

OPINION

Postzygotic diploidization of triploids as a source of unusual cases of mosaicism, chimerism and twinning

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Triploidy is one of the most frequent chromosomal errors responsible for reproduction failure. This paper encompasses, in one conceptual frame, four recent findings in reproduction biology: predominant dispermic origin of triploids, paternal centrosome inheritance, eccentric cleavage divisions of dispermic triploid zygotes and certain intricate cases of mosaicism/chimerism. It is argued that dispermic zygotes, in contrast to digynic ones, are characterized by cytogenetic phenomenon described here as postzygotic diploidization of triploids (PDT). PDT embraces three main developmental scenarios: (i) the maintenance of the triploid state accompanied by regular segregation of $2n$ cells and the $2n/3n$ mixoploid populations; (ii) immediate diploidization with elimination of an odd haploid set of chromosomes and regular appearance of $1n/2n$, $2n/3n$ and other mixoploids and (iii) tripolar spindle formation leading to gross aneuploidy, cell death with occasional survival of $2n+1$ or $2n+1+1$ trisomics and uniparental disomics. According to the PDT concept, a trisomy and disomy might occur due to generalized karyotype instability of dispermic triploids. PDT may provide a natural explanation for the regular appearance of $2n$ homozygous androgenic moles, various $2n/3n$, $2n/2n$ molar/twin complexes without necessitating the concept of the ‘empty’ oocyte fertilization. Convincing evidence for a reservoir of anuclear oocytes does not exist. Peculiar implications are expected in the case of two rounds of diploidizations or involvement of triploid cell derivatives in the twinning process. Cryptic mosaic/chimeras and unusual twins intermediate between monozygotic (MZ) and dizygotic (DZ) are expected. Thus, PDT could have an explanation for the broad spectrum of odd reproductive cytogenetic events and might provide additional alternatives and definite predictions.

Key words: diploidization/mole/mosaicism/triploidy/trisomy/twins

Introduction

During the last two decades experimental findings in reproductive genetics have shown that three relevant basic processes—meiosis and gametogenesis, fertilization, and early embryonic development are ‘remarkably imprecise’ (Hassold, 1986). Data from eleven cytogenetic studies in IVF and embryo transfer indicated that ~35% of zygotes are chromosomally abnormal. The level of chromosomal abnormalities analysed by fluorescence in-situ hybridization (FISH) with specific probes for chromosomes X, Y, 13, 18 and 21 appeared to be higher, 52–61% (Munné *et al.*, 1998). The first detailed FISH analysis throughout all stages of preimplantation development showed that overall, 48.1% of embryos were mosaic. The frequency of mosaic embryos increased from 2–4 cell to 5–8 cell and morula stages (Bielanska *et al.*, 2002). In early embryos the level of chromosomal abnormality is 23–40% (Zenzes and Casper, 1992; Evsikov and Verlinsky, 1998).

Triploidy seems to be one of the most frequent chromosomal errors responsible for cleavage and implantation failure. It occurs in humans in nearly 1% of all conceptions and in >10% of all spontaneous abortions. Recent molecular studies of triploidy have confirmed and extended the main previous conclusions that were based on cytogenetic analysis (Uchida *et al.*, 1985): (i) most triploids are paternal in origin, (ii) there is a parent-of- origin effect on the phenotypes of triploids and (iii) a substantial part of diandric triploids constitute partial hydatidiform moles (Zaragoza *et al.*, 2000). Normozoospermic males produce diandric triploids predominantly by dispermy, while most triploids produced by oligozoospermic males occur by diplospermy, from fertilization by unreduced $2n$ sperm (Macas *et al.*, 2001; Egozcue *et al.*, 2002).

In addition to the observed differences in the developmental profile of digynic and diandric triploids connected with imprinting, we would like to draw attention to another important consequence of paternal dispermic triploidy: a

phenomenon described here as the postzygotic diploidization of triploids (PDT).

Paternal centrosome inheritance and dispermic triploidy

I argue that postzygotic diploidization of triploids (i) could have a direct or indirect relationship to the broad spectrum of unusual cytogenetic events described recently in human reproductive biology, and (ii) shows that predictions can be made within the conceptual frame of this phenomenon.

