due to other ethnicity-related factors, may be at increased risk of having molar pregnancies. Accordingly, providers of gynecologic care (clinicians and pathologists) need to be aware of modern diagnostic techniques that can refine the diagnosis of molar pregnancies.

Non-molar Specimen

Biparental diploidy
Early Conceptus

Early Gestation

[Blaustein's Pathology of the Female Genital Tract, 6th edition (Figure 19.4)]
Immature villi of early (first-trimester) placenta

Immature normal villi
### Types of Early Products of Conception Specimens

**Hydatidiform moles:**
- Complete hydatidiform mole (CHM)
  - Early CHM
- Partial hydatidiform mole (PHM)

**Non-molar entities (can simulate hydatidiform moles):**
- Early abortus (EA)
- Hydropic abortus (HA)
- Abnormal villous morphology (AVM)
  - Non-molar genetic alterations (e.g. trisomy)
- Mosaic/chimeric conceptions

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### Complete Hydatidiform Mole (CHM)

Androgenetic diploidy
(~80% homozygous)
Complete Hydatidiform Mole (CHM)
Complete Hydatidiform Mole (CHM)

- Enlarged, edematous villi
- Central cisterns
- Trophoblastic inclusions
- Circumferential trophoblastic hyperplasia (variable amount and degree of cytologic atypia; >>PHM)
- Atypical exaggerated implantation site is common
- Early embryonic development can occur
  - fetal nRBCs, fetal endothelial cells, stromal macrophages, amnion, yolk sac
- Androgenetic diploidy
Early Complete Hydatidiform Mole

- Redundant bulbous villous growth pattern
- Hypercellular myxoid villous stroma
- Labyrinthine network of villous stromal canaliculi
- Karyorrhectic debris within stroma
- Focal trophoblastic hyperplasia on villi and undersurface of chorionic plate (>PHM)
- Atypical exaggerated implantation site is common
- Androgenetic diploidy

Partial Hydatidiform Mole (PHM)

Diandric triploidy
(~90% heterozygous)
Partial Hydatidiform Mole (PHM)

[Blaustein’s Pathology of the Female Genital Tract, 6th edition (Figure 20.15)]
Triploidy and the Partial Molar Phenotype (PHM): Diandric Triploidy = PHM (Digynic Triploidy ≠ PHM)

Partial Hydatidiform Mole (PHM)

- 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
- Mild trophoblastic hyperplasia
- Evidence of fetal development
  - Stromal vessels with fetal RBCs, chorionic plate, amnion, cord, gestational sac or embryo/fetus
- Diandric triploidy
Very Early Abortus

Early Abortus
## Early Abortus

- Gestational sac with a shell of hyperplastic trophoblast (at earliest phase)
- Immature branching chorionic villi with radiating (polarized) columns of hyperplastic trophoblast (when more developed)
- Trophoblast is relatively abundant, dimorphic, and can appear circumferential but lacks the atypia seen in CHM
- Biparental diploidy

<table>
<thead>
<tr>
<th>Hydropic Abortus</th>
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<tr>
<td><img src="image1.jpg" alt="Image 1" /></td>
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</table>
Hydropic Abortus

- Variably enlarged edematous villi
- Generally no trophoblastic hyperplasia (if hyperplastic, trophoblast is polarized and radiating)
- No trophoblastic inclusions
- Biparental diploidy

Abnormal Villous Morphology (AVM) [trisomy]
**Abnormal Villous Morphology**

- Some irregularly shaped villi
- Some hydropic change
- Some focal/mild trophoblastic hyperplasia (often syncytiotrophoblastic “snouts”)  
- Findings suggest PHM but fully developed diagnostic features are lacking
- Can be associated with non-molar type genetic abnormalities (trisomy, monosomy)
- Biparental diploidy

**Androgenetic/Biparental Mosaicism/Chimerism**  
(placental mesenchymal dysplasia +/- features of CHM)
Mosaic/chimeric Conception

- Variably sized and shaped villi, often with stromal hypercellularity
- Generally no trophoblastic hyperplasia, but focal hyperplasia can be seen in some examples
- Features can suggest a hydatidiform mole (focal CHM in cases with trophoblastic hyperplasia)
- Androgenetic/biparental diploidy (admixture of androgenetic diploid and biparental diploid cells in individual villi, variable from area to area)

Morphologic Overlap of Molar and Non-Molar Specimens
Value of Refined Diagnosis of Molar Specimens

- 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 (genetically identical metastatic GTD after triploid PHM has been observed)
- 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

Methods for Distinction of Molar and Non-molar Specimens

<table>
<thead>
<tr>
<th>Diagnostic technique</th>
<th>CHM</th>
<th>PHM</th>
<th>AVM</th>
<th>HA/EA</th>
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<tbody>
<tr>
<td>Morphology</td>
<td>+/–</td>
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<tr>
<td>Ploidy</td>
<td>Diploid</td>
<td>Triploid*</td>
<td>Diploid</td>
<td>Diploid</td>
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<tr>
<td>FISH</td>
<td>2 signals</td>
<td>3 signals*^</td>
<td>2 signals (3 signals)^</td>
<td>2 signals</td>
</tr>
<tr>
<td>p57 IHC</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Molecular genotyping</td>
<td>Androgenetic diploidy</td>
<td>Diandric triploidy</td>
<td>Biparental diploidy</td>
<td>Biparental diploidy</td>
</tr>
</tbody>
</table>

