Gestational Trophoblastic Diseases

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Abbreviations

EPS	Exaggerated placental site
ETT	Epithelioid trophoblastic tumor
FIGO	International Federation of Gynaecology and Obstetrics
GPC3	Glypican 3
GTD	Gestational trophoblastic disease
GTN	Gestational trophoblastic neoplasia
hCG	Human chorionic gonadotropin
hPL	Human placental lactogen
PSN	Placental site nodule
PSTT	Placental site trophoblastic tumor
STR	Short tandem repeats
WHO	World Health Organization

1. What Are the Characteristic Morphologic Features of Complete Hydatidiform Mole?

Well-developed complete moles, presenting at the end of first trimester or during the second trimester, typically have easily recognizable morphologic features, including diffuse villous enlargement, marked villous hydrops with cistern formation, and circumferential trophoblastic hyperplasia (Fig. 7.1) [1, 2]. The villous contours are usually smooth and round, but surface invaginations resulting in trophoblastic pseudoinclusions may also be seen. Cytological atypia is often present in villous and implantation site trophoblastic cells. The villous stroma is usually hypocellular due to marked edema and is devoid of any vessels or fetal red blood cells.

However, the morphologic changes are more subtle in very early complete moles: the villous size is usually within the normal range, and the villi are polypoid, irregularly shaped with less frequent trophoblastic pseudoinclusions. The trophoblastic proliferation and hydropic changes are not fully developed yet, instead the villous stroma appears hypercellular and myxoid with stellate fibroblasts and prominent karyorrhectic debris. Rarely primitive fetal vessels and even nucleated red blood cells may be seen [3, 4].

2. Can Complete Mole Be Diagnosed in the Absence of Significant Villous Hydrops?

The morphologic changes of an early nonmolar gestation and a very early complete mole (evacuated early during the first trimester) may show significant overlap in terms of chorionic villous size and villous stromal cellularity. Villous hydrops is frequently absent in very early complete moles, and there is no cistern formation. Instead, the villous stroma is usually hypercellular and may appear myxoid with stellate fibroblasts and prominent karyorrhectic debris. Trophoblastic proliferation—even if it is only mild or moderate—is circumferential or random in very early complete mole, in contrast to the polarized trophoblastic proliferation of early normal pregnancy. Spontaneous nonmolar hydropic abortions may show significant villous enlargement and edema, occasionally even with cistern formation, mimicking complete or partial mole on the morphologic level. High index of suspicion is required to triage early gestations with subtle morphologic findings for appropriate ancillary studies to rule out a complete molar gestation.

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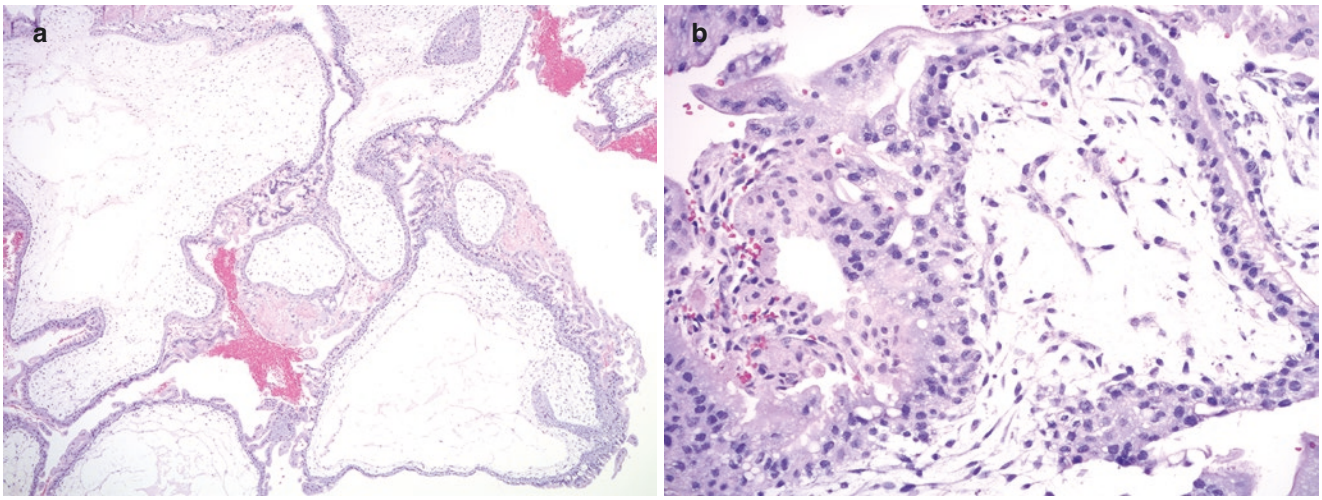

Fig. 7.1 Complete hydatidiform mole. (**a**) Well-developed complete mole shows villous enlargement with marked villous hydrops and circumferential trophoblastic proliferation. (**b**) Very early complete moles lack significant villous hydrops, instead the villous stroma is hypercellular and myxoid with karyorrhectic debris

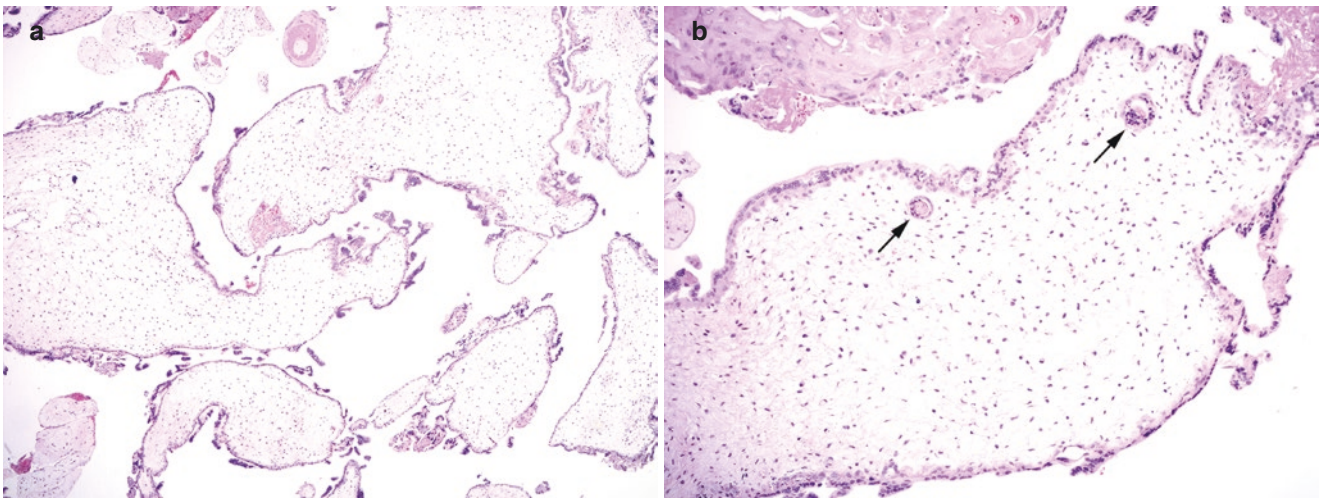

Fig. 7.2 Partial hydatidiform mole. (**a**, **b**) The chorionic villi are enlarged, hydropic, and have irregular contours with trophoblastic pseudoinclusions (**b**, arrows)

3. What Are the Characteristic Morphologic Features of Partial Hydatidiform Mole?

Partial hydatidiform mole usually shows two populations of chorionic villi—large, hydropic villi in the background of a small, fibrotic, or normal appearing villous population. The villous contours are irregular, scalloped, with surface invaginations and frequent round to oval trophoblastic pseudoinclusions (Fig. 7.2) [2, 5–7]. Trophoblastic hyperplasia is typically mild to moderate and focal, and the trophoblast lacks significant cytological atypia. Cistern formation may be seen in nearly 60% of cases, which in combination with a maximum villous size of ≥2.5 mm has been shown to have a 90% positive predictive value for partial mole, when compared with trisomy syndromes and non-molar hydropic abortions [8]. Fetal vessels with nucleated red blood cells are often present, and fetal development may also be seen, showing mild to moderate symmetrical intrauterine growth restriction and specific malformations (e.g., syndactyly) [9].

4. How Can p57 Immunohistochemistry Assist in the Differential Diagnosis of Hydatidiform Moles?

P57 protein is a cyclin-dependent kinase inhibitor, the product of the paternally imprinted, maternally expressed *p57* gene (*CDKN1C*, or *p57KIP2*) located on chromosome 11p15.5 [10]. Gestations containing maternal genetic material (including maternal copy of chromosome 11)—nonmo-

lar hydropic abortions, chromosomal trisomies, digynic triploidy, and partial hydatidiform moles—show normal p57 immunostaining pattern: strong nuclear staining in villous cytotrophoblasts, intermediate trophoblasts, and villous stromal cells [10, 11] (Fig. 7.3). In contrast, complete hydatidiform moles, including very early complete moles, lack maternal genetic contribution, therefore p57 expression is typically absent in the above cell types. Thus, differential p57 expression is useful in the diagnostic distinction between complete mole and its mimics, that is, partial moles and nonmolar hydropic abortions [10–15]. However, the major limitation of p57 immunohistochemistry is its inability to separate partial moles from biparental, diploid nonmolar hydropic abortions, digynic triploid gestations, and chromosomal trisomies, since all of these entities contain a maternal chromosomal complement.

Interpretation of p57 immunostain has been reported as highly reproducible [16, 17]. However, it is not without pitfalls, and discordant/equivocal staining patterns may rarely occur. Care must be given to evaluation of p57 staining in different cell types. P57 expression is always retained in intervillous intermediate trophoblast islands and in maternal decidua, including in complete moles, serving as internal positive control. In contrast, p57 immunostaining is uniformly absent in syncytiotrophoblastic cells, regardless of the genotype. Complete absence (or focal, <10%) of nuclear p57 expression in villous cytotrophoblastic and villous stromal cells is interpreted as negative p57 staining, if satisfactory internal positive control is present.

