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OPINION

Is genetic analysis useful in the routine management of hydatidiform mole?

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Complete hydatidiform mole and partial hydatidiform mole are two abnormal conceptuses that may be identified by clinical, ultrasonographic, gross morphological, histological, and genetic characteristics. Among all these criteria, the specific diagnosis is generally confirmed only upon histological review. However, an accurate diagnosis based on morphological criteria is difficult and several studies have shown that misclassifications are frequent, even for experienced pathologists. An erroneous diagnosis may imply that women are either not enrolled in an adequate β-hCG follow-up with the risk that hydatidiform mole (HM) progresses to choriocarcinoma, or are enrolled in an unnecessary follow-up. A reliable and complementary method to the pathologic interpretation is a genetic study of the conceptus to eliminate the diagnostic dilemma by distinguishing non-molar spontaneous abortions from HM and to define the type of HM. The aim of our study was to review the genetic basis of HM and discuss its relevance in the routine management of the disorder.

Key words: complete hydatidiform mole/gestational trophoblastic disease/partial hydatidiform mole/triploidy

Introduction

Complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM) are chromosomally abnormal pregnancies which may be characterized by clinical, ultrasonographic, gross morphological, histological and genetic criteria (Vassilakos *et al*., 1977; Szulman and Surti, 1978a,b). The distinction between these two entities is important because CHM has a greater malignant potential than PHM and, consequently, the follow-up and the recommendations given to patients may differ.

Although ultrasonography and β-hCG level may be useful diagnostic tools in the identification of hydatidiform mole (HM), the final diagnosis is often confirmed only upon histological review. Nevertheless, several studies have shown that even experienced pathologists may have difficulties in distinguishing this disorder and some atypical cases are not easy to classify definitively on the basis of morphologic criteria alone. For example, it has been found that the concordance rate between pathologists for the diagnosis of molar pregnancies (CHM or PHM) ranges from 55–75% (Javey *et al*., 1979; Driscoll, 1987; Messerli *et al*., 1987). PHM may be particularly difficult to identify because it has features in common with both normal placenta and CHM. Paradinas and co-workers analysed retrospectively 400 cases of HM initially classified as PHM. PHM was confirmed in 50% of cases, CHM in 29%, and in 21% the diagnosis of HM was excluded (Paradinas, 1998). These misclassifications can be attributed to the absence of strict morphological criteria to differentiate the HM and because some characteristics have significant overlap. A useful complement to the pathological interpretation is to assay the ploidy of molar tissue with DNA cytometry analysis or fluorescence in-situ hybridization (FISH), but it may also be associated with a significant rate of misclassification, particularly if no fresh tissue is available and if abundant tissue of maternal origin is present (Bell *et al*., 1999). Moreover, ploidy analysis cannot be used to distinguish a diploid mole from spontaneous abortion which can also exhibit hydropic changes and trophoblast hyperplasia mimicking HM.

Genetic analysis may eliminate this dilemma and the aim of this study is to review the genetic basis of HM and to discuss its relevance in the routine management of the disorder.

Complete hydatidiform mole (CHM)

Histopathology

CHM is characterized by a diffuse trophoblastic hyperplasia with hydrops involving almost all the villi and resembling bunches of grapes, usually with an absence of a fetus or fetal tissue such as blood vessels or amniotic membranes, and isoften associated with cytologic atypia (Figures 1A,B). It was previously believed that CHM never contained fetal tissue but several reports have shown that this is incorrect and some fetal tissues may exist. This observation is not surprising since it has been demonstrated that the stromal tissue of the chorionic villi,

