



REVIEW ARTICLE

Update on the pathology of gestational trophoblastic disease

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Gestational trophoblastic disease can be a challenging area for pathologic evaluation. An update with a focus on pathologic challenges is presented.

Key words: Gestational trophoblastic disease; hydatidiform mole; choriocarcinoma; trophoblastic neoplasms.

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Gestational trophoblastic disease (GTD) means different things to the pathologist and the clinician. The pathologist utilizes a classification based on histopathology, while the clinician uses a clinically based classification, and does not always need to submit tissue for histopathology to appropriately manage the patient, particularly with post-molar GTD (Table 1). Persistent trophoblastic disease after a mole may be a persistent mole, an invasive mole (Fig. 1), or much more rarely, choriocarcinoma (Fig. 2). Management may not require a histopathologic distinction. There are good in-depth reviews of both pathologic and clinical aspects of GTD (1–3). This review focuses on updates and challenges for the pathologist. Challenges include distinction of moles from non-molar gestations, recognition of early moles, distinction of complete moles from partial moles, familiarity with lesions of extravillous trophoblast (EVT), and recognition of forms of GTD that may be seen by the pathologist after therapy.

HYDATIDIFORM MOLE

Hydatidiform moles are divided into complete and partial hydatidiform moles. These are genetically different lesions, with complete moles usually comprised of a diploid complement, both paternally derived, while partial moles are diandric triploids in

most cases. Digynic triploids are not moles. Rarely, complete moles may be biparental, in familial complete moles, associated with mutations of the *NLRP7* gene (4). Genetic variations from the usual chromosomal complements seen in molar disease have been reported. Recognizing moles and distinguishing complete from partial moles is important due to differing risk of persistent trophoblastic disease, which is considerably greater after a complete mole (15–20%) than a partial mole (<5%). Recurrence rates of moles in future pregnancies are the same (1–2%) (2). Histology can be challenging. Below are some specific issues that can arise.

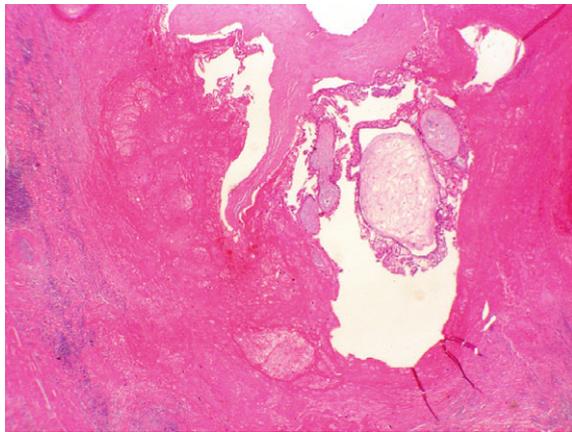
Early complete mole

Hydatidiform mole was previously diagnosed most often in the second trimester, when marked uterine enlargement, passage of ‘grapes’, bleeding, and other clinical manifestations became apparent. With the advent of early ultrasounds in pregnancy, most complete moles are detected in the first trimester, before full development of the pronounced histologic features seen in later complete moles, which show the expected edematous avascular villi with marked circumferential trophoblast proliferation. Earlier complete moles have distinct features as well, and familiarity with these features will aid in detection. Features include redundant bulbous terminal villi, hypercellular myxoid stroma, labyrinthine villous stromal capillaries, focal atypical villous and

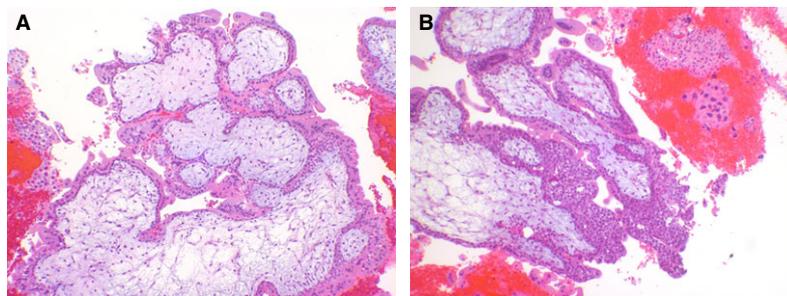
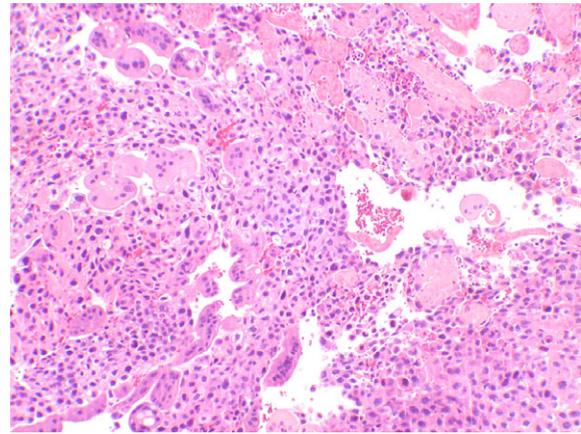
Table 1. Classification of gestational trophoblastic neoplasia