Twin gestations with a complete mole and a coexisting nonmolar fetus may show an admixture of chorionic villi with absent p57 staining and villi showing normal p57 pattern, complicating the interpretation [18]. In addition, rare cases of complete hydatidiform moles may have a retained copy of maternal chromosome 11, resulting in normal p57 protein expression [19, 20], and rare partial moles lack p57 staining due to loss of maternal chromosome 11 [21]. Further, discordant p57 immunostaining pattern—positive p57 in villous cytotrophoblast with negative villous stromal cells, or p57-positive villous stromal cells and negative cytotropho-

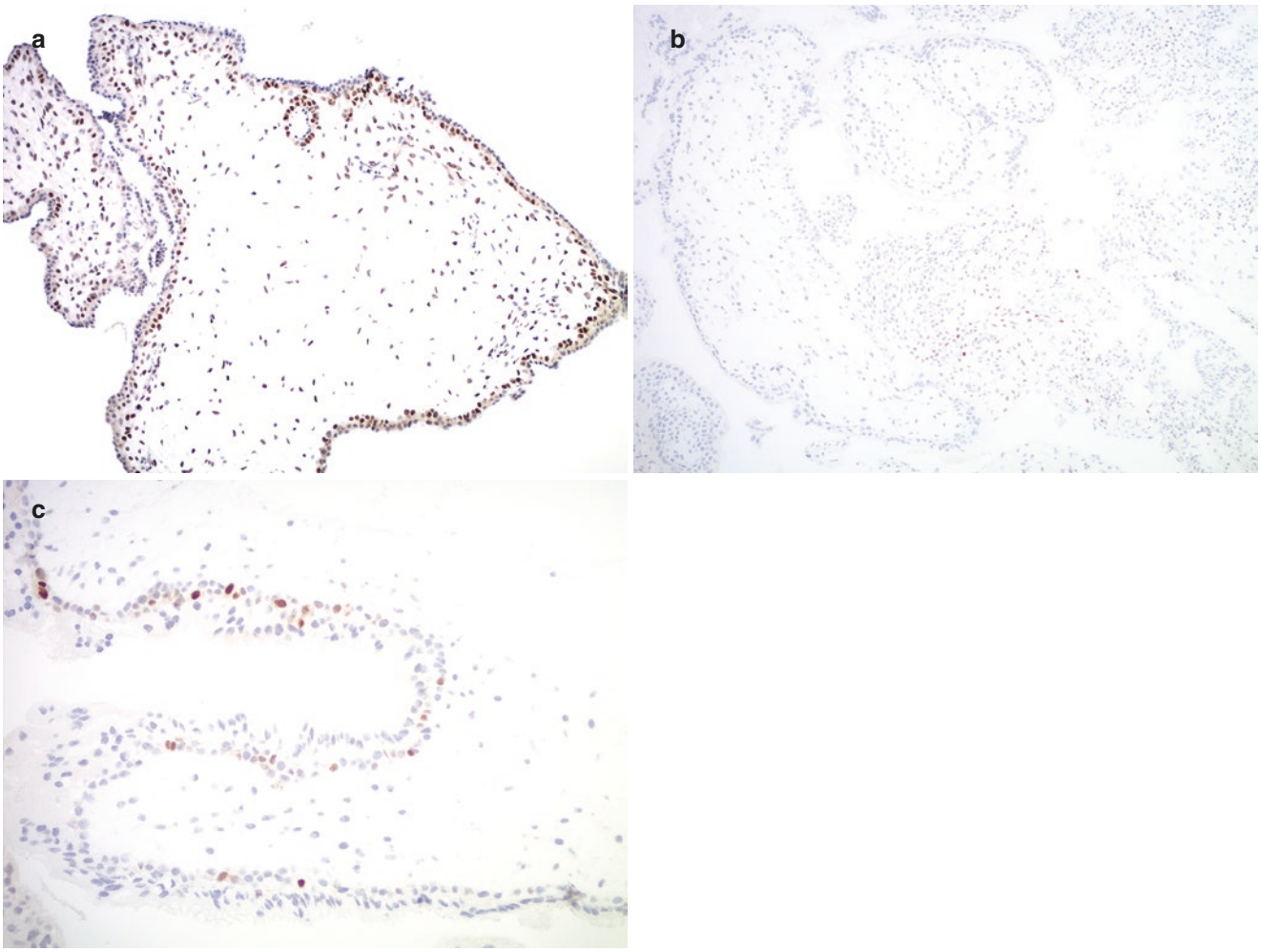

Fig. 7.3 (**a**) P57 immunostaining is retained in villous stromal cells and cytotrophoblast in partial moles. (**b**) In contrast, loss of p57 staining is seen in complete moles. Note the internal positive control in maternal decidua on the right side of image. (**c**) Discordant p57 staining pattern—positive in villous cytotrophoblast and negative in villous stromal cells—may be seen in androgenetic/biparental mosaic or chimeric gestations

blast—has been described in androgenetic/biparental mosaic or chimeric gestations [22, 23].

5. What Is the Genetic Basis of Molecular Genotyping and When Should It Be Performed?

Short tandem repeats (STRs) are highly prevalent and genetically stable, repetitive DNA sequences of 2–7 nucleotides in the human genome. The number of repeats at each STR locus differs between individuals; hence, STR genotyping is used for identity testing in forensics and can also be exploited as part of the routine diagnostic workup for molar gestations [24, 25]. Comparison of the allelic profiles between maternal (decidua) and fetal (chorionic villi) tissues at 15 STR loci provides information about the parental genetic contribution to the villous tissue and the relative proportions of maternal and paternal genetic material, allowing molar gestations to be classified precisely at their genetic level. Unlike some of the other ancillary studies, STR genotyping does not require fresh tissue and can be performed on formalin-fixed paraffin-embedded tissue samples, following dissection of pure maternal and fetal tissues from unstained sections.

Complete hydatidiform moles typically have a diploid, androgenetic-only genome, with the exception of rare tetraploid complete moles and familial biparental complete moles [26, 27]. The majority (80–90%) of complete moles are homozygous, monospermic, resulting from fertilization of an ovum without genetic material ("empty egg") by a single sperm, followed by duplication of its genome [2, 28]. Approximately 10–20% of complete moles are heterozygous, dispermic. Biparental complete moles, containing both paternal and maternal genomes, represent only 0.6–2.6% of all hydatidiform moles and develop due to mutations in maternal-effect genes *NLRP7* and *KHDC3L* [29–33]. STR genotyping analysis of androgenetic complete moles shows paternal-only alleles—either in a homozygous or heterozygous pattern—in at least two informative STR loci (Fig. 7.4).

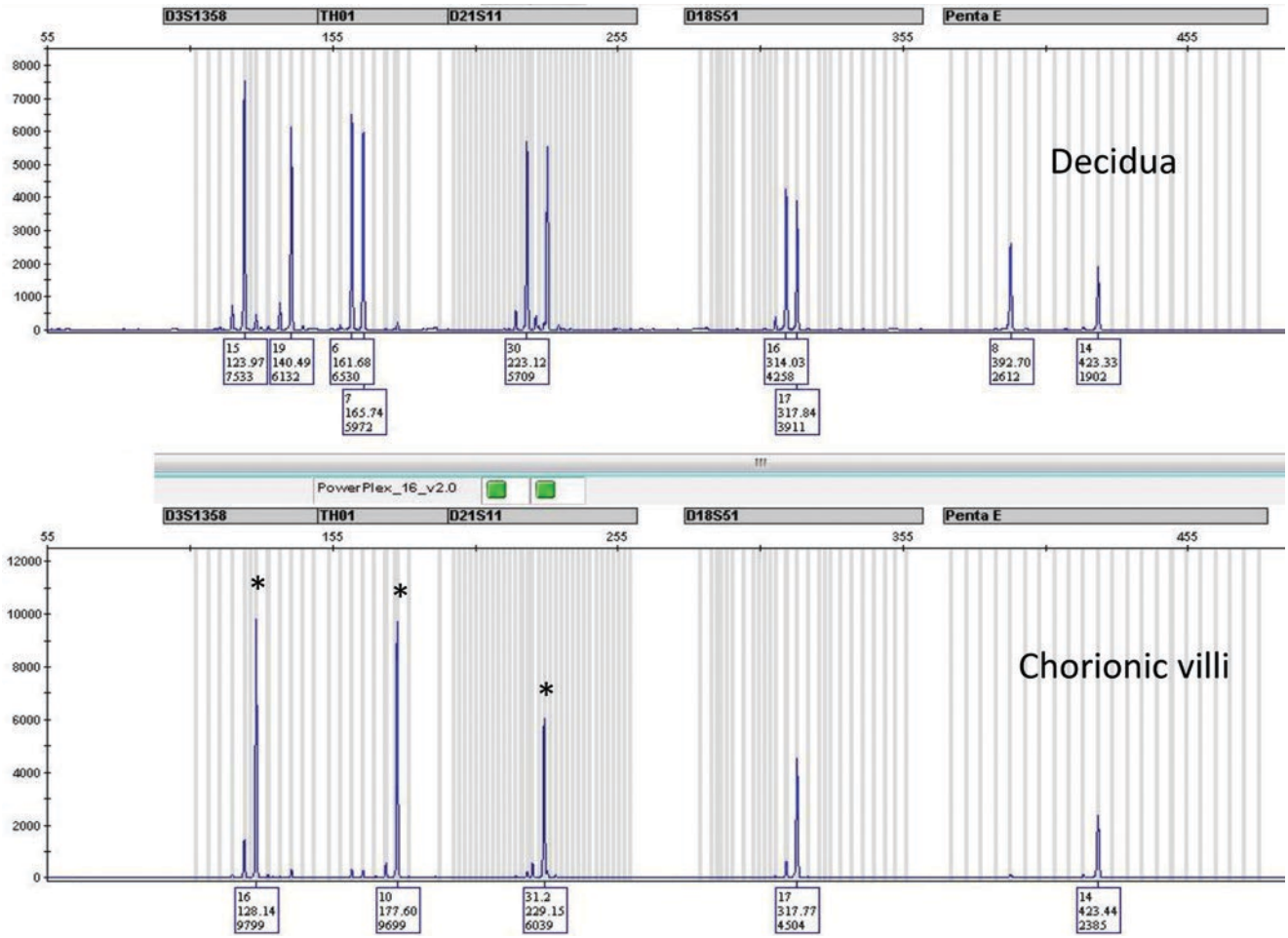

Fig. 7.4 Homozygous (monospermic) complete hydatidiform mole. Molecular genotyping shows unique paternal alleles in duplicate quantity (*) and absence of maternal alleles at multiple STR loci

Partial hydatidiform moles are genetically defined by diandric triploidy, arising from two sperms fertilizing an egg (dispermic/heterozygous partial mole) in most cases, while <10% of cases originate from one haploid sperm fertilizing an egg followed by reduplication of its genome, or from one diploid sperm due to failure of meiosis I or II (monospermic/homozygous partial mole) [24, 34, 35]. Rare tetraploid partial moles have also been reported, containing three haploid paternal chromosome sets [36–38]. Partial moles can be diagnosed on STR genotyping in the presence of two unique paternal alleles in addition to one maternal allele in at least two loci (dispermic/heterozygous partial mole) or one paternal allele in duplicate quantity and one maternal allele at every STR locus (monospermic/homozygous partial mole) (Fig. 7.5). Triandric tetraploid partial moles have a paternal to maternal allele ratio of 3:1 at informative STR loci.