In 1994 it was firmly established that in humans, as in most animals, (excluding rodents) the centrioles and centrosome (regulating syngamy and the first zygotic division) have a paternal origin (Palermo *et al.*, 1994, 1995, 1997). During fertilization the male centrosome is introduced via the sperm tail into the oocyte and remains attached to the sperm head in the process of sperm nuclear decondensation. At syngamy the centrioles duplicate occupying a pivotal position in opposite spindle poles. The centriolar region forms the aster guiding the female pronucleus towards the male pronucleus. The microtubules extending from the centrosome form a bipolar mitotic spindle. As far as microtubules influence other protein fibres, the centrosome acts as the architect of the cytoskeleton. Thus all zygote division is orchestrated by the paternal centrosome (Glover *et al.*, 1993).

Male-derived centrioles were detected up to the blastocyst stage. The female centrosome is inactive (Sathananthan *et al.*, 1996; Palermo *et al.*, 1997; Sathananthan, 1998; Sutovsky and Schatten, 2000). So a digynic triploid has usually normal sperm-derived bipolar centrioles and has a relatively low incidence of chromosome mosaicism in the early embryo divisions. When present, such mosaicism originated at a later embryo division (Palermo *et al.*, 1995). Dramatic problems occur in the case of dispermy, resulting in two pairs of active centrioles in one ovum. This results (in ~50% of cases) in a tripolar spindle, inevitably producing chaotic chromosome distribution and gross aneuploidy.

Angell *et al.* (1986) conducted the first direct cytological observations of the behaviour of tripronuclear fertilized oocytes. Three main developmental outcomes of dispermic triploid zygotes were later confirmed and expanded upon by other investigations (Kola *et al.*, 1987; Plachot *et al.*, 1987, 1992; Pieters *et al.*, 1992; Zenzes and Casper, 1992; Ma *et al.*, 1995; Rosenbusch *et al.*, 1997; Tarin *et al.*, 1999). These were (Figure 1): (i) a mitotic division with bipolar spindle, giving 3n blastomeres and embryos in ~25 % of cases; (ii) exclusion of one haploid genome from the metaphase plate of the first cleavage division (14–32% of cases), resulting in 2n diploid, 2n/3n mosaics and 1n/2n derivatives (variants B–D in Figure 1) and (iii) in ~50–60% of dispermic zygotes a tripolar spindle is formed at the first cleavage division resulting in dramatic abnormalities in chromosome distribution.

In these tripolar zygotes, three sets of chromosomes remained relatively separated, forming a Y-like arrangement in the centre of the oocyte. These zygotes divide first into three cells and then into six cells, whereas diploid zygotes divide into two and then four cells. Only 10–13% of triploids in culture

conditions reach the blastocyst stage (Plachot *et al.*, 1992; Tarin *et al.*, 1999).

Triploid diploidization and its cytogenetic implications

The immediate diploidization of dispermic triploid zygotes was an unforeseen finding. The higher incidence of 'surprisingly diploid' embryos suggested some regulation of the degree of ploidy (Plachot *et al.*, 1992). Since the genes regulating both mitotic and meiotic cell divisions seem universal, it is pertinent to mention here that the phenomenon of human triploidy and its various implications are strikingly similar to the picture of meiotic diploidization of triploid plants Jimson weed (*Datura stramonium*) analysed in the 1920-30s by A.Blakeslee, the founder of the cytogenetics of trisomy and triploidy (see comprehensive discussion in the textbook: Burnham, 1980). Blakeslee firstly found that each chromosome in a trisomic state had a specific phenotype and discovered in the progeny of primary trisomics the secondary trisomics or isochromosomes. He discovered also that fast diploidization of *Datura* triploids occurred during one generation and was accompanied by survival of some single and double trisomics. These plant cytogenetic studies influenced the finding by K.Patau of human trisomies 13 and 18 (Crow, 2002).