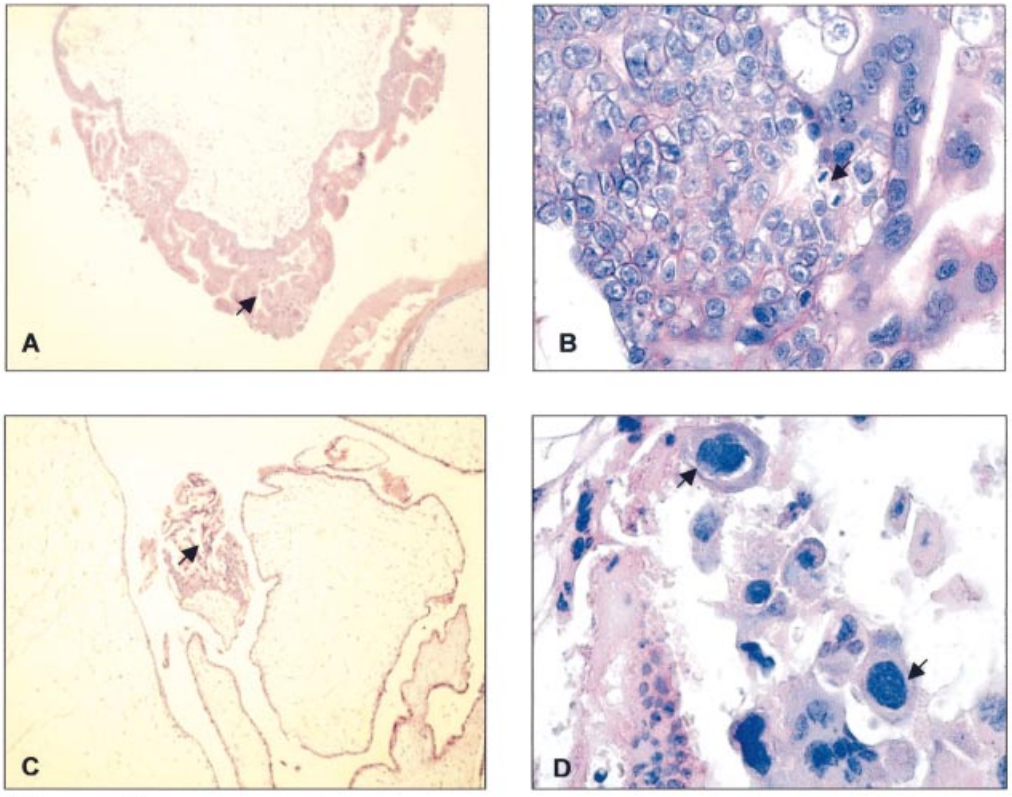

Figure 1

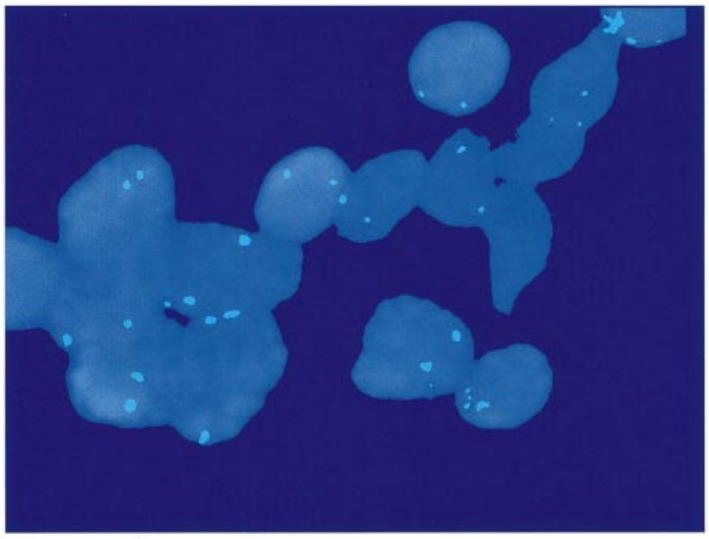

Figure 5

Figure 1. Hydatiform mole. (**A**) Complete hydatidiform mole with trophoblastic hyperplasia (arrow) 100x. (**B**) Complete hydatidiform mole with cellular atypia (same area as Figure 1A) and mitoses (arrow). 400x. (**C**) Partial hydatidiform mole with focal hyperplasia (arrow) 100x. (**D**) Partial hydatidiform mole (PHM) with focal trophoblastic hyperplasia and nuclear atypia (arrow) 400× (same area as Figure 1C). These characteristics have significant overlap with a complete hydatidiform mole and in this case, the diagnosis of PHM was confirmed by genetic analysis.