A biallelic profile on STR genotyping analysis with balanced maternal and paternal contributions indicates a nonmolar abortion. A rare exception, familial biparental complete moles also have a diploid biparental genome, and careful correlation with morphologic findings and p57 immunostaining pattern is required to make the correct diagnosis in such cases. Nonmolar digynic monoandric triploidy constitutes roughly one third of all triploid gestations and may rarely mimic a partial mole morphologically and certainly by ploidy analysis [6, 39]. However, it is not associated with an increased risk of gestational trophoblastic disease or neoplasia and can be distinguished from partial moles by genotyping (Fig. 7.6). Further, STR genotyping can also differentiate between partial mole and chromosomal trisomy syndromes (Fig. 7.7). Gestations with chromosomal trisomy (especially trisomies 7, 8, 13, 15, 16, 18, 21, and 22) may show a significant morphologic overlap with partial moles, as they often have villous hydrops and irregularly shaped villi with trophoblastic pseudoinclusions, and the p57 expression is normal [8, 25, 40–42]. An important potential pitfall of genotyping may be encountered when evaluating a gestation conceived with egg donation, as the chorionic villous tissue does not contain alleles from the recipient mother, hence simulating diandric complete mole on genotyping [43, 44].

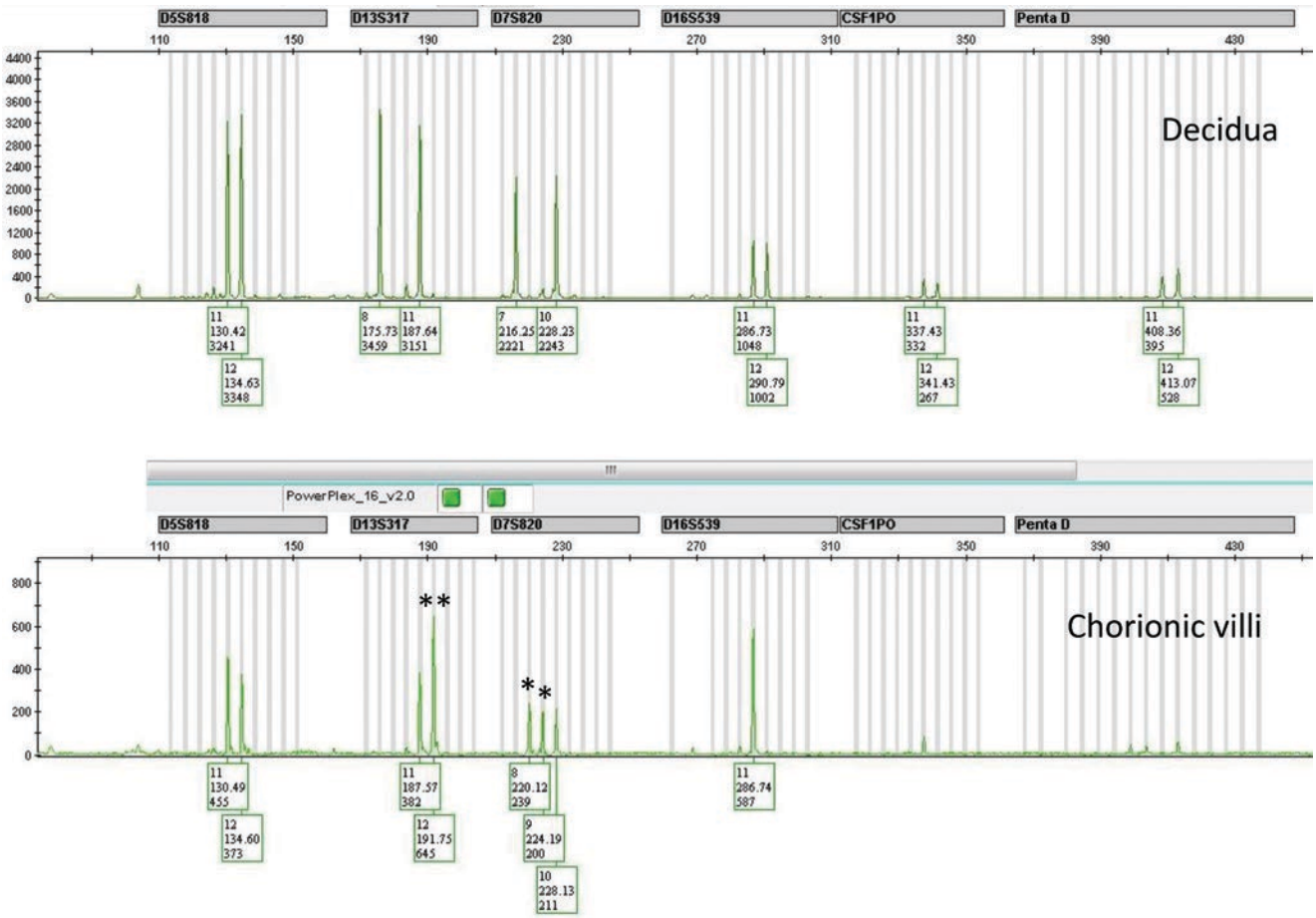

Fig. 7.5 Heterozygous (dispermic) partial hydatidiform mole. Molecular genotyping shows two unique paternal alleles (*) or one paternal allele in duplicate quantity (**) in addition to one maternal allele at multiple STR loci

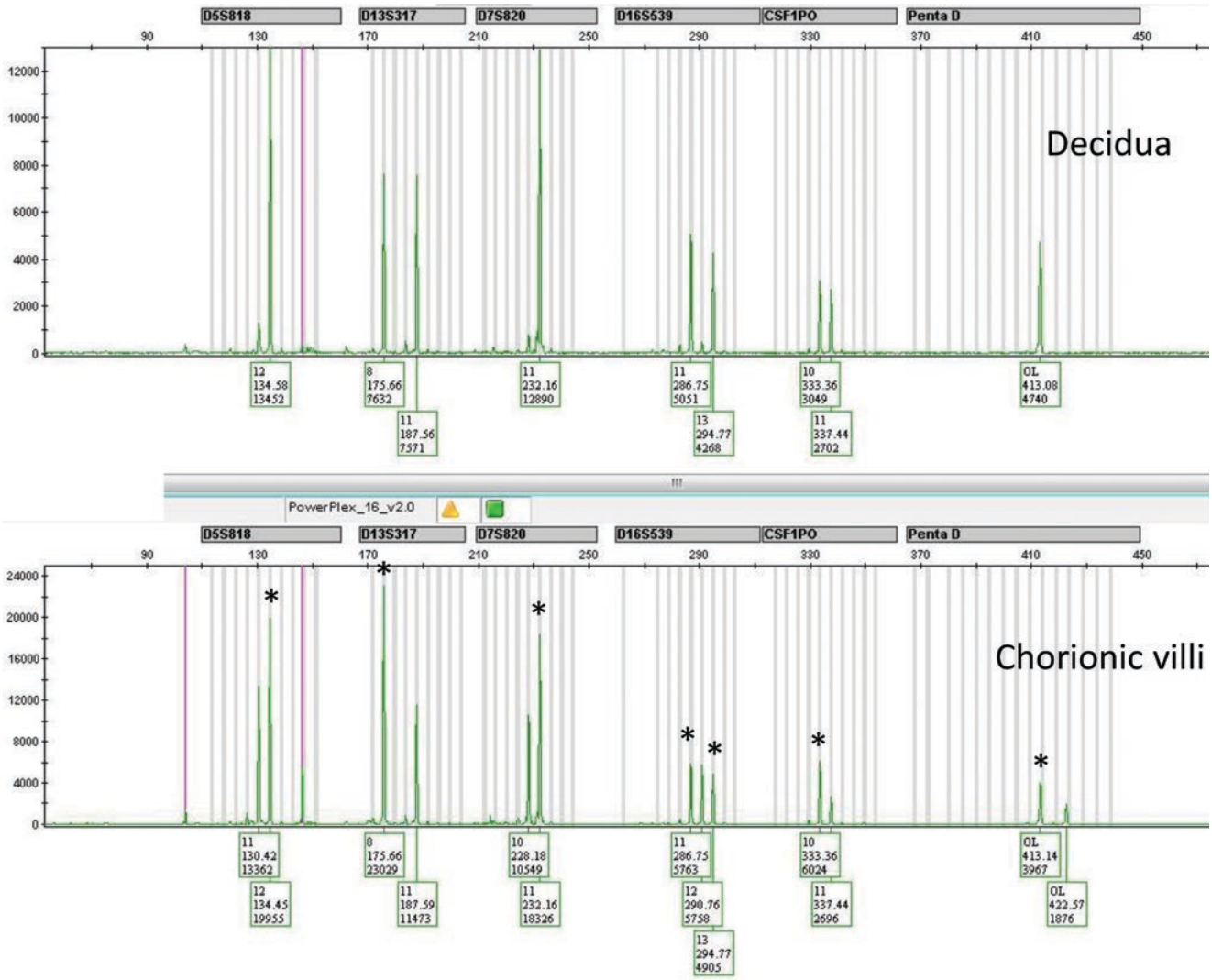

Fig. 7.6 Digynic triploidy. Molecular genotyping shows two distinct maternal alleles (*) or one maternal allele in duplicate quantity (*) in addition to one paternal allele at multiple STR loci