I underscore once more the main post-fertilization developmental profiles of dispermic zygotes (Figure 1): (i) the partial maintenance of the triploid state accompanied by regular segregation of 2n cells and the occurrence of 2n/3n cell/tissue mixoploid populations; (ii) immediate diploidization with elimination of an odd haploid set of chromosomes and appearance of 1n/2n, 2n/3n and other types of mixoploids and (iii) tripolar spindle formation leading to the gross aneuploidy, cell death and an occasional survival of trisomics.

These main three developmental scenarios of dispermic triploids may be overlapped in various cell lineages. But the essential point is their regular (immediate or through some mitotic divisions) diploidization. These premises present a new perspective for an explanation of many relevant striking facts described in human reproductive cytogenetics (see recent discussion: Barinaga 2002; Pearson, 2002;). They also allow the prediction of additional mechanisms for the origin of such events as mixoploidy, chimerism, hydatidiform moles (HM) and twinning. The diploidization of triploid zygotes has universal relevance, occurring also in digynic triploids albeit quite rarely (Palermo *et al.*, 1994). Let us consider some examples.

The 2n/3n mixoploidy

At least some diploid/triploids are viable. Such mixoploids have been known as a clinically recognizable syndrome since the beginning of 1980s (Tharapel *et al.*, 1983). Up to 1993 at least 17 liveborn infants with 2n/3n mosaicism have been described (Carakushansky *et al.*, 1993). Due to selective advantage, 2n cells may quickly replace the original triploid ones.

A 46,XX/69,XXY chromosome complement was identified in a 13-year old boy with mental retardation, club feet, eunuchoid habitus, and underdeveloped genitalia. His triploid cells had two paternal genomes and one maternal genome.

