Influence of diandric and digynic triploid genotypes on early mouse embryogenesis

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Summary

Standard micromanipulatory techniques were used to produce tripronucleate diandric and digynic triploid mouse conceptuses. When these were transferred to suitable recipients, most implanted. A wide range of embryonic stages from the primitive streak to the 15- to 25-somite stage were isolated in both triploid series in otherwise identical recipients. In the diandric triploid series, all of the embryos recovered appeared to be morphologically normal, but considerably smaller than fertilized embryos analysed at similar stages of development. This contrasts with the digynic triploid conceptuses which, though also ranging from the primitive-streak stage to about the 10- to 15-somite stage at the time of their isolation, generally showed poorer embryonic development than the diandric triploids, and were invariably morphologically abnormal. Unlike the situation observed in man, where the placentas of diandric triploid conceptuses commonly display widespread trophoblastic hyperplasia and form the characteristic 'partial' or 'incomplete' type of hydatidiform moles, the extraembryonic membranes of the diandric triploid mouse conceptuses (as well as the digynic triploids) did not appear to be grossly abnormal.

Key words: mouse, diandric triploidy, digynic triploidy, embryogenesis, nuclear transfers.

Introduction

Considering that 1–3% of all recognized human conceptions are believed to be triploid (Carr, 1971a,b; Niebuhr, 1974; Bouè et al. 1975; Beatty, 1978), little is known about the influence of their triploid genotype on their embryogenesis. Until quite recently, difficulties were often encountered in establishing the exact embryological origin of human triploid embryos/fetuses, namely whether they were diandric or digynic in origin. It now appears that about 85% of human triploids result from dispermy/diandry, and only about 15% from digyny (Surti, 1987).

However, because of the technical difficulties encountered in the analysis of human triploid material, we decided to investigate, in an experimental model, whether the presence of a diandric or digynic triploid genome per se had any influence on mammalian embryogenesis. To achieve this end, diandric and digynic triploid mouse conceptuses that had been produced experimentally following the microsurgical insertion of a male or female pronucleus, respectively, into a normal fertilized 1-cell-stage egg were analysed morphologically and cyto genetically during the early post-implantation period. Using this approach, combined with the use of an appropriate paternally derived 'marker' chromosome, we were able to unequivocally confirm the diandric or digynic triploid status of the analysed embryos.

In our study, all the tripronuclear 'hybrid' 1-cell-stage embryos were transferred to pseudopregnant recipients which were autopsied at about midday on the 10th day of pregnancy when normal diploid fertilized embryos would be expected to have achieved the forelimb-bud stage, and possess 25 or more pairs of somites.

As the experimental procedure used resulted in particularly high rates of implantation, it was possible for us to isolate and analyse a high proportion of the transferred conceptuses. The morphological features and developmental stage achieved by each conceptus was also assessed.

Materials and methods

8- to 12-week-old (C57BL × CBA)F1 hybrid female mice were injected with 5 i.u. of pregnant mare's serum gonadotrophin (PMSG) followed 48 h later by 5 i.u. of human chorionic gonadotrophin (HCG). Shortly after the HCG injection, the females were caged, in the preliminary series of experiments, with (C57BL × CBA)F1 hybrid males. Early the following morning the females were checked for the presence of a vaginal plug and the latter was taken as evidence of mating. The morning of finding a vaginal plug was considered to be the first day of pregnancy.

Early-pronucleate-stage fertilized eggs were isolated at
about 10 a.m. in the morning on the day of finding a vaginal plug. In these early fertilized eggs, the female pronucleus is always located in close proximity to the second polar body, while the male pronucleus is located elsewhere in the cytoplasm, but usually towards the periphery of the egg – in the subcortical zone. In the first series of experiments in which F1 hybrid females were mated to F1 hybrid males, male pronuclei were isolated with a small volume of cytoplasm from ‘donor’ eggs and inserted in the presence of inactivated Sendai virus into the perivitelline space of ‘recipient’ 1-cell-stage fertilized eggs using standard micromanipulatory techniques (McGrath & Solter, 1983; Howlett et al. 1987). Evidence of cytoplasmic fusion was always seen within about 1 h of the micromanipulation procedure. The ‘hybrid’ tripronucleate (i.e. diandric) eggs (Fig. 1) were then transferred unilaterally to the oviducts of recipients on the first day of pseudopregnancy (this was subsequently considered the first day of gestation). The recipients were anaesthetized with tribromoethanol (Avertin: Winthrop; dose 0.02 ml g\(^{-1}\) body weight of a freshly prepared 1-2% solution of Avertin dissolved in 0.9% saline).