Figure 5. This shows an example of 46,XX complete hydatidiform mole. Two X-chromosomes specific signals after FISH on placental tissue are seen. Same specimen as Figure 3.

also present in CHM, originates from the embryonic mesoderm. It is presumed that in CHM the embryo dies very early in pregnancy. Recent improvements in the quality of sonography have resulted in the earlier detection of abnormal pregnancy and the evacuation of molar tissue in the first trimester of pregnancy, thus increasing the chances of detecting embryonic development. Several reports have demonstrated cases of androgenetic CHM with embryonic tissue not belonging to a twin pregnancy (Fisher *et al*., 1997; Paradinas *et al*., 1997). In early gestation (6–10 weeks gestation), hydropic villi may not

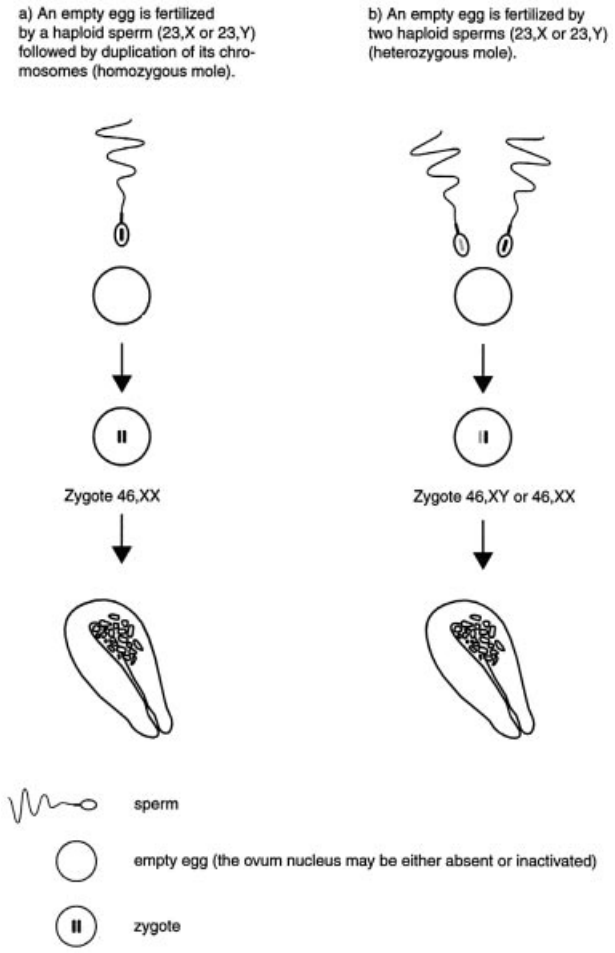

Figure 2. Mechanisms of formation of diploid moles.

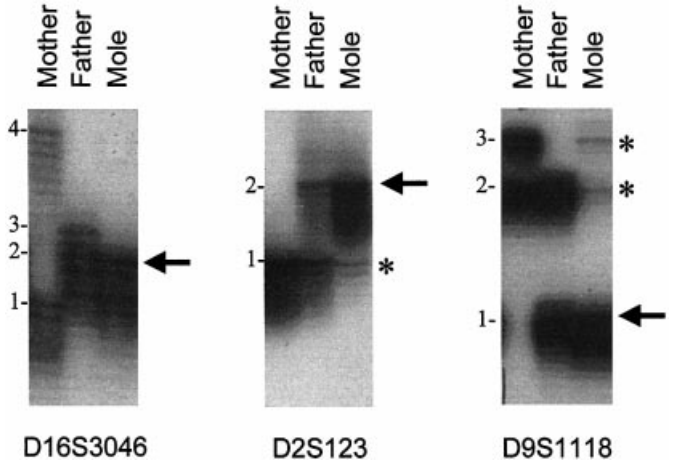

Figure 3. PCR amplified products of 3 microsatellite markers from chromosomes 2,9 and 16 on DNA from maternal, paternal peripheral blood and curettage specimen separated by gel electophoresis (denaturing PAGE). The unique allele observed for marker D16S3046 shows pattern of a single paternal allelic contribution (allele 2; arrow). For markers D2S123 and D9S1118, the unique paternal contribution is shown by the main strong allele (arrow; D2S123-allele2, D9S1118-allele 1). For these 2 markers, additional alleles (stars) of weak intensity can be explained by a minor contamination from maternal cells which is not revealed in marker D16S3046.