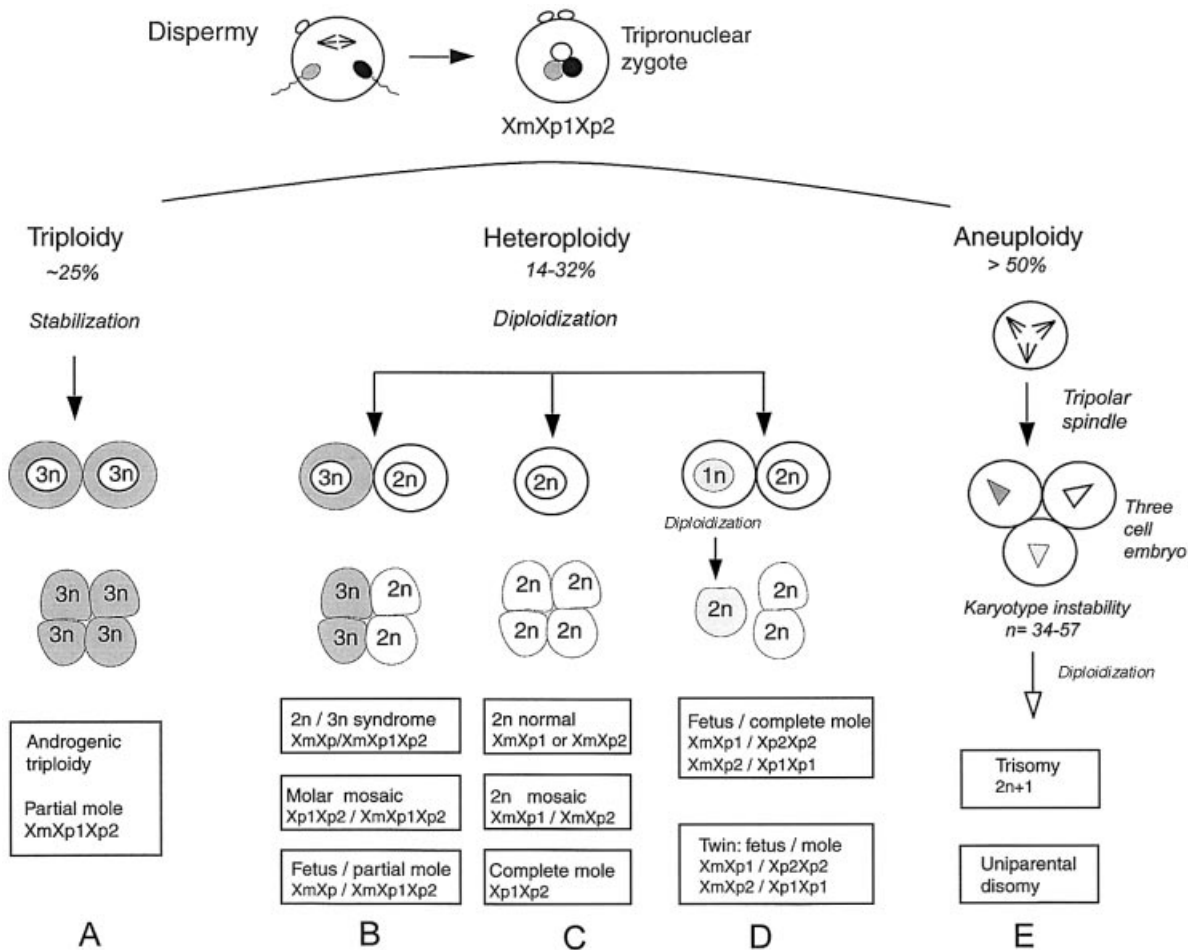


Figure 1. Dispermy, variants of triploid zygote cleavage and their possible developmental outcomes (A-E). The proposed genomic structure of the triploid zygote and its derivatives is indicated, where Xm—maternal and Xp1 and Xp2—two diverse paternal genomes. Small circles are polar bodies; 2n cells are pictured as white, 3n and 1n cells and their derivatives are shadowed. (A) 3n karyotype is stabilized in almost 25% of all cleavage divisions leading to an androgenic triploidy and partial mole; (B, C, D) possible variants of diploidization, occurring in almost 14–32% zygotes; (B) 2n/3n heteroploidy with diverse developmental derivatives; (C) 2n embryos with mosaic and molar outcomes; (D) 1n/2n heteroploids with subsequent endomitosis (haploid diploidization) of the 1n cells and molar derivatives; (E) tripolar spindle formation in almost 50% of all zygotes, appearance of three cell embryos after the first cleavage division, karyotype instability and aneuploidy with possible surviving trisomy and uniparental disomy. Dispermic fertilization may lead also to XmXpY triploid zygotes (not shown) producing after diploidization various sex chromosome mosaics and chimeras. The scheme is based on the following data (Angel *et al.*, 1986; Kola *et al.*, 1987; Plachot *et al.*, 1987, 1992; Pieters *et al.*, 1992; Rosenbush *et al.*, 1997; Tarin *et al.*, 1999).

3n/2n cell ratios were 60:40 in fibroblasts and 4:96 in lymphocytes. The authors suggested an additional fertilization by one of the two first blastomeres (Dewald *et al.*, 1975); but the other possible origin of such 46,XmXp/69,XmXpY mosaics may be dispermic triploidy with its subsequent diploidization (loss of one of the paternal genomes) in the first cell lineages. 2n/3n mosaicism is probably underestimated, since in 70% of cases the triploidy is seen only in fibroblasts; in lymphocytes triploidy is observed usually in <5% of cells. (See discussion in Wulfsberg *et al.*, 1991; Phelan *et al.*, 2001).

Especially informative in the context of this discussion is the finding of the discordance for the level of 2n/3n mosaicism in monozygous (MZ) diamniotic and monochorionic twin girls evaluated at the age of 9 years (Wulfsberg *et al.*, 1991). A skin biopsy of one of the twins showed clear 2n/3n mosaicism, with 65% 46,XX cells and 35% 69,XXX cells. However, all skin cells of the second co-twin were diploid. At the same time both MZ twin sisters manifested a quite typical set of phenotypic abnormalities for this kind of mosaicism (face asymmetry,

cutaneous syndactyly and peculiar pigmental dysplasia). It follows that the twins developed from a single triploid zygote leading in early embryogenesis to similar patterns of 2n/3n mosaicism, which was responsible for their similar phenotypic anomalies. However, the further proliferation of 2n/3n mixoploid cell populations led to differences in each monozygotic partner. Triploid cells were maintained in one twin but disappeared in the second. A similar situation was found in a case of mosaicism for trisomy 16, where mosaic cell populations were noticed in the early embryo but subsequent cytogenetic studies of neonatal tissue did not detect aneuploid cells. This 'occult mosaicism' (Benn, 1998) may be quite usual for the evolution of various mixoploid cell populations.