Pathologic classification	
Hydatidiform mole-complete/partial	
Invasive mole	
Choriocarcinoma	
Placental site trophoblastic tumor (PSTT)	
Epithelioid trophoblastic tumor (ETT)	
Miscellaneous Trophoblastic Lesions-Exaggerated placental site, Placental site nodule/plaque	
Unclassified	
Clinical classification	
Benign Gestational trophoblastic Disease	
Hydatidiform moles	
Malignant Gestational Trophoblastic Disease	
Non-metastatic	
Metastatic w/good prognosis	
Metastatic w/poor prognosis	

Reference: Shih et al. (25).

**Fig. 1.** Invasive mole-Molar villi with adjacent necrosis within myometrium.

extravillous trophoblast hyperplasia, focal extravillous trophoblast hyperplasia, hyperchromatic exaggerated implantation site trophoblast, and stromal karyorrhexis and apoptosis (Fig. 3) (5, 6)

**Fig. 3.** Early complete hydatidiform mole showing bulbous projections from villi (A), myxoid stroma with labyrinthine vessels and proliferating extravillous trophoblast (B).**Fig. 2.** Choriocarcinoma-A biphasic proliferation of cells is seen in the absence of chorionic villi.

Distinguishing complete from partial moles and moles from non-molar gestations

Even with subspecialty expertise, diagnosis of moles by evaluation of hematoxylin and eosin stained slides is challenging, with poor interobserver reproducibility (7). Partial moles have less pronounced histopathologic features than complete moles, with less edema and trophoblast proliferation. The presence of fetal tissue, including nucleated red blood cells or fetal membranes, favors a partial mole over a complete mole, but rare complete moles have evidence of fetal tissue, including a complete mole with a twin, or a mosaic complete mole (6). In partial moles, villi tend to be irregular and scalloped, with trophoblast inclusions, which can also be seen in pregnancies with other chromosomal abnormalities, although those pregnancies will not show trophoblast proliferation (Figs 4 and 5). This differs from the more rounded villi of complete moles. Missed abortions can also show edematous avascular villi, although the atypical trophoblastic proliferation of complete moles is absent. In order to further characterize these lesions, a variety of adjunctive techniques have been employed.

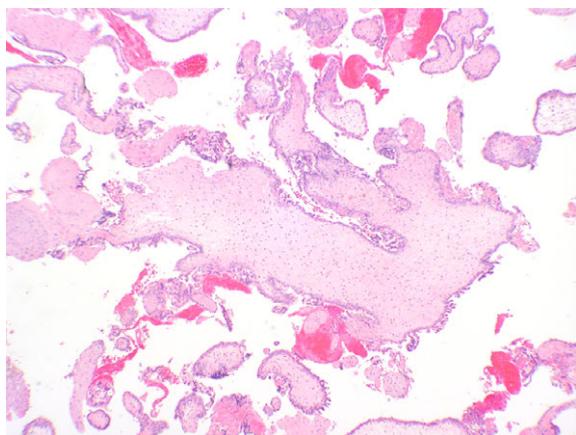


Fig. 4. Partial mole-Villi are scalloped, with less trophoblast proliferation than complete mole, however it is circumferential. There is a mix of molar and non-molar villi. Evidence of fetal tissue (not shown) should be sought.