be apparent and molar stroma may be vascular; subsequently, these early CHMs may be misdiagnosed as PHMs (Paradinas *et al*., 1997).

Origin and genetic constitution of CHM

Genetic studies have demonstrated that CHM are mostly androgenetic and diploid with a 46,XX or 46,XY karyotype. Moles with a 46,YY karyotype have never been described, probably because such pregnancies are not viable. CHM arises from the fertilization of an empty oocyte (the female genome is completely extruded or inactivated) by one or two sperm (Kajii and Ohama, 1977; Szulman and Surti, 1978a,b; Ohama *et al*., 1981). Two mechanisms may explain the genesis of CHM (Figure 2). Theoretically, a third mechanism may involve fertilization through a diploid sperm due to nondivision at meiotic division, but evidence is lacking (Petignat, 2000). Although the chromosomes of CHM are mostly entirely of paternal origin, mitochondrial DNA is of maternal origin (Azuma *et al*., 1991).

CHM and malignancy

Following uterine evacuation, ~20% of patients with a CHM develop a persistent trophoblastic tumour (PTT); of these, ~4% will develop a metastatic tumour (Osathanondh *et al*., 1975; Berkowitz and Goldstein, 1996). Follow-up of these patients includes a weekly determination of β-hCG measurements until undetectable levels for three consecutive weeks, followed by monthly evaluations for 12 consecutive undetectable levels. However, if the patient's β-hCG values reach the normal range within two months after evacuation, follow-up may be limited to six months. Upon completion of follow-up, the patient may choose to conceive at any time (current policy in our institution).

Contribution of genetic studies and perspectives

CHM may be either monospermic if it arises from the doubling of a haploid sperm (homozygous moles), or dispermic if it arises from two haploid sperm (heterozygous moles) (Figure 3) (Ohama *et al*., 1981). Homozygous and heterozygous CHM are two genetically distinct entities which can only be distinguished on the basis of genetic analysis. Studies have suggested that heterozygous mole may have a more malignant potential than its homozygous counterpart. Wake et al. found that three of five patients with heterozygous moles had required treatment for post-molar trophoblastic tumour, compared with only one out of 21 patients with homozygous moles (Wake *et al*., 1984). However, these results have not been confirmed by other investigators who found no significant different risk between both groups (Lawler *et al*., 1982a; Kajii *et al*., 1984; Lawler and Fisher 1987; Lawler *et al*., 1991; Mutter *et al*., 1993). However, it should be mentioned that the number of published cases is small and additional studies are required to determine conclusively whether the heterozygous form is potentially more aggressive. If one form has really a higher malignant potential, it will be important to distinguish between both forms so that the relative risk can be assessed.