Triploidy and trisomy

The occurrence of a trisomy and uniparental disomy associated with it is usually viewed as an event involving a specific

chromosome pair. Most cases of trisomy are due to female (in 10–25% male) meiotic MI errors (Hassold, 1986; Robinson *et al.*, 2001; Hunt and Hassold, 2002). However, I suggest that trisomy sometimes could also occur as an outcome of generalized karyotype instability of the triploid genome (Figure 1E). The process of diploidization suggests the appearance during cell proliferation of single, double or multiple trisomy. Thus at the level of cell populations simultaneous mosaicism both of triploidy and trisomy may be expected. Such events have been described.

A combination of 2n/3n mosaicism with supernumerary sex chromosome, 48,XXYY/71,XXXYY, was found in an 11-month boy with severe developmental retardation (Schmid and Vischer, 1967). The karyotypes of the parents were normal. To explain this unusual karyotypic mosaicism the authors suggested (i) the fertilization of the oocyte by a single aberrant XYY sperm cell (derivative of non-disjunction of the Y-chromosome in MI) and (ii) the occurrence of a triploid line due to the fusion of the second polar body with the one of the first blastomeres. However, a diploidization scenario is also possible, assuming that an original dispermic triploid, XXY, underwent diploidization in one blastomere with subsequent non-disjunction of sex chromosomes in both lineages.

A 2n/3n mosaicism accompanied by trisomy 18 was directly observed in the pre-implantation derivative of a dispermic triploid zygote (Plachot *et al.*, 1987). Triploidy and trisomy 13 were observed in an infant who manifested features of both triploidy and trisomy 13 (Phelan *et al.*, 2001). The distribution of two cell types was the tissue-specific: all cells from the amnion were triploid while all cells from chorionic villi were trisomic; in cord blood 80% of cells were trisomic and 20% triploid; in fibroblasts the percentages were reversed. The karyotype of this unique mosaic was 69,XXY/47,XY,+13. Cytogenetic and DNA microsatellite analysis showed the presence of two non-identical maternal genomes due to an error in MII. The authors postulated two rare postfertilization events: (i) fusion of one blastomere with a second polar body producing the triploid cell lineage, and (ii) non-disjunction of the chromosome 13 in the second blastomere. Alternatively, diploidization in the cell lineages of an original triploid zygote, accompanied by the trisomy 13 may have been responsible. It is pertinent to note that abnormal mitotic divisions and diploidization may also occur in digynic tripronuclear zygotes resulting from ICSI (Macas *et al.*, 1996).

The phenomenon of diploidization of triploids can explain unusual cases of concurrent triploidy and trisomy in chorion cells and in fetuses accompanied by clear dichotomy in the tissue distribution. In one case (English *et al.*, 2000), the cultured cells from both chorion villi and the post mortem placenta showed three cell lines: 46,XX, 47,XX,+6 and 69,XXX, while fetal skin and muscle were entirely 69,XXX, with two maternal and one paternal chromosome sets. The authors consider two possible explanations: (i) 46,XX conception with incorporation of the second polar body, forming a 3n triploid zygote with its immediate mitotic 'segregation' into 2n and 3n blastomeres and subsequent mitotic error in the 2n blastomere producing 46,XX and 47,XX,+6 derivatives or (ii) maternal MII non-disjunction, producing 24,+6 and monosomic 22,-6 female pronuclei. One of these scenarios then produces both aneuploid and normal 46,XX cell lineages (mitotic trisomy rescue) and the other blastomere becomes 69,XXX triploid due to incorporation of the monosomic polar body. It is hard to choose between these unusual and

complicated scenarios. The first requires two fertilization errors—incorporation of second polar body and a mitotic error in early embryogenesis; whereas the second needs three errors—MII maternal error, incorporation of the second polar body and a trisomic mitotic rescue. I suggest an alternative third scenario including an original triploid zygote and its diploidization, accompanied by survival of a trisomic cell lineage. This may also apply to another similar finding of 2n/3n divergent triploidy/trisomy mosaicism (fetus 47,XX+18; placenta 70,XXX+18), which the authors called 'remarkable' in the title (Tuerlings *et al.*, 1993).