These recipients were then autopsied at about midday on the 10th day of gestation, and the number of implantation sites present, resorptions and embryos recovered were noted. Cytogenetic analyses of the extraembryonic membranes – principally the yolk sac, but often including amniotic tissue (in the case of the advanced ‘unturned’ or the ‘turned’ embryos) or of the intact conceptuses (in all other cases) were made using a modification of the technique described by Evans et al. (1972), in order to confirm that they had a triploid chromosome constitution. All of the developmentally more advanced triploid conceptuses were, in addition, analysed histologically. This experimental group has been termed Series 1.

In order to establish unequivocally that the triploids produced were indeed diandric in origin, an additional group of superovulated F1 hybrid females which had previously been mated to fertile homozygous Rb(1.3)I Bnr males (MRC, Harwell) were autopsied on the morning of finding a vaginal plug. Male pronuclei were again isolated from ‘donor’ eggs and transferred into the perivitelline space of ‘recipient’ eggs derived from the same mating combination (i.e. F1 female × Rb(1.3)I Bnr male), as described above, in order to obtain diandric triploidy.

The genotype of Rb(1.3)I Bnr males (2n number = 38) contains two large metacentric chromosomes, being Robertsonian translocations involving chromosomes 1 and 3 (cf. normal mouse 2n number = 40). Consequently, following fertilization by spermatozoa from these males, the paternally derived haploid genome contains 18 acrocentric and 1 large metacentric ‘marker’ chromosome. Thus diandric triploid eggs, which have a normal female chromosome complement associated with two paternally derived haploid chromosome sets derived from the Rb(1.3)I Bnr males, would inevitably contain a total of 58 chromosomes, two of which would be the large metacentric ‘marker’ chromosomes. This experimental group has been termed Series 2.

A further group of superovulated F1 hybrid females were mated to fertile homozygous Rb(1.3)I Bnr males, but in this series female pronuclei were isolated from ‘donor’ eggs and inserted, as described above, into the perivitelline space of ‘recipient’ eggs derived from the F1 female × Rb(1.3)I Bnr mating combination, in order to obtain digynic triploidy. These triploidy eggs (Fig. 2) were then transferred unilaterally to the oviducts of recipients as described above, and autopsies carried out on the 10th day of gestation in order, in due course, to analyse the development potential of these triploid conceptuses. Mitotic spreads from these digynic triploids, with two genetically normal maternally derived pronuclei and one paternally derived Rb(1.3)I Bnr pronucleus would inevitably contain 59 chromosomes, only one of which was a large metacentric ‘marker’ chromosome. This experimental group has been termed Series 3.

An additional group of unilateral oviduct transfers was also carried out in which pronucleate-stage normal diploid fertilized eggs were transferred to recipients on the first day of pseudopregnancy. These embryos acted as controls for the three experimental series described above. This control group has been termed Series 4.
Diandric and digynic triploid mouse embryos

Table 1. Postimplantation development of diandric and digynic triploid conceptuses isolated on the 10th day of gestation following the transfer of tripronucleate eggs to the oviducts of pseudopregnant recipients