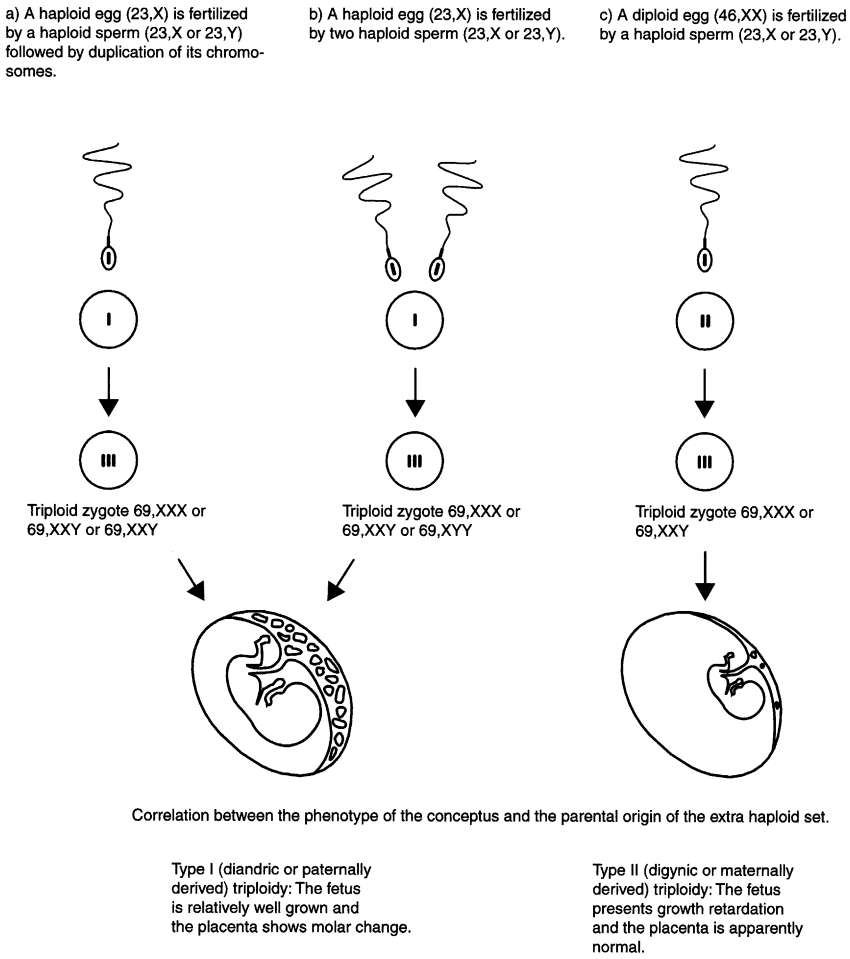

Figure 4. Mechanisms of formation of triploidy.

Partial hydatidiform mole (PHM)

Histopathology

PHM is generally accompanied by a fetus, or shows evidence of a previous existence of a fetus by the presence of erythroblasts or fetal membranes. Trophoblast hyperplasia is very focal and circumferential excess trophoblast often invaginates into the stroma to form characteristic scalloped outlines and round pseudoinclusions (Figure 1C); nuclear trophoblastic atypia may be present (Figure 1D). PHM must be extensively sampled as focal hyperplasia of trophoblast may not be detected and, subsequently, misdiagnosed as a banal hydropic abortus. On the other hand, non-molar pregnancies in certain specific conditions, such as chromosomal abnormalities may have irregular villi and round inclusions like a PHM and may be misclassified as partial mole (Chew *et al*., 2000). The hydropic abortus is completely benign, whereas a significant risk of PTT exists for those patients with a PHM.

Origin and genetic constitution of PHM

Partial moles are generally triploid gestations in which the extra chromosomal load is of paternal or maternal origin; the karyotype is 69,XXY, 69,XXX, or rarely 69,XYY (Jacobs *et al*., 1982; McFadden and Kalousek, 1991; McFadden *et al*., 1993). Triploid PHM may either arise through fertilization of a haploid oocyte by one spermatozoon which doubles its chromosomes after fertilization, or two sperm (one maternal and two paternal contributions), or through the fertilization of a diploid oocyte by one spermatozoon (two maternal and one paternal contribution) (Figure 4) (Lawler *et al*., 1982b; Jacobs *et al*., 1982). A diploid oocyte originates from failure of meiosis I or II. Another mechanism at the origin of diploid oocyte involves the fusion of two ova (‘dieggy’) (Zaragoza *et al*., 2000)

PHM and malignancy

PHM has a lower malignancy potential than CHM, with an incidence of PTT ranging from 4–11% and rarely transforms into choriocarcinoma (Palmer, 1994; Seckl *et al*., 2000). However, even if the risk of PTT is low, the current management of all patients with PHM is routinely to monitor β-hCG levels after evacuation. Follow-up includes a weekly β-hCG determination until undetectable levels are recorded for three consecutive weeks. The β-hCG level is then recorded monthly until normal for six consecutive months and patients are recommended to use contraception during that period (current policy in our institution).