Multiple trisomy

Multiple trisomics are mostly cell lethals and escape observation. Trisomies account for ~60% of cytogenetically abnormal abortions and thus only a small portion of single trisomics survives to birth. The incidence of double trisomy is 0.7% of all karyotyped spontaneous abortions (three tissues were studied: fetal, placenta and villi; Reddy, 1997). A total of 55 different combinations of double trisomy have been observed. All chromosomes except chromosome 1 and 19 were found in double trisomy combinations. Triple trisomy involves mainly chromosome 18, 21 and X.

Multiple trisomy is usually considered as the result of non-disjunctions of two or more pairs of chromosome in the successive cell lineages of the same zygote. It was found, however, that the proportion of numerical trisomy among triploids is ~7%, in comparison with only 2.6% among chromosomally unbalanced fetal losses on a diploid background (Daniel *et al.*, 2001). The authors were inclined to suggest that in triploid fetuses the negative viability effect of additional trisomy and other chromosome abnormalities is less than in diploids. Alternatively, diploidization of triploids may be itself a natural relevant explanation of this interesting observation and finding.

In one unusual case, cytogenetic analysis of amniotic fluid cells from a 31-week-old fetus suffering from polyhydramnios revealed that there were two cell lines, each with either trisomy 13 or trisomy 18. There was no discordance of parent-child transmission between the two cell lines, suggesting that the observed mixoploidy was not chimerism but mosaicism (Abe *et al.*, 1996). It cannot be excluded that in other situations double and triple trisomy have an origin not from independent segregation errors, but rather the occasional maintenance of distinct trisomic clones resulting from abnormal diploidization of a pre-existing triploid zygote.

Uniparental disomy

In tripolar cleavage each of three genomes moves to the metaphase plate and segregates relatively separately from the other two genomes. However, deviations from such whole genomic distributions are quite possible. They may result in an appearance of uniparental disomy. Let us consider, for example, a dispermic triploid X_mX_p1X_p2; A_m1A_p1A_p2 where X and A designate any two pair of chromosome and 'm' and 'p' correspond to the maternal versus paternal chromosome origin. If the diploidization in some cases involves chromosomes from different genomes of the triploid zygote, one may predict that 1/3 of mitotic derivatives for each pair of chromosomes may be uniparental.

Correspondingly in 1/9 double combinations we may expect the uniparental state simultaneously on two chromosome pairs.

This may result in diploid cells with the karyotype Xp1Xp2; Ap1Ap2, where both two pairs would be paternal.

For some chromosomes, postzygotic mitotic errors may be the main cause of the uniparental chromosomal pattern (Eggerman *et al.*, 2001). Uniparental disomy occurring postzygotically is well established in many cases of confined placental mosaicism characterized by a discrepancy between the karyotypes of the fetus and placenta. Placental tissue is more tolerant to the existence of specific trisomies and uniparental disomy is expected in one third of the disomic cell progeny of trisomic cells (Kalousek and Barrett, 1994).

Hydatidiform moles (HM) and twinning

In the process of diploidization of a dispermic triploid, one predicts various types of the 2n/3n and 1n/2n mixoploidy. It was suggested firstly by R.G.Edwards and supported by cytological observations by Angell *et al.* (1986) that, in the case of diploidization, some 2n cell derivatives may develop as an HM. Unusual variants may occur during early embryogenesis if triploid-dependent mixoploidy coincides with the twinning. This may lead to the appearance of twin associations between the hydatidiform mole and co-existing fetus. In some 2n/3n mixoploids the developing diploid fetus may be associated with the triploid partial HM, or conversely, the triploid abnormal fetus with the diploid androgenic mole (Figure 1B,D). Unusual PDT-dependent molar/fetus associations may be rather frequent but ‘‘are either not diagnosed as moles or the fetus has been lost during the first week of pregnancy’’ (Baergen *et al.*, 1996).