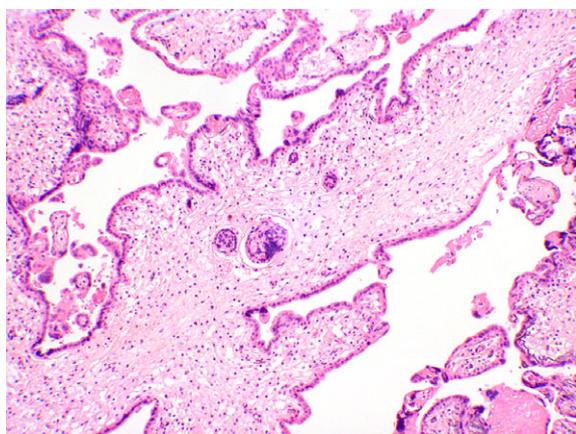


Fig. 5. Trophoblast inclusions-Numerous trophoblast inclusions are seen in this triploid placenta.

P57 immunohistochemistry

P57 immunostaining is helpful, but it is necessary to know how to interpret the results, which are not 'positive' and 'negative'. P57 is a paternally imprinted maternally expressed gene which stains maternal

tissues. This allows decidua to serve as an internal positive control. In complete moles, there is no villous staining, although extravillous trophoblast clusters do stain (Fig. 6A). The explanation for this staining of extravillous trophoblast clusters is unclear, but has been theorized to be due to incomplete or relaxed imprinting of p57, or less likely due to cross-reactivity (8). In partial moles, there is staining of villous stromal cells and cytotrophoblast (Fig. 6B).

Other adjunctive techniques

Ploidy analysis, via flow cytometry, fluorescent *in situ* hybridization or chromogenic *in situ* hybridization, can be helpful, distinguishing diploidy from triploidy. However, ploidy will not distinguish a diploid missed abortion from a diploid complete mole, or a triploid digynic gestation from a triploid diandric partial mole.

Short tandem repeat (STR) genotyping has emerged as the most useful ancillary technique, not only distinguishing moles from their mimics, and complete mole from partial mole, but also able to distinguish gestational from non-gestational trophoblastic disease, as well as recurrent from new molar disease (7, 9). STR genotyping can be performed on formalin-fixed, paraffin-embedded tissue. The limiting factor is the lack of availability of the technique at all centers. As in all techniques, there are rare unusual cases and pitfalls.

Familial hydatidiform mole (biparental complete mole)

A rare clinical form of complete hydatidiform mole is familial recurrent hydatidiform mole, where the moles are diploid but biparental. This is a recurrent autosomal recessive disorder. Histologically, the lesions are similar to sporadic complete moles, and usually are histologically indistinguishable, but the molar features may be less pronounced. P57 immunostaining is the same as for sporadic complete moles (4).

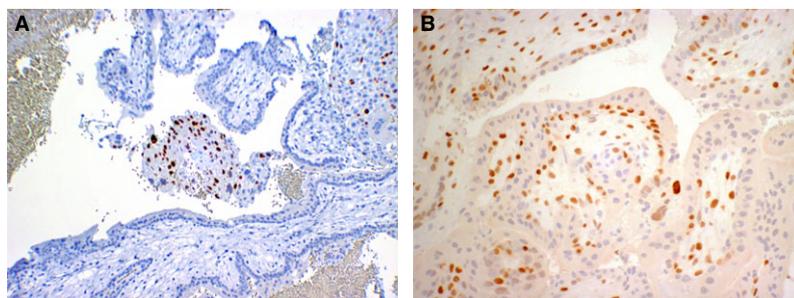


Fig. 6. P57 immunostaining in complete (A) and partial (B) moles. In complete moles (A), there is staining of extravillous trophoblast, but villi are negative. In partial moles (B), there is staining of villous stromal cells and cytotrophoblast.

MIMICS OF HYDATIDIFORM MOLE AND GTD

A variety of lesions may be mistaken for hydatidiform mole or GTD. Features of these are illustrated below:

Placental mesenchymal dysplasia

Placental mesenchymal dysplasia is a rare lesion associated with chromosomal abnormalities, Beckwith-Wiedemann syndrome, and stillbirth. It manifests as placentomegaly with cystic dilatation of stem villi which can be mistaken for molar vesicles, as opposed to molar dilatation of terminal villi (10) (Fig. 7). The cystic dilatation is concentrated near the fetal surface of the placenta, giving it a unique gross appearance.

Trophoblast inclusions

Partial moles are characterized by scalloping of terminal villi. These indentations can be seen on histologic sections as trophoblast inclusions if sectioned tangentially. While a rare one is probably of no significance, if increased over 1–2, trophoblast inclusions can also be a manifestation of other chromosomal abnormalities, including triploidy (11) (Fig. 5), and increased trophoblast inclusions are not a rare finding in spontaneous abortions. These other abnormal gestations will lack the trophoblast proliferation and mix of molar and non-molar villi of a partial mole.

