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Histological comparison of partial hydatidiform mole and trisomy gestation specimens

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Summary

The distinction between partial hydatidiform mole (PHM) and trisomy gestation is not always straightforward histologically and it is unclear which morphological features, alone or in combination, provide the greatest diagnostic accuracy. We performed a comparative review of 89 products of conception (POC) specimens including 54 PHMs and 35 trisomy gestations, assessing the following in each case: trophoblastic atypia, cistern formation, multifocal trophoblast proliferation, lace-like trophoblast, villous enlargement, large trophoblast inclusions, scalloped villous shape, stromal apoptosis, small round villous inclusions, and fibrillary stromal collagen. There was a significant difference in the presence of trophoblast atypia, cistern formation, multifocal trophoblast proliferation, lace-like trophoblast, large trophoblastic inclusions, small round villous inclusions, fibrillary collagen (all $p<0.01$), and apoptosis ($p=0.028$), between PHM and trisomy cases. Fibrillary collagen was more common in trisomy specimens whereas the other features were more common in PHMs. There was no significant difference in villous enlargement or scalloped villous shape between the two groups. The combination of cistern formation, multifocal trophoblast proliferation and large trophoblast inclusions correctly classified 83 (93.3%) of cases where the presence of at least two features was considered diagnostic of PHM. While cytogenetic analysis is arguably the gold standard for diagnosis, this study demonstrates that histological assessment permits accurate distinction of PHM and trisomic gestations in the great majority of cases.

Key words: Partial hydatidiform mole; trisomy; morphological features; histological features; cistern; multifocal trophoblast proliferation; large trophoblast inclusions; fibrillary collagen.

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INTRODUCTION

Sporadic miscarriages affect one in four women and subsequently products of conception (POC) represent one of the most common specimens submitted to many diagnostic histopathology laboratories.[1] Although not performed routinely, cytogenetic analysis has established that approximately 20% of first trimester miscarriages show autosomal trisomy, most often involving chromosomes 16, 22, 21, 15, 18 and 2.[1] The risk of trisomy is linked to advancing maternal age and since the proportion of pregnancies in women over 35 years has risen from 5% to 20% over the past 20 years, the incidence of trisomy gestations has also increased.[2] Approximately 8–10% of miscarriage specimens demonstrate triploidy and the majority of these are diandric and show histological features of partial hydatidiform mole (PHM).[1] Unlike trisomy gestation, PHM carries a low but significant risk of gestational trophoblastic disease and therefore patients require appropriate counselling and follow-up including monitoring of serum β-hCG levels.

While the distinction between PHM and trisomic pregnancy in POC specimens is important in terms of clinical management, this is not always straightforward histologically, and there continues to be interobserver and intraobserver variation in the diagnosis of molar and non-molar gestations.[3–6] Immunohistochemistry for p57 protein has greatly facilitated the accurate diagnosis of complete hydatidiform mole (CHM), including early CHM,[6,7] but this does not help to distinguish triploid from trisomic pregnancies since both show retained p57 expression in the cytotrophoblast and villous stromal cells. Cytogenetic analysis, using classical cell culture, DNA cytometry, fluorescence *in situ* hybridisation (FISH), and/or polymerase chain reaction (PCR), is considered the gold standard for diagnosis but these techniques are not readily available in most surgical pathology laboratories. Thus, routine histological assessment remains the mainstay of diagnosis in most cases.

The characteristic morphological features of PHM are well described but in individual cases these can overlap significantly with those of non-molar gestations, particularly trisomy.[4,6,8,9] Indeed, in a recent review that tabulated the differential diagnostic features of molar and non-molar pregnancies, only cistern formation appeared to be an absolute discriminator favouring PHM over trisomy.[10] However, cisterns are not identified in all cases of PHM and it is not clear which additional features may be useful in this diagnostic context. Therefore, in this study we have performed a comparative analysis of cytogenetically verified PHM and

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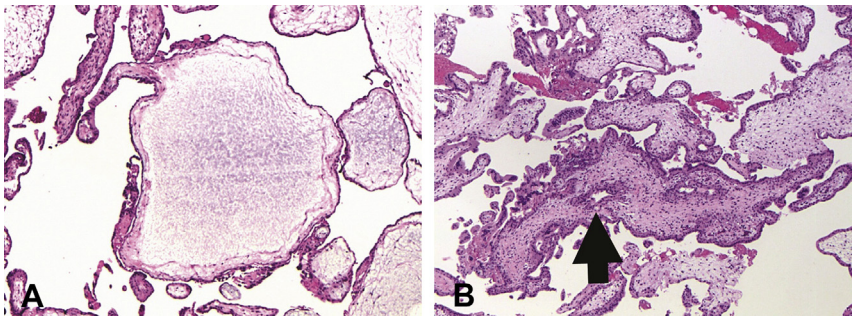

Fig. 1 Cisterns (A) and multifocal trophoblast proliferation (B) in a partial hydatidifrom mole. Large trophoblastic inclusions (arrow) are also present.

trisomic gestations to determine which combination of histological changes might offer the best discrimination between these types of POC.