6. What Is the Recommended Diagnostic Algorithm for Hydatidiform Moles?

Recently proposed pathology diagnostic algorithms for hydatidiform moles integrate morphologic evaluation, p57 immunohistochemistry, and STR genotyping [2, 15, 34, 45]. Morphologic evaluation has been shown to have inherent limitations and poor interobserver reproducibility due to the well-documented morphologic overlap between the various entities, yet it remains the critical first step in triaging specimens for ancillary studies. If the morphologic features are suspicious for hydatidiform mole (either complete or partial), one approach suggests p57 immunohistochemistry as the next step. Negative p57 immunostain in villous cytotrophoblast and villous stromal cells is confirmatory of complete mole, but if p57 is positive in the above cell types, molecular genotyping should be performed to rule out a partial mole. Another approach recommends molecular genotyping on all cases with morphologic suspicion for hydatidiform mole and performing p57 immunostain only if there is a discrepancy between the morphology and genotyping result—for example, rare cases of biparental complete mole, mosaicism/chimerism, complete mole arising from a twin gestation, or egg donor gestation. The advantage of the latter approach is the one-step process in most cases, and the ability to identify the clinically more aggressive heterozygous (dispermic) complete moles [46–48]. The two algorithms may also be combined to improve cost-effectiveness and turn-around time: cases with morphologic suspicious for complete mole can be first subjected to p57 immunostain and are only analyzed further by genotyping if the p57 expression pattern is normal. However, if the mor-

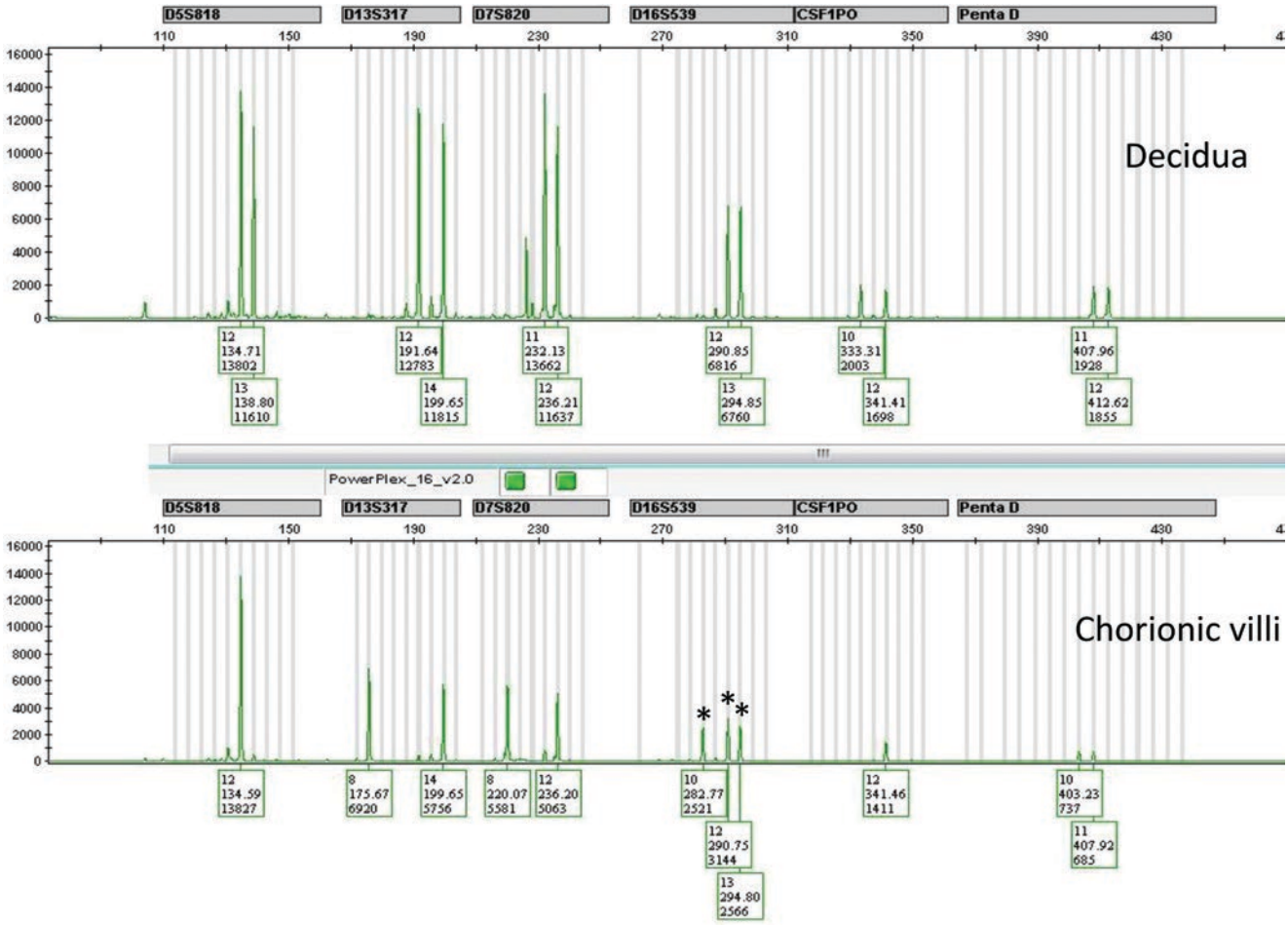

Fig. 7.7 Trisomy 16. Molecular genotyping shows three alleles (*) at an STR locus on chromosome 16 (D16S539). All other loci show balanced biparental alleles

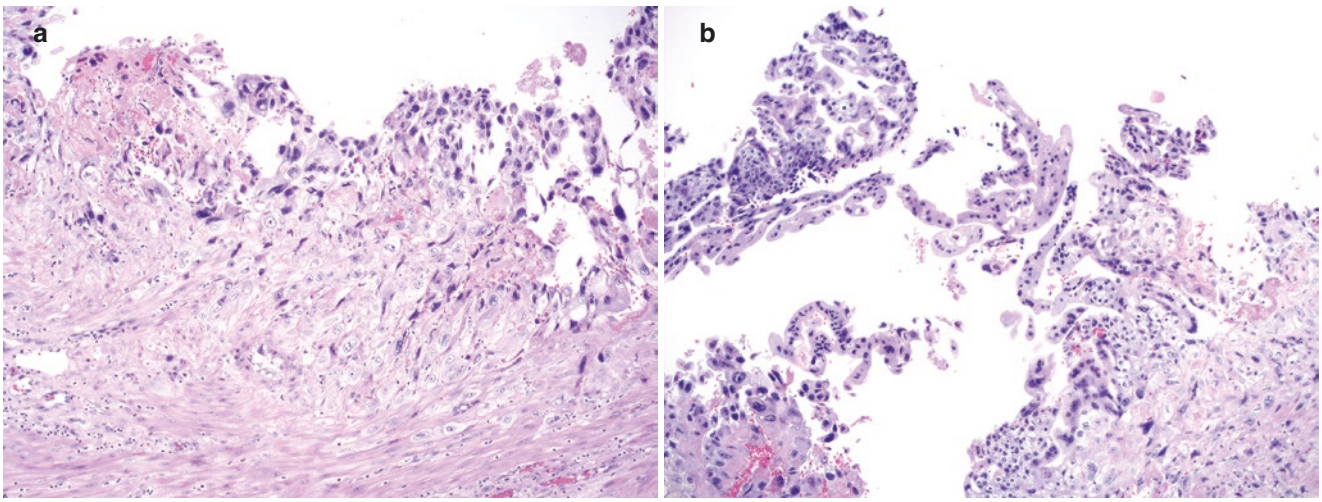

Fig. 7.8 (**a**, **b**) Cytological atypia is often seen in implantation site intermediate trophoblast in association with complete hydatidiform mole and may mimic choriocarcinoma

phology suggests partial hydatidiform mole, genotyping is pursued directly without p57 immunohistochemistry.

7. What Is the Risk of Persistent Gestational Trophoblastic Disease After Molar Pregnancy?

Hydatidiform moles, unlike their nonmolar morphologic mimics, are associated with increased risk of persistent gestational trophoblastic disease (GTD) or gestational trophoblastic neoplasia (GTN) and require careful clinical follow-up of the patient. Precise diagnostic distinction between complete and partial moles is essential for prognostic assessment, as there is a significant difference in their risk of postmolar GTD. Complete moles have a 20–25% risk of progression into persistent/invasive mole and 3–5% risk of gestational choriocarcinoma, while the risk of persistent/invasive mole and choriocarcinoma following a partial molar gestation is 4–5% and 0.2%, respectively [2, 49]. In addition, further genetic subclassification of complete hydatidiform moles by genotyping into homozygous (monospermic) versus heterozygous (dispermic) ones has also been found to have prognostic implications: heterozygous complete moles have a significantly higher frequency of postmolar GTD compared to homozygous complete moles [46, 50–53]. The most recent study reported persistent GTD following 37% of heterozygous and 11.6% of homozygous complete moles ($p = 0.0009$) [54].