Contribution of genetic studies and perspectives

Studies of the genetic origins of triploid PHM have demonstrated that fetal and placental phenotypes of triploid pregnancies correlate with parental origin and permit the definition of two types (Jacobs *et al*. 1982; McFadden and Kalousek 1991; McFadden *et al*. 1993; Zaragoza *et al*., 2000).

Type I triploid fetus or paternally-derived or diandric triploidy

The fetus is of normal size for gestational age and the placenta is abnormally large and cystic (Figure 4). In most cases, the fetus dies a few weeks after conception and, to our knowledge, no fetus of paternal origin has survived until term.

Type II triploid fetus or maternally-derived or digynic triploidy

The fetus is growth-retarded and the placenta is abnormally small without cystic formation (Figure 4). Some cases of triploid fetuses of maternal origin have been previously described as born alive with a survival of a few months (Fryns *et al*., 1977).

Currently, it is unclear if type I (diandric) and type II (digynic) triploidies have different malignant potential and if the distinction will have a direct impact on the management and counselling of patients. However, it appears that PHM type I is more aggressive than type II. Seckl *et al*. (2000) studied 3000 patients with PHM, three of whom developed a choriocarcinoma; genetic analysis showed that all three were PHM type I.

Unusual cases

Twin pregnancy consisting of an HM and a normal fetus

The occurrence of such a pregnancy is infrequent with an incidence estimated in the range of 1/10 000 to 1/100 000 pregnancies, but it is assumed that this diagnosis is grossly underrated. Firstly, because fetal loss may occur in early pregnancy without leaving gross evidence of 'a vanishing twin' and, secondly, because the differential diagnosis between HM co-existent with a fetus and partial mole is difficult and such situations are commonly mistaken for PHM (Petignat *et al*., 2001). First reports with small series indicated that the distinction is important given that the risk of PTT is much higher (~50% of cases) in a twin pregnancy combining a CHM and a normal fetus than in singleton CHM (Steller *et al*., 1994; Fishman *et al*., 1998; Petignat *et al*., 2002). However, a recent, larger study did not confirm these results and found that patients with a twin pregnancy involving an HM showed no increased risk of PTT over singleton CHM (Sebire *et al*., 2002).

CHM with biparental contribution

Several investigators have reported cases of CHM with genetic markers consistent with a normal conception. The constitution has both a paternal and maternal contribution to the genome, but are pathologically identical as classical androgenetic CHM (Jacobs *et al*., 1980; Davis *et al*., 1984; Kovacs *et al*., 1991; Fisher *et al*., 1997; Moglabey *et al*., 1999; Fisher *et al*., 2000). Such cases suggest that there may be more than two subgroups of CHM and other mechanisms could be involved that cause molar placental changes. Recently Fisher and colleagues have studied an HM of biparental origin (by comparing microsatellites polymorphism) and found no evidence of chromosomal uniparental disomy, suggesting that HM in these cases may results from uniparental disomy of only a small region of the paternal genome (Fisher *et al*., 2000). Cases with biparental origin could be a valuable tool in identifying the imprinted gene involved in molar development.

HM with unusual ploidy

Although the majority of PHM are triploid, uncommon DNA content such as haploid, diploid, tetraploid and mosaicism PHM have been reported (Vejerslev *et al*., 1987; Lage *et al*., 1992). Cases of triploid, tetraploid and mosaicism CHM have been reported also. These unusual ploidies associated with both partial and CHM render their differentiation and classification exceptionally difficult. However, the most important factor appears to be the ratio of paternal and maternal chromosomes and not the ploidy of the tissue (Vejerslev *et al*., 1987). For example, tetraploidy without maternal genome has the pathologic features of CHM and if maternal genome is present, the histopathology resembles a PHM. The implications of this rare condition in terms of malignancy are still unknown, but it seems that the degree of molar change correlates with both the proportion of paternal contribution and the risk of PTT.