<table>
<thead>
<tr>
<th>Group</th>
<th>No. recipients</th>
<th>No. embryos transferred</th>
<th>No. implantations (%) transferred</th>
<th>No. resorptions (%) implants</th>
<th>No. conceptuses isolated</th>
<th>Triploidy confirmed cytogenetically</th>
<th>Diandric/digynic triploidy confirmed cytogenetically</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (F_1) hybrid females (\times) (F_1) hybrid males (diandric triploid conceptuses)</td>
<td>6</td>
<td>41</td>
<td>33 (80.5)</td>
<td>12 (36.4)</td>
<td>21</td>
<td>19</td>
<td>NA*</td>
</tr>
<tr>
<td>2. (F_1) hybrid females (\times) Rb(1.3)1Bnr 'marker' male</td>
<td>7</td>
<td>39</td>
<td>34 (87.2)</td>
<td>9 (26.5)</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>(a) Diandric triploid conceptuses</td>
<td>5</td>
<td>28</td>
<td>24 (85.7)</td>
<td>6 (25.0)</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>(b) Digynic triploid conceptuses</td>
<td>3</td>
<td>18</td>
<td>17 (94.4)</td>
<td>3 (17.6)</td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. (F_1) hybrid females (\times) (F_1) hybrid males (diploid fertilized controls)</td>
<td>-</td>
<td>-</td>
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</table>

* Impossible to confirm diandric status unequivocally in the absence of a paternally derived 'marker' chromosome.

In order to study the morphological appearance of embryos and their extraembryonic membranes in situ, a small group of recipients into which \(F_1\) (female) \(\times\) Rb(1.3)1Bnr (male) diandric triploid embryos had been transferred unilaterally, were autopsied at about midday on the 9th day of gestation. Approximately half of their implantation sites were fixed intact and their contents examined histologically. The conceptuses isolated from within the other implantation sites were examined cytogenetically in order to determine their ploidy and confirm their diandric origin.

Results

(A) Postimplantation development of diandric and digynic triploids

Series 1. The development of \(F_1 \times F_1\) diandric triploid embryos

The results of this first series of experiments are presented in Table 1 (Group 1). The implantation rate in this series was 80.5%, and 19 out of 21 of the conceptuses isolated at about midday on the 10th day of gestation from the operated sides had a triploid chromosome constitution. However, in the absence of an appropriate paternally derived 'marker' chromosome, it was not possible to establish unequivocally whether these conceptuses were in fact diandric or digynic in origin. Nevertheless, the age of the eggs at the time of micromanipulation – being at the early pronucleate stage, and the location of the female pronucleus – being in close proximity to the second polar body, allowed the male pronucleus to be recognized with little difficulty. However, in those instances where there was any doubt about the identity of the male pronucleus, the egg was automatically excluded from the study.

The two conceptuses isolated from the side into which embryos had been transferred that had a diploid chromosome constitution were probably eggs that had undergone micromanipulation, but in which the 'donor' nucleus failed to incorporate and take part in the subsequent development of the conceptus. Alternatively, these diploid conceptuses might have resulted from the recipients' own eggs that had been activated parthenogenetically. The latter is the less likely scenario for the reasons indicated below (see section C). Since the developmental stage achieved by the triploid conceptuses in this series appeared in all respects to be identical to that achieved by the diandric triploids from Series 2 (see below), the findings from these two series have been combined, and are for convenience presented at the end of the next section.

Series 2. The development of \(F_1\) (female) \(\times\) Rb(1.3)1Bnr (male) diandric triploid embryos

The results of this series of experiments are presented in Table 1 (Group 2a). The implantation rate in this series was 87.2%, and all of the conceptuses isolated on the 10th day of gestation from the operated sides had a triploid chromosome constitution. However, in the presence of an appropriate paternally derived 'marker' chromosome, it was possible to establish whether these conceptuses were diandric or digynic in origin. In fact, analysis of the metaphase spreads from these triploid conceptuses unequivocally revealed that they all were diandric in origin. All of these metaphase spreads had a total of 58 chromosomes present, two of which were the paternally derived large metacentric 'marker' chromosomes (see Fig. 3).