8. What Is the Significance of Trophoblast Atypia Associated with Molar Gestation?

Cytological atypia is often present in villous and implantation site trophoblastic cells in complete hydatidiform mole (Fig. 7.8). In addition, "in situ" (or "intramolar") choriocarcinoma in the presence of molar chorionic villi has also been reported (see question #9) and may be encountered in a uterine curettage. In some cases, the follow-up endometrial curettage after the initial evacuation of complete mole may only show aggregates of proliferating atypical trophoblasts without residual molar villi, mimicking choriocarcinoma [55]. Similarly, if hysterectomy is performed, the specimen may show an invasive mole and contain foci of myoinvasive trophoblastic proliferation with marked cytologic atypia, with or without associated molar villi, concerning for choriocarcinoma. These lesions likely occur on a spectrum of morphologic continuum, and currently no definitive cut-off exists for size or other distinct parameters for "in-situ" or early choriocarcinoma. As a practical approach, in cases with indeterminate histologic features, a diagnosis of "atypical trophoblastic proliferation consistent with persistent trophoblastic disease or gestational trophoblastic neoplasia" can be rendered. According to the current World Health Organization classification, patients with persistently elevated serum beta-hCG levels are considered to have persistent gestational trophoblastic disease/neoplasia and require chemotherapy based on clinical parameters, without the need of tissue diagnostic confirmation [56].

9. Can Gestational Choriocarcinoma Be Diagnosed in the Presence of Chorionic Villi?

Traditional morphologic diagnostic criteria of choriocarcinoma include a biphasic growth pattern with mononuclear trophoblast rimmed by a layer of multinucleated syncytiotrophoblast, severe cytological atypia, and high mitotic activity, in the absence of chorionic villi. However, "emerging" or "in situ" choriocarcinoma in the presence of chorionic villi (particularly molar villi) with exuberant trophoblastic hyperplasia and marked cytological atypia has also been reported [55, 57–60]. Rare cases of intraplacental choriocarcinoma have also been documented in a full-term placenta [61–66]. Some patients with intraplacental choriocarcinoma may present with a distant metastasis, and the primary lesion may only be discovered after careful reexamination of the placenta. These lesions are often small—measuring less than 1 cm—therefore thorough sectioning of the placenta (at 5–10 mm intervals) and sampling of any hemorrhagic mass lesions are recommended.

10. Why Is It Important to Differentiate Between Gestational and Nongestational Choriocarcinoma and What Ancillary Studies Are Useful in the Diagnostic Workup?

Trophoblastic differentiation in tumors may arise through three different pathogenetic mechanisms: (1) Gestational trophoblastic tumors (including gestational choriocarcinoma) develop from an antecedent gestation: a term pregnancy, abortion, or a hydatidiform mole [67–69]. (2) Germ cell tumors of the ovary, testis, or rarely extragonadal sites may be histologically entirely or partially composed of trophoblastic elements (pure or mixed choriocarcinomas) and are not associated with a prior gestation [70, 71]. (3) Focal trophoblastic differentiation, morphologically presenting as choriocarcinoma or as scattered syncytiotrophoblastic giant cells, has been reported in somatic carcinomas, including endometrial, cervical, ovarian, bladder, and lung primaries, and is thought to arise from the somatic component through clonal progression [72–78].

Gestational choriocarcinoma typically presents during the reproductive years (mean: 30 years), following a normal pregnancy, complete hydatidiform mole, or abortion in 50%,

22.5%, and 20% of the cases, respectively [68, 69]. The time interval between choriocarcinoma and the antecedent gestation is 1–3 months on average after a term pregnancy and 13 months following a complete mole, although rarely it may be over 20 years [79]. The serum beta-hCG level usually exceeds 10,000 mIU/mL and may even be over 1,000,000 mIU/mL [80]. The tumor typically forms a bulky, extensively hemorrhagic, and necrotic mass lesion within the uterus or in the fallopian tube or ovary in association with an ectopic pregnancy [81]. Nongestational choriocarcinoma of germ cell origin in female patients is very rare, usually occurs in children or in young adults, involves the ovary, and may contain other nonchoriocarcinomatous components as part of a mixed germ cell tumor [82, 83]. Patients present with an adnexal mass and abdominal pain mimicking an ectopic pregnancy [70]. In children, isosexual precocity may also occur.

Morphological features of choriocarcinoma, regardless of its pathogenesis, include a bi- or triphasic growth pattern with markedly atypical mononuclear trophoblastic cells and multinucleated syncytiotrophoblasts (Fig. 7.9). Brisk mitotic activity, abundant tumor necrosis, and hemorrhage are characteristic. Somatic carcinomas with trophoblastic differentiation typically also contain a distinct somatic carcinoma component (e.g., adenocarcinoma); however, this may only be focal and may not be present in a small biopsy specimen. Immunohistochemistry may be useful in confirming trophoblastic differentiation but cannot determine the pathogenesis of choriocarcinoma (see Chap. 15).

The prognosis of gestational choriocarcinoma is excellent; it is highly chemosensitive responding well to single agent methotrexate (low risk disease) or EMA-CO combination chemotherapy (high-risk disease) [68]. In contrast, nongestational choriocarcinoma of germ cell origin is more aggressive, often invading into adjacent structures and is more resistant to traditional chemotherapy. Patients with nongestational choriocarcinoma require multiagent chemotherapy regardless of the tumor stage and risk factor scores. Further, somatic carcinomas with trophoblastic differentiation typically have a poor prognosis with little response to chemotherapy [74].

In some cases, the tumor origin—gestational vs nongestational (germ cell or somatic)—can be determined based on clinicopathological features: choriocarcinoma in a prepubertal patient is nongestational (germ cell derived). Older patient age, postmenopausal status, and a relatively lower level of serum beta-hCG (usually <10,000 mIU/mL) are in favor of somatic (nongestational) origin. Uterine location and a recent gestational event (especially complete hydatidiform mole) are essentially consistent with gestational origin. However, in cases with equivocal clinicopathological parameters, especially in those of extra-uterine location (tubo-ovarian, or presenting as a distant metastasis), STR genotyping can provide definitive determination of gestational vs nongestational etiology by identifying the presence or absence of unique paternal alleles not present in the patient's paired normal tissue [70, 84, 85].

11. What Are the Morphologic Characteristics of Placental Site Trophoblastic Tumor (PSTT)?

PSTT commonly involves the uterine corpus as a nodular, round solid mass of 1–10 cm in size with fleshy, light tan to yellow cut surface. Focal hemorrhage and necrosis are grossly seen in nearly half of the cases [86, 87]. Deep myometrial involvement is seen in 50% of the patients, and

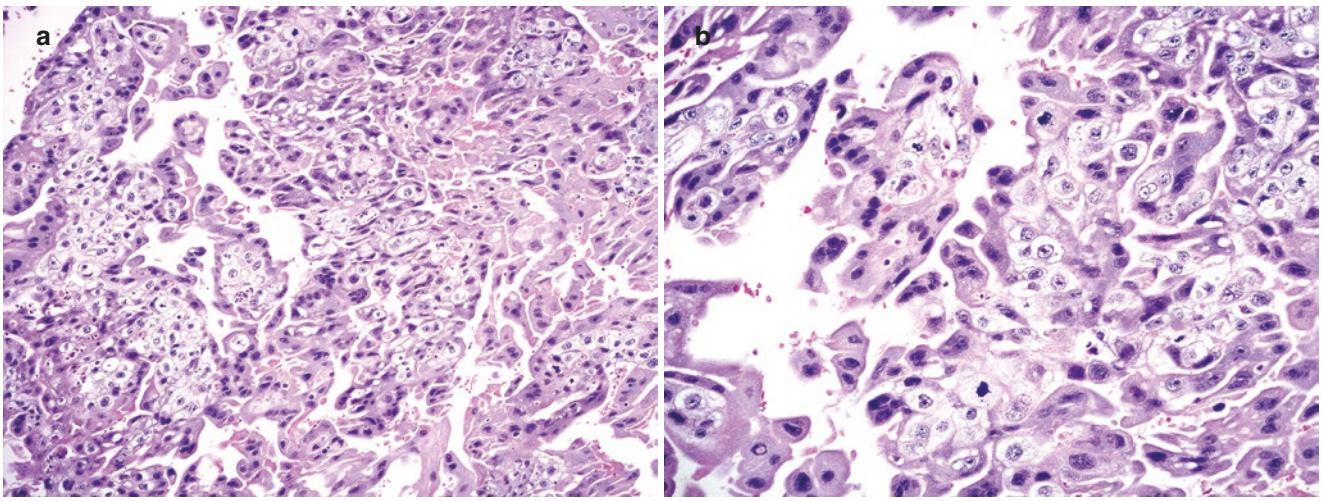

Fig. 7.9 Gestational choriocarcinoma. Biphasic growth pattern (**a**) and marked cytological atypia with frequent mitotic figures are characteristic (**b**)

transmural invasion is present in about 10% of the reported cases. Microscopically, PSTT consists of nodular proliferation of large, polyhedral to round, predominantly mononuclear intermediate trophoblastic cells that form cords, nests to large sheets. Scattered large multinucleated tumor cells are common. At the tumor periphery, the cells typically infiltrate and separate existing myometrial smooth muscle fibers (Fig. 7.10). Cytologically, the tumor cells are epithelioid with amphophilic, eosinophilic, or clear cytoplasm. Focal spindled tumor cell morphology may also be seen in some cases. The tumor has large convoluted nuclei with marked hyperchromasia, nuclear grooves, and pseudoinclusions are present in most cases. Tumors with round small nuclei with pale chromatin patterns can also be seen. Nucleoli are generally present and may be prominent. Mitotic count is usually between 2 and 4/10 HPF in most cases [86–88]. The tumor cells may replace venous walls while maintaining the overall vascular architecture. The tumor cells express human placental lactogen (hPL), hCG, MUC-4, HSD3B1, CD10, HLA-G, GATA 3, and Mel-CAM (CD146) [89]. hPL staining is generally strong and diffuse in over 2/3 of the cases. In contrast, hCG and inhibin are positive only in scattered multinucleated tumor cells. GPC3 (glypican 3) is another trophoblastic cell marker that can be used to separate PSTT from nontrophoblastic tumors [90]. Cytokeratin AE1/3 and CK18 are strongly expressed in the tumor cells, while p63, SALL4, and P40 are negative [91, 92]. Ki-67 is expressed in the range of 10–30% of the tumor cells [93].