MATERIALS AND METHODS

Following institutional ethics approval, 89 POC specimens comprising 54 PHM and 35 trisomy gestations accessioned between 2006 and 2014 were selected from the surgical pathology files of King Edward Memorial Hospital, Perth. All cases represented spontaneous miscarriages or medical terminations of pregnancy (MTOP). The PHM cases were sequentially identified POCs in which the reporting pathologist considered the histological findings to be suggestive or diagnostic of PHM, and in which subsequent FISH analysis confirmed triploidy. The trisomy cases included MTOPs in which cytogenetic analysis had already been performed following antenatal screening, or miscarriage specimens in which FISH was performed in view of the histological suspicion of trisomy, or difficulty in distinguishing trisomy from PHM.

The haematoxylin and eosin (H&E) stained slides from each case were reviewed by two pathologists and evaluated for the following morphological criteria of PHM.[11] Each feature was assessed as being either present or absent without further qualification. We did not include dual villous populations (the admixture of apparently normal villi and enlarged atypical hydropic villi) in the analysis since initial assessment indicated that this was not a useful discriminating feature.

1. Trophoblast atypia. This was assessed in cytotrophoblast (CT), syncytiotrophoblast (ST) and intermediate trophoblast (IT), and defined as threefold variation in nuclear size, nuclear enlargement with pleomorphism, and/or hyperchromasia. Atypia was recognisable at medium magnification (×100). Areas showing degenerative changes were excluded.
2. Cisterns. These were defined as completely acellular cavities containing oedema fluid within the centre of villi that were sharply demarcated from the surrounding villous stroma (Fig. 1A).[12] The cavities occupied at least 50% of the cross sectional area of the terminal villi.[10] Well-formed cisterns were counted even if the villi showed degenerative changes. Pseudo-cisterns resulting from folds in the placental membranes or stem villi were carefully excluded.
3. Multifocal trophoblast proliferation. This required the presence of two or more clearly separate foci of trophoblast proliferation, usually including CT and ST, distributed along the surface of villi (Fig. 1B). Circumferential trophoblast was also included although this was seldom identified.
4. Lace-like trophoblast. This was characterised by prominent intracytoplasmic lacunae (vacuolation) developing in IT and ST, typically in an extravillous distribution and recognisable at ×40 to ×100 magnification.
5. Villous enlargement. This was defined as villi measuring at least 2.5 mm in their longest axis (corresponding approximately to one field diameter at ×100 magnification).
6. Large trophoblastic inclusions. These resulted from sectioning across scalloped or irregular villous outlines resulting in irregularly shaped inclusions of various sizes but >0.2 mm diameter (Fig. 2A).
7. Scalloped villous shapes. These were characterised by multiple regular invaginations in the contours of enlarged villi.
8. Stromal apoptosis. This comprised karyorrhectic debris typically present within the superficial stroma adjacent to the CT and sometimes related to degenerate capillary-type vessels.
9. Small round villous inclusions. These were perfectly rounded inclusions no greater than 0.2 mm diameter that did not appear in continuity with the surface trophoblast (Fig. 2B).
10. Fibrillary collagen. This comprised wavy collagen bundles orientated along the long axis of lager villi (Fig. 3). At high magnification a cross-banded appearance was sometimes evident.

Fluorescence *in situ* hybridisation

The Vysis Aneuvysion probe kit (Abbott Molecular, USA) was used to enumerate copy numbers of chromosomes X, Y, 13, 18 and 21 in each sample. Disaggregated nuclei from paraffin-embedded tissue were prepared for FISH analysis using an established in-house method. Tissue core samples were obtained from paraffin-embedded blocks in which chorionic elements had been identified and marked on corresponding H&E stained slides as previously described.[13] The chorionic tissue was collected using a 20-gauge blunt needle inserted into the relevant area of the block and the tissue cores were then transferred on to a petri dish and finely minced with a disposable scalpel before being transferred to a micro-centrifuge tube. Paraffin was removed at room temperature with three 15-min changes of xylene, mixing the sample every 5 min. This was followed by a wash in three changes of 100% ethanol after which the cell pellet was allowed to dessicate before enzymatic digestion in 500 μL of freshly-made proteinase K solution (0.25 mg/mL proteinase K in

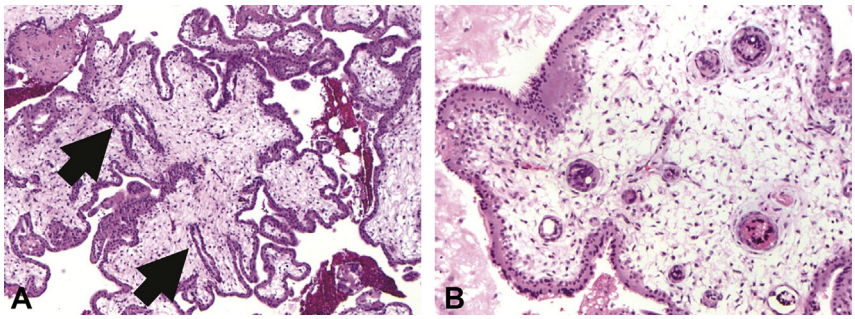

Fig. 2 Large trophoblast inclusions in a partial hydatidiform mole (A, arrows) show irregular or elongated appearances and are >0.2 mm diameter. In contrast, small round trophoblast inclusions in a trisomy pregnancy (B) are perfectly rounded and <0.2 mm diameter.