Of the 44 diandric triploid conceptuses isolated (i.e. 19 'assumed' diandric triploids from Series 1 and the 25 diandric triploids whose genotype had been confirmed cytogenetically from Series 2), the developmental stages achieved varied widely. Nine were in the form of an empty gestational sac, similar in many respects to the 'triploidy syndrome' first described in detail by Wroblewska (1971). The other embryos recovered,
however, all appeared to be morphologically normal in appearance but significantly smaller than normal fertilized embryos at similar stages of development. Thus, three embryos were at the advanced-egg-cylinder or early-primitive-streak stage, thirteen were at the early-headfold/early-somite stage, fifteen were at the advanced ‘unturned’/partially ‘turned’ stage with about 10–15 pairs of somites present, while the four developmentally most advanced embryos had all ‘turned’, and were at the forelimb-bud stage with about 20–25 pairs of somites.

Series 3. The development of F1 (female) × Rb(1.3)1Bnr (male) digynic triploid embryos
The results of this series of experiments are presented in Table 1 (Group 2b). The implantation rate in this series was 85-7% and all of the conceptuses isolated on the 10th day of gestation from the operated sides had a triploid chromosome constitution. Analysis of the metaphase spreads from these triploid conceptuses unequivocally revealed that they all were digynic in origin. All of these metaphase spreads had a total of 59 chromosomes present, only one of which was the paternally derived large metacentric ‘marker’ chromosome (see Fig. 4).

As in the diandric triploid series described above, the developmental stages achieved by the digynic triploids varied widely, though only two empty gestational sacs were recovered. All of the other conceptuses recovered were substantially smaller in size than fertilized embryos isolated at a similar developmental stage. More importantly, however, they were all clearly morphologically abnormal. Three of the embryos recovered were at the primitive streak stage, eight were at the early headfold/early somite stage of development, while the developmentally most advanced five embryos recovered in this series were at the advanced ‘unturned’ stage, with about 10–15 pairs of somites present.

Series 4. The development of F1 × F1 normal (diploid) fertilized embryos
The results of this series of experiments are presented in Table 1 (Group 3). The implantation rate in this series was 94-4%, and 14 out of 17 pronucleate-stage embryos that were transferred to recipients were recovered on the 10th day of gestation. All were morphologically normal ‘turned’ forelimb-bud-stage embryos with more than 25 pairs of somites present. Cytogenetic analysis of their extraembryonic membranes confirmed their diploid chromosome constitution.

(B) Analysis of the extraembryonic tissues of the diandric and digynic triploids
In an additional preliminary study in which F1 × F1
Diandric and digynic triploid mouse embryos

Fig. 5. Representative histological section through the middle of an implantation site containing a presumed diandric triploid morphologically normal advanced-primitive-streak/early-headfold-stage embryo. The embryonic region (e), amnion (a) and allantois (b) are all clearly seen. Possibly due to the oblique plane of section, the ectoplacental cone region (arrow) appears to be rather smaller than expected. Bar, 250 µm.

Fig. 6. Representative sagittal histological section through an advanced 'unturned' digynic triploid embryo with about 8–10 pairs of somites. The cephalic region (c), primitive heart tube (h), somites (s) and base of the allantois (a) are clearly seen. This embryo was the most normal of the digynic triploids that were studied histologically. Bar, 200 µm.

diandric triploid pronucleate-stage embryos were transferred unilaterally to the oviducts of two pseudopregnant recipients, the latter were autopsied on the 9th day of gestation. Approximately half of the implantation sites consisting of five decidua and their contents were retained intact and analysed histologically, while the remaining conceptuses were isolated intact from within the other implantation sites and analysed cytogenetically. Since all of the latter group had a triploid chromosome constitution, it seemed reasonable to assume that the histological analysis had in fact been performed on a group of diandric triploid embryos. These embryos, which were mostly at the advanced-egg-cylinder-/early-primitive-streak stage of development, and their extraembryonic tissues, appeared in all respects to be morphologically normal. The developmentally most-advanced embryo in this series had about 8–10 pairs of somites present, and also appeared to be morphologically normal (see Fig. 5).