Chorangioma with trophoblastic proliferation

Chorangiomas are fairly frequent in placentas, and represent hemangiomas within the parenchyma.

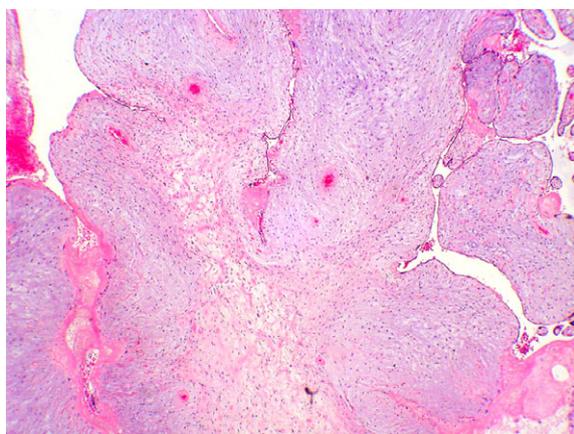


Fig. 7. Placental mesenchymal dysplasia—Stem villi are enlarged with a myxoid stroma and prominent vessels.

Unless large, they are usually of no clinical significance. Some of these lesions are associated with trophoblast proliferation around the periphery, or tracking along lobules of the lesion (Fig. 8), and the literature has termed these lesions ‘chorangiocarcinomas’. This is an unfortunate misnomer, as there have been no reports of persistent trophoblastic disease in mothers or their infants (12).

Florid trophoblastic proliferation in early pregnancy and tubal ectopic pregnancy

An area of challenge is the exuberant trophoblast proliferation that can be seen in early intrauterine gestations, as well as in tubal ectopic pregnancy. The trophoblast proliferation in early non-molar gestations has been described as ‘polar capping’, with the trophoblast at one end of the villus, the side of the direction of implantation. It is possible for a tubal hydatidiform mole to occur, but it is exceptionally rare. P57 Immunostaining may help if this issue arises in the fallopian tube or early intrauterine gestation. If products of conception are examined early enough for there to be no chorionic villi (pre-villous trophoblast), the question of choriocarcinoma may arise. In the absence of availability of STR genotyping, the distinction may fall on clinical evaluation, however this is a rare issue.

INTRAPLACENTAL CHORIOCARCINOMA

Intraplacental choriocarcinoma is not seen often, and this may be due to the lack of a grossly recognizable lesion in some cases. It is rare, representing about 0.03% of GTD in one series (13). It is generally found on examination of third trimester

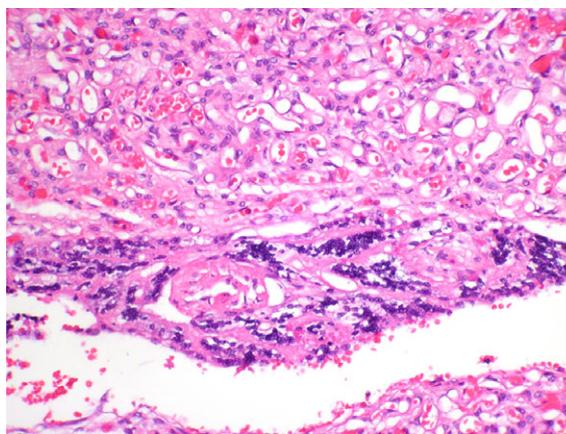


Fig. 8. Chorangioma with trophoblast proliferation—Trophoblast is seen in the center between lobules of a chorangioma.

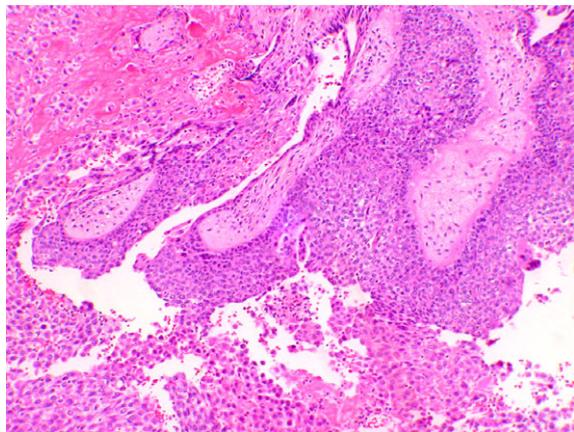


Fig. 9. Intraplacental choriocarcinoma-Exuberant trophoblast proliferation around a cluster of villi in a third trimester placenta that was otherwise normal. Necrosis is not uncommon.

placentas. While usually asymptomatic, vaginal bleeding, melena as well as respiratory and neurologic symptoms have been reported (13). Histologically, there is marked trophoblast proliferation around one or a cluster of villi, while the rest of the placenta is normal (Fig. 9). This is an important lesion to recognize and report to the clinicians, as there may be metastatic disease in both mothers and infants (13). Intraplacental choriocarcinoma may be the precursor lesion of gestational choriocarcinoma after a term gestation.