Discussion

As mentioned previously, the diagnosis of HM is difficult and the histological criteria may be insufficient to distinguish CHM from PHM and HM from a non-molar abortion exhibiting hydropic change and trophoblast hyperplasia. Pathologists may be in doubt of the histological diagnosis (particularly pathologists inexperienced in the diagnosis of molar pregnancies) because, in many cases, products of conceptions show some histological evidence of hydropic change and the pathologist has to make a decision as to whether it is a molar or a non-molar pregnancy. Clinicians require an accurate diagnosis of these entities for both prognosis and patient management and a diagnosis reflecting uncertainty such as 'cannot rule out molar pregnancy' or 'lesion suspicious for HM' is insufficient. These difficulties have probably increased over recent years with the advent of more sophisticated prenatal screening tests and the use of high frequency probes for transvaginal sonogram which allow an earlier detection of abnormal pregnancy and earlier evacuation.

Alternative approaches have been developed to provide more objective diagnostic criteria to distinguish different forms of trophoblastic disease. An adjunct to histological diagnosis may be to determine the cell ploidy by means of flow cytometry or fluorescent in-situ hybridization (FISH). The combination of morphology and DNA content may be a useful aid in the differential diagnosis of molar pregnancy and improve the pathologists' concordance (Conran *et al*., 1993). However, cytometry or FISH results will not aid distinction of CHM from non-molar gestation given that there is a significant

overlap in the histologic and ploidy characteristics between these two entities (Bell *et al*., 1999). Recently, an immunohistopathological staining technique using $p57^{kip2}$ expression analysis has been reported as a good diagnostic adjunct complementary to histology and ploidy analysis to distinguish CHM from other types of conceptuses. This method could be easier to perform and interpret than genetic analysis in a pathology setting; however additional studies are required to determine the specificity and sensitivity of the technique (Zaragoza *et al*. 2000).

In the past, the laborious cytogenetic preparation of cell cultures and the potential misinterpretation on analysis of size and staining variants have precluded routine genetic studies. Currently, new molecular biology tools have made feasible the analysis of both molar and parental DNA on a routine basis. Such examinations may be performed, for example, by PCR amplification of several microsatellites markers of DNA and by comparing the sequences in the molar tissue and in genitors. In Figure 3, the analysis of the alleles (electrophoretic band) of each DNA indicates that the genetic content of the mole is of monospermic origin. This analysis is however semi-quantitative and does not inform about the ploidy of the molar tissue. This information can be determined by (FISH) using chromosome-specific DNA probes (Figure 5). Both methods are rapid and less costly than cell culture, can be assessed on either fresh tissues or paraffin-embedded specimens, thus providing accurate information on the genetic constitution of the conceptus. By combining histopathology, FISH and DNA analysis of the microsatellites, it is possible to distinguish CHM from PHM, or banal hydropic abortion from an HM.

We consider that the management of HM requires an accurate diagnosis that should be based on a histopathology and conclusively supported by a genetic analysis. Ideally, a routine genetic examination should be done as an adjunct to pathological examination each time that a trophoblastic disease is suspected. Some will argue that there is no founding for this routine analysis but although this diagnostic pathway is certainly more costly, we consider that the efforts justify the benefit for the patient's management. Ultimately, it should prevent the development of choriocarcinoma, because although most of these patients can be successfully treated with current chemotherapy, there are still those who will die from this disease or receive inadequate treatment, usually because of a delayed or erroneous diagnosis (Seckl *et al*., 2000). The reliability of the diagnosis is crucial for appropriate counselling and to determine if a patient falls into a 'short-term' or 'long-term' follow-up to minimize the period during which patients are recommended to use contraceptive methods. In some instances, for example in women having a 'lesion suspicious for HM', the diagnosis could be confirmed or ruled out, thus avoiding an unnecessary follow-up. This may be of particular importance in 'older patients' having difficulties in conceiving and for whom a one-year wait may be extremely distressing.

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