12. Can Placental Site Trophoblastic Tumor (PSTT) Be Diagnosed in the Presence of Chorionic Villi?

PSTT does not occur with a concurrent pregnancy but develops months to years after term pregnancy, abortion, or hyda-

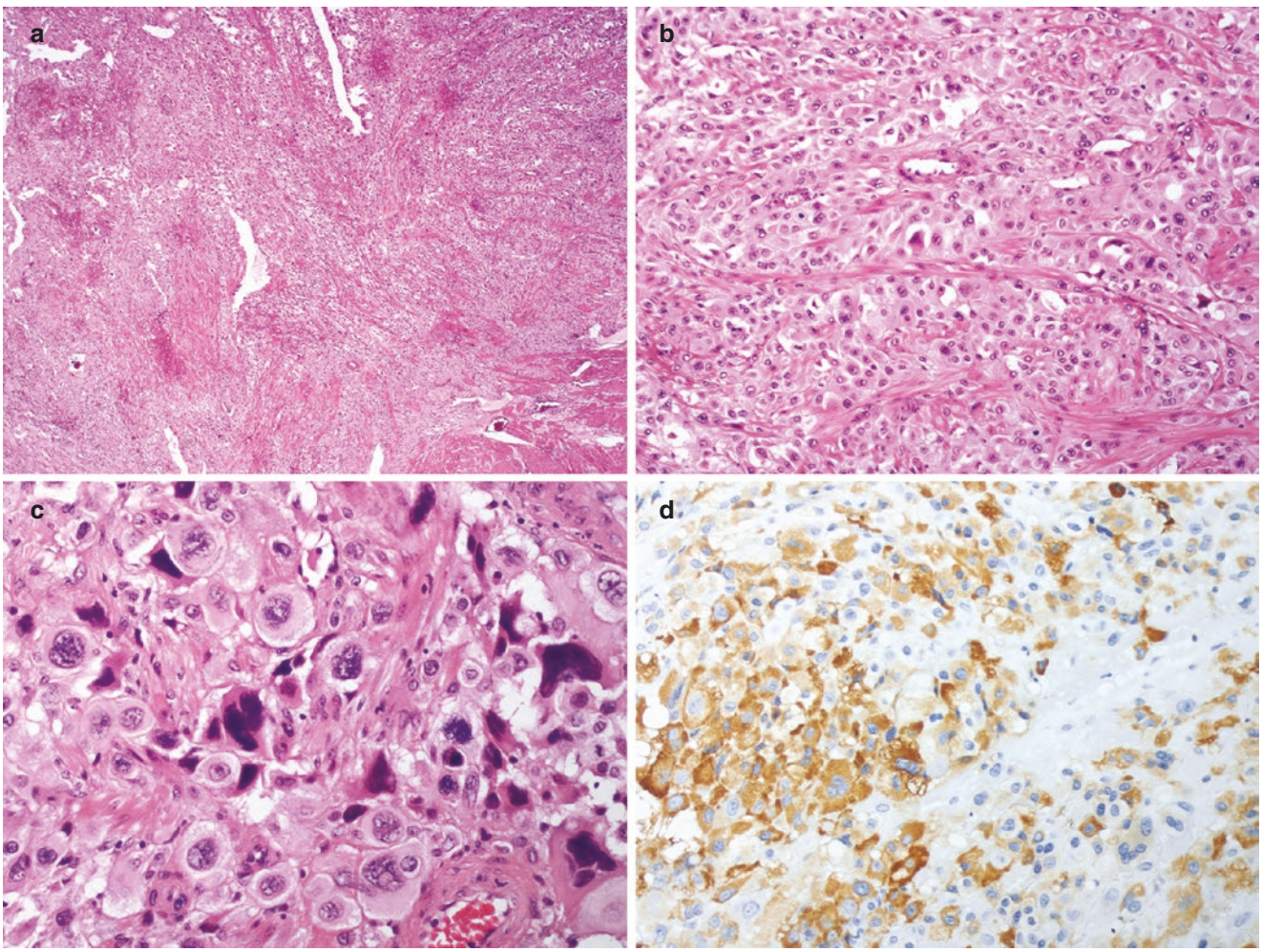

Fig. 7.10 Placental site trophoblastic tumor (PSTT). Mass proliferation of intermediate trophoblasts involving endomyometrium with infiltrating tumor borders (**a**, **b**). Proliferation of large epithelioid tumor cells with eosinophilic, amphophilic, or clear cytoplasm is characteristic (**c**). Cytological atypia, nucleomegaly, and pleomorphism along with frequent mitotic figures are common (**c**). The tumor cells are diffusely positive for hPL (**d**)

tidiform mole. However, the related reactive lesion, exaggerated placental site (EPS), occurs with a concurrent gestation and may show alarming features simulating PSTT (see also question #20). EPS is usually evacuated by curettage and is not visible on gross examination. The histological features in separation of EPS from PSTT include absence of mass lesion, presence of chorionic villi, mononuclear trophoblastic cells admixed with evenly distributed multinucleated forms, absence of mitotic activity, and a low level of Ki-67 labeling index (<2%). In contrast, PSTT is a space-occupying mass lesion, and patients usually present with vaginal bleeding or amenorrhea and mild elevation of serum hCG, months or years after term pregnancy or abortion. Ki-67 immunostain typically demonstrates a higher labeling index (5–30%) [94]. In curettage specimens, in which a diagnostic separation of EPS from PSTT cannot be decided [95], imaging study or close follow-up of the patient with serum hCG monitoring is recommended.

13. What Are the Morphologic Characteristics of Epithelioid Trophoblastic Tumor (ETT)?

ETT forms invasive, discrete nodules or cystic hemorrhagic masses of 0.5–5 cm with white-tan to brown cut surfaces with varying amounts of hemorrhage and necrosis. Close to half of the cases arise in the uterine cervix or lower uterine segment [96, 97]. Ulceration and fistula formation are not uncommon. Microscopically, the tumor consists of nodular, expansile proliferation of medium to small trophoblastic cells forming nests, cords, and large sheets. Well-circumscribed tumor border is typically observed (Fig. 7.11) [98]. Extensive or "geographic" necrosis and focal calcification are common. Eosinophilic hyaline-like material may be present in the center of tumor nests or between tumor cells [88, 96]. The tumor cells are relatively uniform with moderate amounts of finely granular, eosinophilic to clear

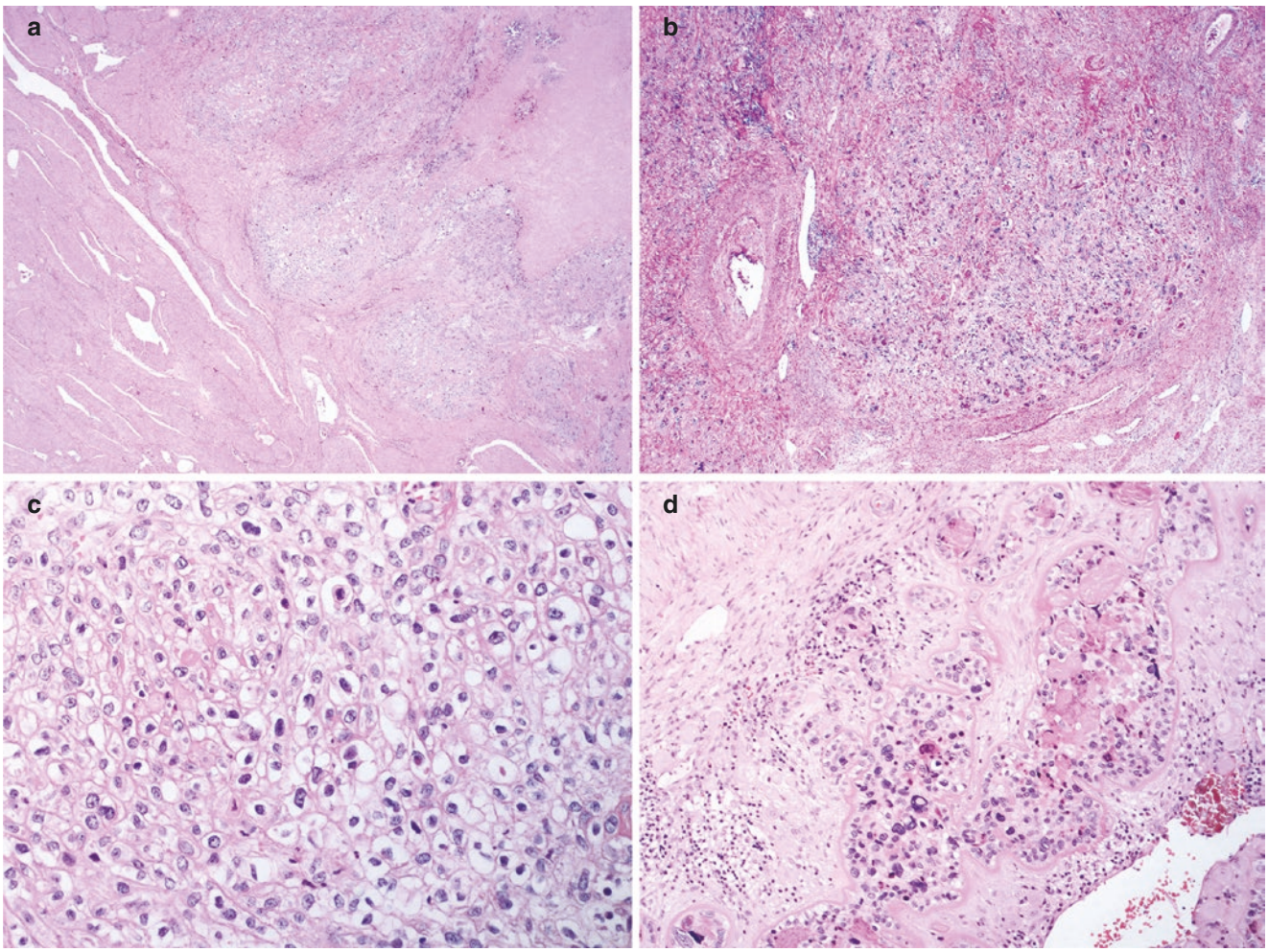

Fig. 7.11 Epithelioid trophoblastic tumor (ETT). Nodular expansile proliferation of cohesive epithelioid cells with well-circumscribed tumor borders is characteristic. Geographic tumor cell necrosis is common (**a**, **b**). ETT shows nested proliferation of medium- to small-sized chorionic-type intermediate trophoblastic cells with pink to clear cytoplasm and mild to moderate cytological atypia (**c**). ETT may histologically simulate squamous cell carcinoma including infiltrating nested growth and cervical mucosal colonization mimicking high-grade squamous intraepithelial lesion. Eosinophilic keratin-like material can be seen within tumor nests (**d**)