A placenta with a partial HM manifesting 2n/3n mosaicism was identified by molecular analysis (Ikeda *et al.*, 1996). Molar region was mixoploid consisting of 2n and 3n cells and phenotypically normal tissue had a mainly diploid constitution. As a possibility the authors suggested that this mixoploid placenta originated from a triploid conceptus ‘‘followed by postzygotic loss of a paternal haploid set’’. This principally corresponds to the diploidization scenario B (Figure 1).

Recently an unusual 2n/3n mosaic molar complex associated with a normal female fetus (died *in utero*) was described. The normal and cystic villous tissues were diffusely intermixed. The genetic analysis showed the normal villi to be diploid, but heterozygous, and the molar villi triploid. Two placental tissues had the same genotype. The authors suggested an interesting fertilization scenario in which a tetraploid oocyte (prior to polar body extrusion) became fertilized and the resulting 5n zygote immediately separated into 3n and 2n major cells (Zhang *et al.*, 2000). Although this may be correct, a more plausible explanation is diploidization of the triploid in accordance with the scenario, Figure 1D. Authors discuss this explanation but deny it, asking ‘‘how to understand such an exclusion of one complete set of chromosomes from a triploid conceptus’’. However, the direct cytogenetic observations showed that dispermic tripronuclear zygotes after first metaphase regularly give rise to 1n/2n daughter cells (Plachot *et al.*, 1992; Rosenbusch *et al.*, 1997). Thus the 2n clone may develop into a normal fetus, and 1n clone, after endoreduplication produce 2n androgenic molar diploid clones with 2n/3n molar mosaicism in the placenta. Exclusion of maternal genome leads to normal/molar complex.

The estimated incidence of twin associations consisting of a HM and a co-existing fetus is 1 per 22 000–100 000 pregnancies, according to the long term observations at the New England Trophoblastic Disease Center (Steller *et al.*, 1994).

Among nine such patients treated, the prevalence of complete HMs was evident—8 out of 9 cases (Steller *et al.*, 1994). In a comprehensive relevant study of 72 cases of twins associated with HM, the most frequent combination was again a normal diploid fetus and an androgenic diploid mole (Matsui *et al.*, 2000). Thus among 27 fetuses, 25 had a normal diploid karyotype; the remaining two cases manifested both triploidy and trisomy (as expected in triploid-dependent mixoploidy). Among 12 tested androgenic moles, 10 were homozygous (such as Xp1Xp1) and only two appeared heterozygous (Matsui *et al.*, 2000).

In a similar study, nine twin associations of complete HM and co-existing fetuses were described, with the fetuses showing an even sex distribution (XX and XY), whereas all complete HM appeared to be paternal homozygotes (Choi-Hong *et al.*, 1995). These striking findings need an explanation. Why are the moles in the twin-mole associations predominantly diploid and homozygous? It is hard to explain this unusual bias by the usually accepted dizygotic origin of such associations (Matsui *et al.*, 2000). Apparent natural explanation follows from the diploidization concept.

Approximately 80% of complete HM are androgenic diploids. Among them, nearly 75% are homozygous for paternal chromosomes (Lindor *et al.*, 1992; Kovacs *et al.*, 1991). Two mechanisms are usually envisaged for this kind of diandric diploidy: (i) penetration by a haploid sperm of an anuclear (‘empty’) oocyte with subsequent endoreduplication of the male pronucleus. Only 46,XX conceptions will survive, 46,YY are nonviable; (ii) fertilization of an anuclear oocyte by two haploid sperms, resulting in 46,Xp1Xp2 and 46,XpY karyotypes, the so-called heterozygous mole. It is noteworthy that both of these mechanisms implicitly assume some regular ‘reservoir’ of empty or anuclear oocytes. Convincing evidence for this does not exist. Enucleated oocytes are feasibly produced in embryo/genetic experimental situations. However, they have not been described as a regular feature of an oogenesis, capable of being fertilized in natural conditions.