In all of the subsequent studies in which both diandric and digynic triploid embryos were isolated on the 10th day of gestation, the gross morphological appearance of the extraembryonic membranes present did not appear to be different in any obvious way from that expected to be seen in normal fertilized embryos isolated at similar developmental stages (see Fig. 6). However, it should be noted that, in quantitative terms, since these embryos were substantially smaller than normal fertilized embryos isolated at similar developmental stages, the relatively small volume of the extraembryonic tissues present appeared to be entirely consistent with the small size of these embryos.

(C) Implantation sites in the non-operated (i.e. contralateral) uterine horns

In approximately one third of recipients that were autopsied on the 10th day of gestation, implantation sites were present in the contralateral uterine horns. In none of the 10 implantation sites of this type analysed were either embryos or extraembryonic tissues recovered. In addition, in four instances more implants were present in the ipsilateral uterine horns (i.e. on the same side into which embryos had been transferred) than embryos were transferred. These 'additional' implants in fact result from the parthenogenetic activation of the recipients' own eggs which had probably been stimulated to develop by the general anaesthetic given at the time of the oviduct transfer. This is a well-established phenomenon (see Kaufman, 1975, 1983; Kaufman & Sachs, 1975). It is also possible that the two diploid embryos recovered from the operated side in Series 1 (see Table 1, Group 1) might have resulted
Figs 7, 8. Representative transverse histological sections through the cephalic region (Fig. 7) and cardiac region (Fig. 8) of an apparently morphologically normal diandric triploid forelimb-bud-stage mouse embryo with about 20–25 pairs of somites present. The genetic status of this embryo was confirmed following an analysis of its extraembryonic membranes.

from the parthenogenetic activation of the recipients’ own eggs. This is unlikely, however, since neither embryos nor extraembryonic membranes were recovered from the implantation sites in the non-operated uterine horns. This hypothesis is also consistent with the findings in previous studies in which parthenogenetically activated eggs were transferred to intact (i.e. non-ovariectomized) pseudopregnant recipients. In these studies, a decidual reaction is usually evoked, but no embryonic derivatives are usually recovered (see Kaufman & Gardner, 1974; Kaufman, 1983).

(D) Histological appearance of the diandric and digynic triploids

Diandric triploids
As indicated elsewhere, the developmentally most advanced diandric triploid embryos recovered from recipients on the 10th day of gestation were morphologically normal and had about 20–25 pairs of somites. A total of nine embryos in this series were examined histologically. Representative sections through the cephalic and cardiac regions of the developmentally most advanced embryo in this series which was serially sectioned are illustrated in Figs 7 and 8. Representative histological sections through approximately the same regions in a control fertilized embryo are illustrated in Figs 9 and 10. A total of six control embryos from this series were examined histologically. As the extraembryonic tissues in all of these advanced-somite-stage embryos were analysed cytogenetically to confirm their triploid status, no histological sections were taken of intact decidua.

Digynic triploids
As the digynic triploids were, as a group, developmentally less well advanced than the diandric triploids, only six embryos were examined histologically. The somite-stage embryos that were dissected from within their extraembryonic membranes were invariably found to be morphologically abnormal. The cephalic regions of all of the most advanced embryos recovered, which had about 10–15 pairs of somites present, were clearly morphologically abnormal. Most of these embryos showed evidence of peripheral oedema, possibly indicative of their impending death. A representative sagittal section through the most advanced relatively normal embryo from this series is illustrated in Fig. 6. One common feature that was observed in almost all of the primitive-streak- and headfold-stage embryos was the presence of an enlarged allantois. The significance of this has yet to be established, but a similar observation has been noted previously (see Surani & Barton, 1983).