Table 7.1 Immunohistochemistry in trophoblastic tumors

	Choriocarcinoma	PSTT	ETT	PSN	APSN
hCG	+ (Diffuse)	+ (Rare cells)	+ (Rare cells)	–/+	–/+
hPL	+	+ (Diffuse)	+ (Rare cells)	+ (Rare cells)	+ (Rare cells)
CD146	+	+ (Diffuse)	–	–	–
GATA3	+	+	+	+	+
P63	+/–	–	+	+	+
SALL4	+	–	–	–	–
Ki-67	>40%	>5%	>10%	<5%	5–10%
HLA-G	+	+	+	+	+
Cyclin E	+	?	+	–	–/+
Inhibin	+	+	+	+	+
HSD3B1	+	+	+	+	+
GPC3	?	+	?	+	?
Cytokeratin	+	+	+	+	+
P40	+/–	–	+	+	+

cytoplasm, distinct cell membranes, round nuclei, and small but distinct nucleoli. Moderate nuclear atypia is generally present, and the mitotic count ranges from 0 to 47 with an average of 2 per 10 HPF [96]. Pseudo-decidualized benign stromal cells are often present at the tumor periphery. Colonization of the cervical mucosal surface or endocervical glandular epithelium by tumor cells may simulate high-grade squamous intraepithelial lesion [96]. ETTs express cytokeratins (CK18, AE1/3), HSD3B1, HLA-G, p63, cyclin E, p40, inhibin-alpha, and GATA3. Mel-CAM and hPL are expressed only in individual cells, and the Ki-67 labeling index is 10–25% in most cases [93]. SALL4 is not expressed in ETT [91]. Cyclin E is strongly positive in ETT [99] as opposed to negative staining in placental site nodule (PSN). DNA genotyping offers a definitive separation of ETT from somatic carcinomas including cervical squamous cell carcinoma [100, 101].

14. How Can Immunohistochemistry Assist in the Differential Diagnosis of Gestational Trophoblastic Neoplasia?

Trophoblastic tumors arise from various subtypes of placental trophoblasts, and each has distinct pathological and clinical features attributable to the proliferative ability of their constituent trophoblasts and therefore express some common trophoblastic markers but retain distinct expression of other immunomarkers. Gestational choriocarcinoma is a trophoblastic neoplasm recapitulating the primitive cells of the previous stage of the placenta. The intermediate trophoblast at the implantation site is considered the cell type seen in placental site trophoblastic tumor (PSTT) and exaggerated placental site reaction (EPS), whereas the intermediate trophoblast at the chorion laeve is considered the cell type found in epithelioid trophoblastic tumor (ETT) and placental site nodule (PSN) [102]. Therefore, a battery of appropriate immunohistochemical markers is useful in their differential diagnoses (Table 7.1).

15. What Is the Recommended Immunohistochemical Panel for Differentiating Between Squamous Cell Carcinoma and ETT?

Around 50% of ETTs arise in the cervix or lower uterine segment and can occur many years after a remote gestation in peri- and postmenopausal women [103, 104]. The single most important differential diagnosis is cervical squamous cell carcinoma [1, 96]. Absence of history of squamous intraepithelial neoplasia or HPV infection, lack of true squamous differentiation (true keratin formation or cell bridges), and presence of pseudodecidualized stromal cells at the tumor periphery are helpful features in favor of ETT. The recommended immunohistochemical panel for separating cervical squamous cell carcinoma from ETT includes trophoblastic markers (H3D3B1, HLA-G, inhibin-alpha. Mel-CAM, and hPL) [96]. The Ki-67 index is generally much higher in squamous cell carcinoma (>50%) in contrast to the relatively low index (<25%) in ETT [88]. Negative or patchy p16 immunostain is seen in ETT in contrast to the strong block-like staining pattern in HPV-related cervical squamous cell carcinoma [99].

16. What Are the Main Clinical, Prognostic, and Therapeutic Differences Between Gestational Trophoblastic Tumors?

Choriocarcinoma is the most common gestational trophoblastic tumor. Most often it presents with vaginal bleeding, but an extrauterine hemorrhagic event may be the primary

presenting symptom in some patients as a result of metastasis to lung (60–75%), liver (15–20%), central nerve system (15–20%), and gastrointestinal tract (10–20%) [105, 106] (see also question #10). High levels of serum hCG over 10,000 mIU/ml are invariably present. The latency period between the antecedent pregnancy and the diagnosis of gestational choriocarcinoma may be several months to as long as 14 years in rare cases [67, 107]. PSTT may develop after any type of gestation, but term pregnancy is the most common antecedent event reported in about 2/3 of the cases, while complete mole and missed abortion are seen in 16% and 13% of the cases, respectively [87, 108–110]. The patient age at presentation ranges from 20 to 63 years, with a mean of 31 years [86, 87, 110]. Vaginal bleeding and uterine enlargement are the most common findings, followed by amenorrhea and abdominal pain [109, 111, 112]. Mild to moderate elevation of serum hCG was seen in nearly 80% of the cases with values ranging from 5 to <100,000 mIU/ml (average 680–9422 mIU/ml) [87, 110, 113, 114]. Epithelioid trophoblastic tumor (ETT) occurs in patients 15–66 years of age (mean of 37.1 years). Vaginal bleeding or menometrorrhagia are the most common symptoms but amenorrhea also occurs. Mild to moderate elevation of serum hCG of less than 2500 mIU/ml is detectable in over 80% of the cases [96, 115]. Compared with gestational choriocarcinoma and PSTT, 50% of ETTs arise from the uterine cervix or lower uterine segment [96, 97].

Gestational choriocarcinoma is not a surgical disease. Once the diagnosis is established, the WHO/FIGO risk scoring scheme [116] is used for triaging patients into low- and high-risk categories for clinical management using single versus multiagent chemotherapy regimens [117–119]. Unlike choriocarcinoma, PSTT and ETT are not chemosensitive but require hysterectomy [112, 120], and therefore, the WHO/FIGO risk scoring system is not appropriate. Patients wishing to keep fertility must be carefully counseled in terms of prognostic risks. High-risk patients are treated with adjuvant chemotherapy and those with metastatic disease should receive combined chemotherapy (EMA-CO or EMA-EP) after surgical removal of all visible lesions to maximize the chance of cure [121]. PD-L1 blockade has been shown recently to be a promising treatment option for PSTT among other gestational trophoblastic tumors [122, 123].

17. What Are the Characteristics and Significance of Mixed Trophoblastic Tumors?

Mixed trophoblastic tumor is defined at the microscopic level by the presence of two or three histological subtypes of gestational trophoblastic tumors, including choriocarcinoma, PSTT, and ETT. Mixed trophoblastic tumor is very uncommon and has been reported to involve the uterus, fallopian tube or ovary [124–126]. The patient age ranges between 15 and 60 years (median 34 years) [127] . Abnormal vaginal bleeding is the most common presenting symptom. The preceding gestational event is normal pregnancy in more than half of the patients, and antecedent molar pregnancy has been reported in 30% of the cases. Serum hCG level is usually mildly elevated for mixed ETT and PSTT and mildly to moderately elevated for tumors with a choriocarcinoma component. High levels of hCG are mainly observed in patients with lung metastasis of mixed trophoblastic tumor with a choriocarcinoma component [97, 128]. Solid mass lesions of 2–8 cm in size are characteristic. Hemorrhage and necrosis are frequently present. Extensively hemorrhagic areas may correspond to the presence of choriocarcinoma components. Microscopically, distinct areas of PSTT, ETT, or choriocarcinoma are seen with characteristic histomorphology of each subtype. Mixed choriocarcinoma and ETT is the most common combination [97, 124, 129].

18. Can Gestational Trophoblastic Tumors Be Diagnosed in Postmenopausal Women?

Gestational choriocarcinoma presents at a wide range of patient age but mainly in the reproductive years with a mean age of 30 years [130]. The tumor rarely develops in postmenopausal patients [79, 131–134]. The oldest patient was documented in a 73-year-old woman who developed a choriocarcinoma 38 years after her last pregnancy [135]. The age of patients with PSTT at presentation ranges from 20 to rarely 63 years with a mean of 31 years [86, 87, 110]. Epithelioid trophoblastic tumor (ETT) occurs in patients of 15–66 years of age (mean of 37.1 years). Comparing with choriocarcinoma and PSTT, a significantly higher percentage of ETT has been reported in peri- and postmenopausal patients [103, 136, 137].