This clear difficulty is rarely discussed considering the high frequency of molar pregnancies: 1 in every 1500 pregnancies in USA, with relative risk up to 2.5% in the age between 35 and 39 years (Lindor *et al.*, 1992). Meanwhile, the predominance of complete 2n androgenic moles 46, Xp1Xp1 associated with normal diploid twins of both sexes can be explained if we assume the division of an original tripronuclear zygote in the above mentioned 1n/2n scenario. 2n clones may produce a normal fetus and 1n clones after endoreduplication generate complete androgenic HM (Figure 1D). Diploidization—dependent complete moles do not need ‘empty oocyte’ for their occurrence. The principal series of events may be the following: (i) 1n/2n clones from the original dispermic triploids XmXp1Xp2 may have after diploidization karyotypes Xp1/XmXp2 or Xp2/XmXp1; also the clones from the Y-carrying triploids XmXpY may produce relevant 1n/2n derivatives Xp/XmY; (ii) 2n clones give normal fetuses of both sexes and (iii) haploid cells after endoreduplication (or haploid diploidization) yield only paternal-derived homozygous complete moles. Apparently, this sequence of events may also give an Xm haploid derivative that subsequently becomes diploid after endoreduplication and may yield maternal teratoma (Surti *et al.*, 1990).

The significant confirmation of this conclusion might be the recent molecular genetic studies of unusual mole/fetus asso-

ciation of a genotype Xp1Xp1/XmXp1 where both the mole and fetus had identical paternal genome and monospermic origin (Makrydimas *et al.*, 2002). The authors suggest: (i) heterochronous (precocious) male pronucleus mitotic division leading to temporary zygotic triploidy Xp1Xp1Xm, (ii) appearance of two blastomeres- normal XmXp1 and haploid androgenic Xp1, and (iii) subsequent diploidization of androgenic blastomere resulting in complete Xp1Xp1 mole.

Cryptic mosaics/chimeras and unusual twins

The appearance of regular 2n/3n mosaics in the cell progeny of triploids is a well-established phenomenon. Meanwhile, an interesting situation is expected in the case of two successive rounds of diploidization of triploid zygotes. This event may lead to an appearance of genetically different 2n clones or cryptic 'chimeras'. If these involve same-sexed 2n clones, they usually escape phenotypic identification. However in the case of diploidization of triploids carrying a Y chromosome (XmXpY), it is possible to expect both cryptic same-sexed chimeras XmY and XpY and unlike-sexed chimeras combining XmXp and XmY. They would have the same maternal and different paternal genomes and might show hermaphroditism, dependently on the distortion of XX and XY cells.

XX/XY hermaphrodites have been described since the end of the 1970s (Fitzgerald *et al.*, 1979; Dewald *et al.*, 1980). Cytogenetic evidence of a chimeric hermaphrodite having 46,XX/46,XY karyotype (ratio 38:12 in lymphocytes) was confirmed using microsatellite DNA analysis (Giltay *et al.*, 1998). As 'the most likely mechanism' of its origin, the authors suggest (i) a single haploid oocyte dividing parthenogenetically into two haploid blastomeres, (ii) followed by their fertilization by distinct male gametes (iii) fusion of two zygotes into a single individual. Apparently, the occurrence of similar chimeras XmXp/XmY with one maternal and two paternal genomes via two events of diploidization of the original triploid zygote XmXpY may also provide an explanation of this unique finding.

Finally, triploid-derived chimeric 2n embryonal cell populations could be involved in the twinning process. A triploid zygote XmXp1Xp2 may produce XmXp1 and XmXp2 cell derivatives, which may develop into mosaics/chimeras or into twins (Figure 1C). Such unusual twins, having common maternal and distinct paternal genomes, will mimic dizygotic (DZ) twins, but are predicted to be more similar in some traits. They were previously termed SZ or Sesquizygotic twins (Golubovsky and Golubovskaya 1984; Golubovsky 2002). Using the same 'diploidization logic', dispermic triploids with Y-chromosome XmXpY may also generate unlike-sexed pairs of twins with identical maternal but different paternal genomes.

We would like to highlight that consideration of postzygotic diploidization of triploids, especially dispermic ones, will lead to a better understanding of some otherwise unusual but complicated cases of the chromosomal mosaicism and human reproductive genetics.

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