PITFALLS IN FOLLOWING SERUM BETA-HCG

Beta-hCG testing can give false positive results unrelated to GTD. This can occur in some tumors (14), end-stage renal disease, with some drugs, with heterophile antibody (15), or by misinterpretation of results. A variety of conditions can give false positive urine hcg results (16). False negative results

have been reported with GTD (17), and clinical judgment is always important.

A form of hCG, hyperglycosylated hcg (hCG-H) is made by extravillous cytotrophoblasts, as opposed to usual hCG, which is produced by syncytiotrophoblasts. hCG-H is related to normal implantation, and is associated with aggressiveness as well as chemosensitivity of choriocarcinoma, where it tends to be higher (18). It is lower in less aggressive/more chemoresistant GTD.

Quiescent GTD is a presumed inactive form of GTD with low levels of true hCG over several months, with disease undetectable, and the hCG unresponsive to chemotherapy. hCG subanalysis reveals no hyperglycosylated hCG (18). Long-term low b-hCG with no detectable disease may revert to normal at some point, but in one series (19), a large proportion of non-reverters were subsequently discovered to have PSTT or epithelioid trophoblastic tumor (ETT).

POST-EVACUATION GTD WITH FACTORS COMPLICATING HISTOPATHOLOGIC DIAGNOSIS

Tissue received by the pathology laboratory for evaluation of persistent GTD after mole can be challenging, and this can be complicated by factors such as insufficient history, or prior therapy. Curettings may only contain scant individual trophoblast cells, sometimes atypical, in blood clot. Theoretically, that finding could represent persistent mole, invasive mole, choriocarcinoma, PSTT/ETT, or a new intrauterine gestation. Lu et al. (20) described four cases of GTD where masses persisted after chemotherapy, two moles, one 'GTD', and one choriocarcinoma. After chemotherapy, the tissue resembled ETT, and the authors suggested that chemotherapy may have eliminated the more primitive choriocarcinoma cells, allowing evolution into a more chemoresistant phenotype of extravillous trophoblast.

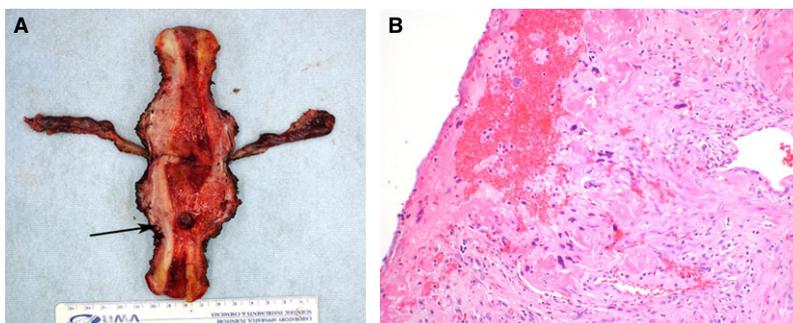


Fig. 10. Persistent GTD – A nodule is seen in the lower uterine segment (arrow) (A). It was mainly comprised of fibrin and blood clot, with rare atypical trophoblast cells dispersed within (B).

A lack of history can also hamper diagnosis. A hysterectomy for GTD was received by the laboratory for a persistent mass (Fig. 10A). Histologic evaluation showed rare atypical cells within blood clot (Fig. 10B). The initial history was that the patient had had a mole evacuated somewhere else a few months prior, and there was something ‘funny about it’. The patient’s serum hCG was reportedly low. Based on the greater immunostaining with human placental lactogen than with beta-hCG of the atypical cells from the hysterectomy, the initial impression was of a possible lesion of extravillous trophoblast, such as PSTT. Several weeks later, the prior slides from the molar pregnancy were available for review. They showed a complete mole with a more than usual amount of trophoblast proliferation, with greater atypia. The original diagnosis suggested that this mole may have been evolving into choriocarcinoma, and that the criteria for this were not well established. The reclassified final diagnosis on the hysterectomy was ‘GTD’, no longer thought to represent a lesion of extravillous trophoblast. This case emphasizes the importance of review of prior contributory pathologic material.