19. What Are the Distinguishing Features Between Epithelioid Trophoblastic Tumor (ETT) and Placental Site Nodule (PSN)?

The cell of origin for both PSN and ETT is chorion leave-type intermediate trophoblast. PSN represents the reactive, nonneoplastic end of the spectrum, while ETT is a malignant neoplasm [88]. PSN is often an incidental finding in an endometrial biopsy or curettage, or patients may present with irregular uterine bleeding. PSN frequently involves the lower uterine segment or endocervix and may also be present in endocervical curettings. The time interval between the most recent pregnancy and PSN can range from months to several years. Microscopically, it is composed of a well-

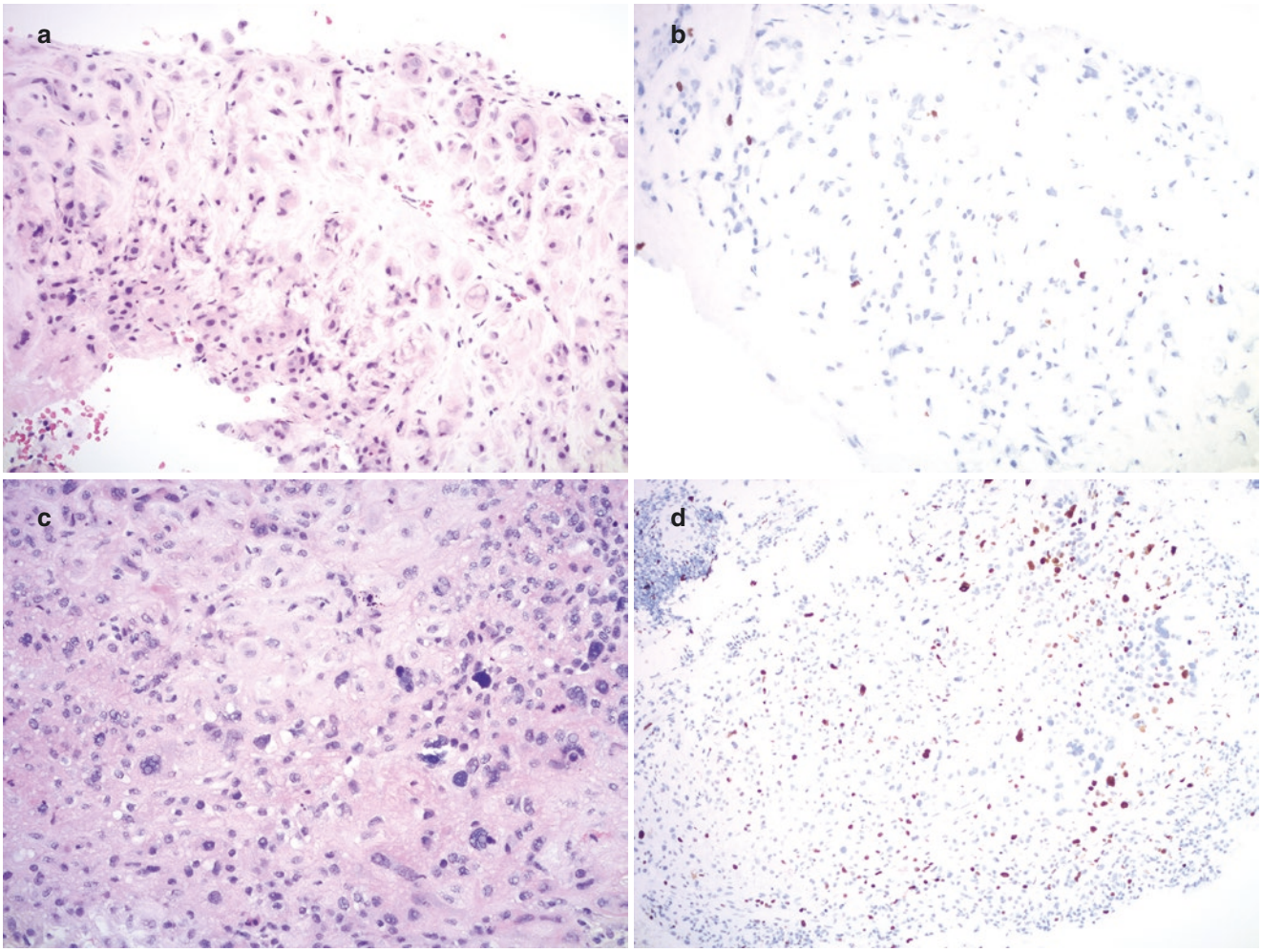

Fig. 7.12 Placental site nodule is composed of haphazard arrangement of mononuclear or multinucleated chorion laeve-type intermediate trophoblasts in a hyalinized matrix (**a**). The Ki-67 proliferation index is low (**b**). Atypical placental site nodule shows increased cellularity and nuclear atypia (**c**), along with elevated Ki-67 proliferation index (**d**)

circumscribed proliferation of haphazardly arranged mononuclear, and multinucleated intermediate trophoblasts in a hyalinized matrix, usually measuring less than 5 mm in size [138]. The cellularity is variable and typically shows zonation with a peripheral cellular zone surrounding a hypocellular, hyalinized center (Fig. 7.12). Mild nuclear atypia and nuclear pseudoinclusions may be seen, but mitotic figures are rare or absent.

In contrast, patients with ETT usually present with abnormal vaginal bleeding and have mildly elevated serum hCG. ETT forms a mass lesion—recognizable on imaging studies and on gross examination, shows increased cellularity, moderate nuclear atypia, geographic necrosis, and increased mitotic activity [55, 96, 97] (see also question #13). Distinction between ETT and PSN may be particularly difficult in small, fragmented biopsy or curettage specimens, especially if no imaging data are available. The immunoprofile of both ETT and PSN is similar: cytokeratins (e.g., CK8, CK18, CAM 5.2, AE1/AE3) are typically positive in addition to diffuse immunoreactivity for p63, p40, inhibin-alpha, and GATA3. Human placental lactogen (hPL) usually shows only weak, focal positivity. The proliferation index by Ki-67 immunostain is <5% in PSN, whereas ETT shows >10% Ki-67 labeling. Cyclin E immunostain has been reported to be strongly, diffusely positive in ETT, while PSN is negative or only weakly, focally positive for cyclin E [99], although the stain is not widely available in pathology laboratories for routine diagnostic workup.

A potential pathogenetic link between ETT and PSN and an intermediate precursor lesion, atypical placental site nodule (APSN) have been also proposed. APSN measures 5–10 mm and shows increased cellularity and nuclear atypia compared with typical PSN. In addition, mitotic figures may be present, and the Ki-67 proliferation index is between 5%

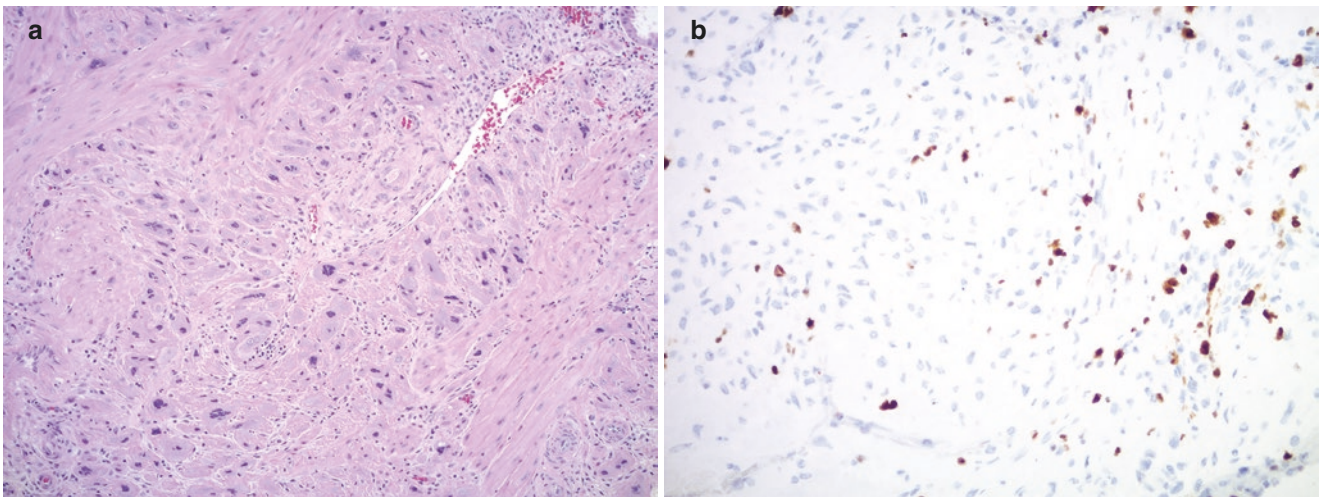

Fig. 7.13 Exaggerated placental site. Large intermediate trophoblastic cells (mono- and multinucleated) infiltrate the myometrium without forming a mass lesion (**a**). The Ki-67 proliferation index is low (**b**)

and 10%. Association with gestational trophoblastic tumors (ETT or PSTT) was reported in 14% of APSN in a large series of 21 patients [139].

20. What Are the Distinguishing Features Between Placental Site Trophoblastic Tumor (PSTT) and Exaggerated Placental Site (EPS)?

Exaggerated placental site (EPS) is a reactive proliferation of intermediate trophoblast at the implantation site in a concurrent normal, ectopic, or molar pregnancy. EPS typically does not form a grossly recognizable mass lesion. Microscopically, it infiltrates the underlying myometrium dissecting between individual smooth muscle fibers but does not alter the architecture of the endomyometrium. It is composed of large mononuclear intermediate trophoblastic cells with abundant eosinophilic cytoplasm, admixed with a variable number of evenly distributed multinucleated trophoblasts (Fig. 7.13). The trophoblastic cells may be single or form cords, nests, or confluent sheets. Necrosis and mitotic figures are absent, but focal degenerative changes, fibrin deposition, and hyalinization may be seen. Nuclear pleomorphism and hyperchromasia, especially in the multinucleated trophoblastic cells, may be present and may raise concern for PSTT.

Both EPS and PSTT have an infiltrative growth pattern—including invasion and replacement of vascular walls, similar to the physiologic process during normal implantation—and are composed of an admixture of mononuclear and multinuclear intermediate trophoblasts. However, EPS does not form a mass lesion on gross examination or on imaging studies and is invariably associated with a concurrent pregnancy. In contrast, PSTT usually presents with vaginal bleeding several months after the antecedent pregnancy (median interval: 12–18 months) and forms an infiltrative mass lesion (see also question #12) [87]. In addition, even distribution of the multinucleated cells, presence of chorionic villi, and lack of necrosis and mitotic activity are in favor of EPS. The immunohistochemical profiles of EPS and PSTT show a significant overlap: both are positive for pan-trophoblastic markers (CKs, inhibin, GATA3) and markers of implantation-type intermediate trophoblast (hPL, Mel-CAM) [15, 89]. P63 and p40 immunostains are typically negative in both EPS and PSTT. Ki-67 usually shows a low proliferation index (<2%) in EPS, while it usually exceeds 10% in PSTT [94].

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