Discussion
Recent experimental studies have indicated that the male and female genomes are not equivalent, but have complementary roles during mammalian embryogen-
Diandric and digynic triploid mouse embryos

Fig. 9, 10. Representative transverse histological sections through the cephalic region (Fig. 9) and cardiac region (Fig. 10) of a normal fertilized embryo from the control study at an approximately similar stage of development to the diandric triploid embryo illustrated in Figs 7 and 8. Figs 7–10 are all illustrated at the same magnification. Bar, 250 μm. Note that while the morphological features of these two embryos are remarkably similar, the overall dimensions of the diandric triploid embryo are substantially smaller than those of the normal fertilized embryo. Key to Figs 7–10. f, forebrain; h, hindbrain; o, region of first branchial arch; a, common atrial chamber; v, ventricle; t, transverse section through tail region.

esis. While the presence of both genomes is clearly essential for normal fetal development to term, the female genome is thought to play an essential role in controlling preimplantation embryonic development and embryogenesis, whereas the male genome is believed to play an essential role in the establishment of the extraembryonic tissues (such as the yolk sac and trophoblast) and the placenta (McGrath & Solter, 1983, 1984; Barton et al. 1984; Surani et al. 1984, 1986). These conclusions have been drawn from the interpretation of the findings from an extensive series of studies involving nuclear transplantation, and from earlier studies in which the development potential of parthenogenetic, gynogenetic and androgenetic embryos had been analysed. In almost all of these studies, diploid mouse embryos were produced where the source of the two haploid pronuclei, and consequently their genetic origin, was known. Furthermore, the eggs’ cytoplasm in these experimental studies was also from a known source, being obtained, for example, following the enucleation of normal fertilized eggs or from enucleated parthenogenetically activated eggs (Kaufman et al. 1977; Mann & Lovell-Badge, 1984; Surani & Barton, 1983; Surani et al. 1984, 1986). A similar differential effect has also recently been demonstrated genetically in mice (Cattanach & Kirk, 1985). These and other studies would appear to indicate that complex interactions probably occur between some parental alleles. However, the molecular basis for the differences in their expression is unclear.

In the present study, however, we were more interested in the influence of a diandric or digynic triploid chromosome constitution on early embryogenesis in the mouse. The limited clinical data from human spontaneous abortus studies had indicated that a clear relationship existed between the genetic constitution of a triploid conceptus and the histological/morphological features of its placenta. However, only minimal information was available on the effect of these triploid genotypes on the development of the embryo/fetus (Niebuhr, 1974). We were, however, aware that, in man, the presence of two paternally derived haploid sets of chromosomes, in the absence of a maternally derived genome (i.e. a diandric diploid), generally results in the production of gross abnormalities in placental morphology, with the formation of a ‘complete’ type of hydatidiform mole – a condition which is invariably associated with the absence of an embryo or indeed any embryonic tissues (Szulman & Surti, 1978a, b; Szulman, 1987a, b). Diandric triploid conceptuses, on the other hand, most of which probably result from dispermy, have a different embryological
origin (Kaufman, 1988) and different histopathological features (Szulman & Surti, 1978a,b). In these conceptions, the placentas show localized areas of hydropic change and often display widespread trophoblastic hyperplasia (Szulman, 1987a) giving rise to the 'partial' or 'incomplete' type of hydatidiform mole. These are almost invariably associated with the presence of either an intact embryo/fetus or recognizable embryonic derivatives. In the case of the digynic triploids, however, the placenta shows no characteristic abnormal features, though it may show evidence of localized hydropic changes, similar to those occasionally encountered in the placentas of spontaneous abortuses with a normal diploid chromosome constitution. Usually an embryo/fetus is present (Jacobs et al. 1982). These authors noted that the placentas of some digynic triploids were partially molar, and that all of these resulted from first meiotic errors in which failure of extrusion of the first polar body had occurred.