LESIONS OF EXTRAVILLOUS TROPHOBLAST

There are three types of EVT, the EVT of the trophoblastic columns, implantation site EVT, and chorionic EVT. These different types of EVT have different immunohistochemical profiles, as do the lesions arising from them (Table 2) (21). This category of lesion can be diagnostically challenging, due to overlap of histologic features, as well as lack of familiarity. EVT has also been termed ‘intermediate trophoblast’ in the literature, and some authors continue to utilize this terminology, but this is based more on histopathologic features than on the cells representing an intermediate stage of development.

Placental site nodules/plaques

Placental site nodules and plaques are frequently incidental findings in curettings, and it is unclear if

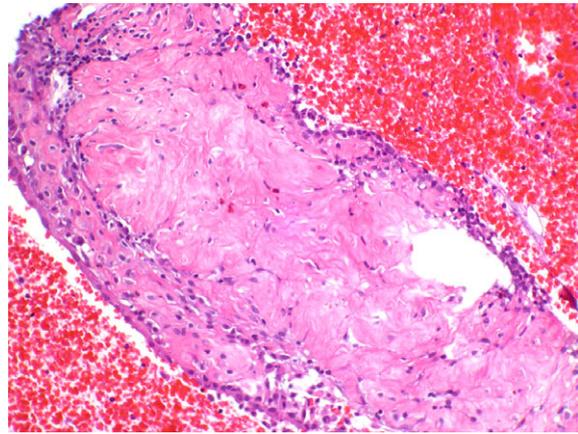


Fig. 11. Placental site nodule—This nodule found in a curettage demonstrates the low cellularity of these lesions.

they actually contribute to abnormal uterine bleeding. They are thought to be the ‘graveyards’ of prior implantation sites, often remote. The nodules and plaques are of low cellularity, predominantly composed of eosinophilic material (Fig. 11). A potential pitfall, although rare, are atypical placental site nodules (APSN) (22). On one series, 3/21 lesions termed APSN were associated with GTD on follow-up, developing into either PSTT or ETT. These patients did not have elevated beta-hCG. The histologic features of the APSN were intermediate between PSN and PSTT/ETT, with lesions larger than the usual PSN, which rarely exceed 4 mm. APSNs also had increased cellularity, mild atypia, and an increased ki-67 index compared to usual PSN (22).

Exaggerated placental site

Exaggerated implantation sites occur at the time of a gestation, either normal or molar. It is comprised of implantation site trophoblasts infiltrating decidua and between bundles of uterine smooth muscle. When seen in smooth muscle, particularly on curettings and if the chorionic villi have been passed, the concern of PSTT is sometimes raised. Exaggerated placental site (EPS) (Fig. 12) has more multinucleation than PSTT, and a ki-67 index of 0,

Table 2. Immunohistochemistry of lesions of extravillous trophoblast

	Cell type	CK18 AE1/3 EMA	Mel-CAM	Inhibin	hPL	PLAP	hCG	P63	Ki-67
EPS	Implantation EVT	+	+	+	+	Rare	–		0
PSN	Chorionic EVT	+	occ	+/-	occ	+	occ	+	5%
PSTT	Implantation EVT	+	+	+	+	+/-	Rare	–	14%
ETT	Chorionic EVT	+	occ	+/-	occ	occ	occ	+++	18%

EPS, exaggerated placental site; PSN, placental site nodule; PSTT, placental site trophoblastic tumor; ETT, epithelioid trophoblastic tumor; EVT, extravillous trophoblast; occ, occasional.

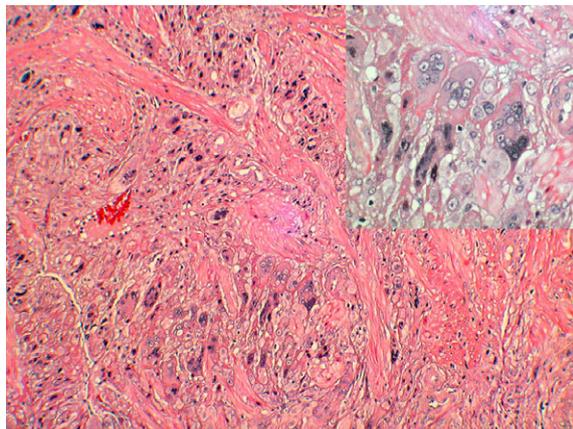


Fig. 12. Exaggerated placental site. EVT is seen dissecting between smooth muscle bundles. Many of the cells are multinucleated (inset).

as opposed to PSTT. EPS does not form masses like PSTT. In addition, the presence of chorionic villi helps establish the diagnosis.