*cannot distinguish diandric from digynic triploidy
^ triploidy (PHM) and trisomy (AVM) confused with use of limited probes
• *p57* is a paternally imprinted maternally expressed gene

• Immunohistochemical analysis of *p57* protein expression exploits distinct genetic features of hydatidiform moles for diagnosis

**Utility of *p57* For Diagnosis of Hydatidiform Moles**

Non-molar Specimen: Biparental Diploidy

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

Maternal Chr 11

Paternal Chr 11 (2 copies)

Positive in villous stromal cells, cytotrophoblast, and intermediate trophoblast (nuclear expression)

Complete Hydatidiform Mole: Androgenetic Diploidy

No maternal DNA

Paternal Chr 11 (2 copies)

Negative in villous stromal cells and cytotrophoblast (intermediate trophoblastic cells +)
Molecular Genotyping for Diagnosis of Hydatidiform Moles

- Identity testing applied to microdissected formalin-fixed paraffin-embedded tissue sections
- PCR amplification of polymorphic short tandem repeat (STR) loci on multiple chromosomes plus amelogenin locus (XY identification)
- Capillary electrophoresis
- Comparison of DNA patterns of villous and decidual tissue components
  - Determine ploidy (number of alleles and ratios)
  - Determine source of alleles (maternal vs paternal)
STR = 4 bp

- PCR primers flank the repeat regions
- Amplification of both alleles (maternal and paternal)
- Fluorescent PCR products analyzed by capillary electrophoresis
**Non-molar Specimen: Biparental Diploidy**
(1 maternal and 1 paternal chromosome complement)

- Informative loci have 2 distinct alleles, with 1:1 ratio

**Complete Hydatidiform Mole: Androgenetic Diploidy**
(2 paternal and no maternal chromosome complements)

- Homozygous (~80%)
  (monospermy)

- Heterozygous (~20%)
  (dispermy)
Partial Hydatidiform Mole: Diandric Triploidy
(2 paternal and 1 maternal chromosome complements)

Decidua

Villous

Heterozygous (~90%)
(dispermy)

Homozygous (~10%)
(monospermy)

Triploid but Indeterminate Loci
(?diandric versus digynic)

Decidua

Villous

Presence of 2:1 allele ratio or 3 alleles establishes triploidy
but alleles in double dosage are shared with maternal ones
Non-informative Loci

Decidua

Villous

Homozygous loci with shared alleles
Heterozygous loci with both alleles shared
Molecular Genotyping:
Androgenetic Diploidy = CHM

Androgenetic diploidy due to monospermy:
- multiple loci with paternal alleles only
- single peak at each locus = homozygous pattern
Overall negative
(focally positive in <10% of villi)
Molecular Genotyping: Androgenetic Diploidy = CHM
Diagnostic Clue
(morphologic & molecular genetic)

(Courtesy of Dwayne Lawrence, M.D.)
Molecular Genotyping:
Androgenetic Diploidy = CHM

Androgenetic diploidy due to monospermy:
• multiple loci with paternal alleles only
• single peak at each locus = homozygous pattern
Androgenetic Diploid Early CHM

(Courtesy of Dwayne Lawrence, M.D.)
Molecular Genotyping: Diandric Triploidy = PHM

Diandric triploidy due to dispermy:
- multiple loci with 3 alleles or 2 alleles with ratio = 2:1 (source unknown)
- 1 locus with novel allele in double dosage (paternal:maternal allele ratio = 2:1)
Molecular Genotyping: Biparental Diploidy with Trisomy 7,13,20 (paternal) = Non-molar AVM

<table>
<thead>
<tr>
<th>Decidua</th>
<th>Villous</th>
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<tbody>
<tr>
<td>120</td>
<td>160</td>
</tr>
<tr>
<td>3000</td>
<td>4000</td>
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- Two alleles at multiple loci (paternal:maternal allele ratio = 1:1)
- Two loci with 3 alleles = trisomy 7 & 13 (additional markers: trisomy 20)
Molecular Genotyping: Androgenetic/biparental Mosaic/chimera

Decidua

Villous (1)

Villous (2)
Algorithmic Approach to Diagnosis of Hydatidiform Moles

Possible Hydatidiform Mole

p57 immunohistochemistry

- p57 negative (villous stroma, cytotrophoblast)
- p57 positive (or equivocal/aberrant/discordant) (villous stroma, cytotrophoblast)

Morphology

- Morphology appropriate
- Morphology equivocal

Molecular genotyping

- Androgenetic diploidy
- Diandric triploidy
- Biparental diploidy

- Complete hydatidiform mole (including early form)
- Partial hydatidiform mole
- Non-molar