We believe that this report represents the first unequivocal demonstration that some diandric triploid mouse embryos are capable of developing to at least the 20- to 25-somite stage, and that these embryos appear, on gross inspection and on histological analysis, to be morphologically normal. In the absence of histological analyses of the developmentally less advanced embryos, however, while they appeared on gross inspection to be morphologically normal, it is clearly impossible for us to state unequivocally at this stage that they were in fact so. Further detailed analyses will have to be undertaken, to investigate this aspect in more detail. This is in marked contrast to the findings in relation to the digynic triploids which, when isolated from recipients at a comparable stage of gestation were, as a group, generally more retarded in their development than the diandric triploids. More significantly, at least in the case of the primitive streak and developmentally more advanced embryos, they were invariably morphologically abnormal. The abnormalities observed in the digynic triploid advanced-somite-stage embryos produced in the present study were remarkably similar to those observed in the LT/Sv strain digynic triploid advanced-somite-stage embryos produced by the monospermic fertilization of ovulated primary oocytes (Kaufman & Speirs, 1987; see also Kaufman, 1983; Kaufman & Howlett, 1986; O’Neill & Kaufman, 1987). In both groups of digynic triploids, the most common abnormalities encountered were confined to the neural tube, due to defects in its morphogenesis, often resulting in closure defects involving the cephalic region. In the most advanced somite-stage digynic triploid embryos, vascular stasis was often encountered in the cephalic region, sometimes associated with peripheral oedema, though in all instances spontaneous contractions of the heart were seen. It is believed that these vascular problems may have been indicative of the impending death of these embryos. These findings in relation to the digynic triploids are in general agreement with those of Surani & Barton (1983, see also Surani, 1985, 1986) who produced digynic triploid mouse embryos by suppressing the extrusion of the second polar body. These embryos were invariably retarded in their development compared to the controls. The most advanced embryos obtained had about 15-16 pairs of somites, but had ‘a variety of abnormalities’ and showed evidence of problems associated with the process of ‘turning’, and were all in poor condition ‘and deteriorating’ at the time of their isolation. In the less well developed embryos, the presence of a prominent amnion and abnormal allantois was noted.

Direct extrapolation from the human placental findings would seem to indicate that the extraembryonic derivatives in the diandric triploid mouse conceptuses might, in a proportion of cases, show evidence of widespread trophoblastic hyperplasia (Szulman, 1987a; Szulman & Surti, 1978a,b) or abnormalities of the extraembryonic tissues. However, no very obvious differences were observed in our study between the extraembryonic tissues of the diandric and the digynic triploids. Furthermore, considering that both groups of triploids were substantially smaller than normal fertilized embryos analysed at comparable stages, the extraembryonic membranes present (on a volume-for-volume basis) were not noticeably different from those expected in controls isolated at similar developmental stages.

While the extraembryonic findings in our triploid mouse embryos may not, presumably due to species differences, be comparable to the situation observed in man, the influence of a diandric or digynic triploid genome on some aspects of embryogenesis may nevertheless be similar, particularly in relation to the production of severe intrauterine growth retardation which is invariably seen in human triploid conceptuses (C. Gosden, personal communication).

The proportion of the implantation sites analysed on the 10th day of gestation that contained resorptions in the diandric and digynic triploid series was relatively high. Possibly had the autopsies been carried out at an earlier stage in the postimplantation period, the resorption rate in one or both series might have been substantially lower. Additional studies will clearly have to be undertaken in order to investigate this matter further.

In future studies, we also plan to investigate whether a relationship exists between the genotype of a diandric triploid mouse embryo (which may be 60,XXX, 60,XXY or 60,XYy) and that of digynic triploids (which may be 60,XXX or 60,XYy) and their morphological features and development potential. While we appreciate that a greater diversity of sex chromosomal constitution exists in the diandric compared to the digynic triploids (XMY1XP1YP2, XMY1YP1YP2 and XMYYPYPYP2 as compared to XM1XPXM2 and XM1XM2YP, respectively), it is difficult to see how this could modify the survival of the triploid embryos. It is possible that one explanation for the present findings might be that certain parental alleles, which are, in some way, involved in controlling the development of these embryos, are present in different ratios.

Insufficient data are available from the analysis of human triploids at the present time to indicate whether
a consistent abnormality or group of abnormalities is associated with any of the various genotypes indicated above, apart from the fact, discussed earlier, that 69,XY triploids appear to die (for reasons yet to be established) during the early postimplantation period and are consequently only very rarely encountered.

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