Placental site trophoblastic tumor

Placental site trophoblastic tumor is composed of extravillous trophoblast of implantational type. It is monophasic (Fig. 13), unlike the biphasic choriocarcinoma, and dissects between bundles of smooth muscle, where it can resemble EPS. In addition to the presence of a ki-67 index of about 14%, PSTT is more extensive than EPS, and PSTT is mass forming. PSTT is usually non-aggressive in behavior, but metastatic disease has occurred. PSTT is more chemoresistant than choriocarcinoma.

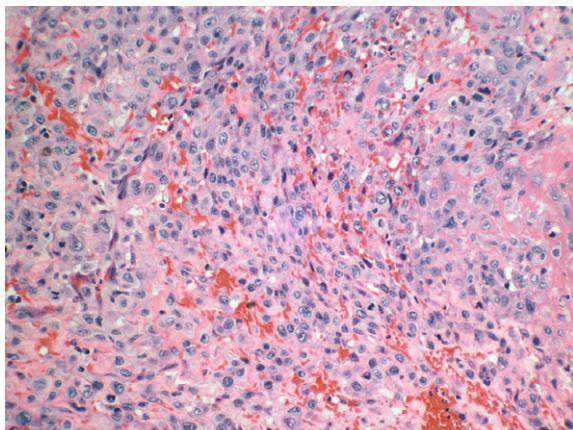


Fig. 13. Placental site trophoblastic tumor-The lesion is comprised of a monomorphous population of extravillous trophoblast.

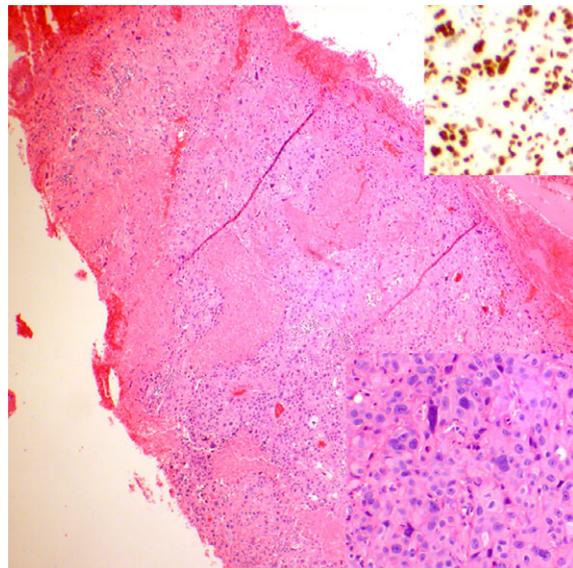


Fig. 14. Epithelioid trophoblastic tumor. The lesion shows geographic necrosis, and is comprised of a monomorphic proliferation of EVT (lower right), with a ki-67 index (upper right), in the range of 18%.

Epithelioid trophoblastic tumor

Similar in behavior to PSTT, ETT is derived from chorionic type extravillous trophoblast, and so has a different immunoprofile than PSTT (Table 2). An important characteristic is the presence of geographic necrosis (Fig. 14). Like PSTT, it tends to be chemoresistant.

Extrauterine lesions of extravillous trophoblasts:

Lesions of extravillous trophoblast can rarely occur in the fallopian tube, ovary or in paratubal tissue. This must be kept in mind for these rare lesions, and they should be distinguished from non-gestational choriocarcinoma in the adnexal region. A classification system for these rare lesions has been devised (23).

Future directions in GTD

Although still experimental, some feasibility has been established for identifying GTD and distinguishing it from non-gestational hCG-secreting tumors by evaluating circulating cell-free DNA (24).

CONCLUSIONS

Gestational trophoblastic disease continues to be a challenge for pathologists. Familiarity with the

histology and use of adjunctive techniques for unusual variants and early lesions will assist in the evaluation of these specimens.

DISCLOSURES

None.

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