The genetic constitution of multinuclear blastomeres and their derivative daughter blastomeres

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The presence of multinuclear blastomeres (MBN) has been widely reported in in-vitro cultured embryos. Multinucleation at the first mitotic division and affecting both blastomeres is considered abnormal and such embryos are not transferred. The objective of this study was to use fluorescent in-situ hybridization (FISH) and probes specific for chromosomes X, Y and 18 to examine the genetic constitution of embryos developing from the 2-cell stage in which both blastomeres were bi- or multinuclear. Initially, 2-cell embryos in which both blastomeres were bi- or multinuclear were cultured further. Of 101 embryos, 89 (88.1%) cleaved further and were analysed at the 3- to 8-cell stage on day 2 or 3. Among embryos analysed, 30.4% contained only mononuclear diploid blastomeres, 35.9% had a combination of mononuclear diploid and non-diploid blastomeres, and 33.7% had non-diploid blastomeres, indicative of chaotic division. Results obtained were similar with embryos derived from in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Also, no significant differences were found between 2-cell embryos with bi- or multinuclear blastomeres or between slowly or normally cleaved embryos. Twelve (11.9%) embryos arrested at the 2-cell stage on day 3; of these, one had diploid blastomeres and the others were abnormal and highly polyploid. Subsequently, 59 embryos were analysed at the 2-cell stage. Initial observations related to the high number of nuclei in metaphase at the moment of spreading, notably when multinuclear blastomeres were observed. Genetic analysis showed 44.7% of embryos to be susceptible to analysis; the genetic constitution corresponded in both blastomeres to a diploid status. A combined diploid blastomere and abnormal blastomere was found in 4.3% of embryos; both blastomeres were abnormal in 51%. These data show that the genetic constitution of bi- or multinuclear blastomeres, and the daughter cells developing from them, are not always abnormal.

Key words: FISH/multinuclear blastomeres

Introduction

Although a normal human embryo has a single nucleus in each blastomere, it has been reported that, after in-vitro culture, multinuclear blastomeres (MBN) are observed following the first division (Tesarik et al., 1987; Winston et al., 1991; Hardy et al., 1993). The presence of MBN in human embryos is considered abnormal and is possibly related either to insufficient culture medium (Winston et al., 1991), to adverse effects on the cytoskeleton from cooling during the in-vitro fertilization (IVF) procedure (Pickering et al., 1990), or to the detrimental effect of hypoxic intrafollicular conditions of the oocyte on the chromosome and spindle organization (Van Blerkom et al., 1997). The presence of multinuclear blastomeres in embryos resulting from in-vivo conception was reported as early as 1954 by Hertig et al., indicating that the phenomenon is not related only to in-vitro culture conditions.

Different mechanisms leading to multinuclear blastomeres have been suggested: (i) karyokinesis without cytokinesis; (ii) partial fragmentation of nuclei; or (iii) defective migration of chromosomes at mitotic anaphase (Lopata et al., 1983; Tesarik et al., 1987; Winston et al., 1991; Hardy et al., 1993). Munné et al. (1993), using fluorescence in-situ hybridization (FISH) demonstrated that all these mechanisms can be involved. The same sex chromosomes were present in each of the nuclei of multinuclear blastomeres and in sibling mononuclear blastomeres, indicating that cytokinesis did not occur in some blastomeres. Partial fragmentation occurred in some multinuclear blastomeres since the number of sex chromosomes in the different nuclei was similar in sibling mononuclear blastomeres. In some multinuclear blastomeres chromosomes were distributed non-randomly, suggesting an abnormality of the mitotic spindle.

Few data exist about the further developmental potential of multinuclear blastomeres and the genetic constitution of their daughter cells. We have observed, as has been recently described by Kligman et al. (1996), that multinucleation occurring at the first mitotic division and affecting both blastomeres, is consistent with further development. As no clear information was available concerning the genetic constitution of these embryos, they were not considered for transfer.

The aim of the present study was to determine the genetic constitution of embryos developing from 2-cell embryos in which both blastomeres were bi- or multinuclear.

Materials and methods

Ovarian hyperstimulation, ovulation induction, oocyte retrieval, insemination or injection, fertilization assessment and embryo development

Embryos were obtained from patients participating in the IVF programme of the Centre for Reproductive Medicine at the Dutch-speaking Brussels Free University. Ovarian stimulation and ovulation
induction were as described previously (Smits et al., 1988, 1992). Oocyte retrieval was carried out by ultrasound-guided puncture 36 h after human chorionic gonadotrophin (HCG) administration. The retrieved oocytes were cultured and subsequently inseminated (Staessen et al., 1995) or injected (Van Steirteghem et al., 1993, 1995).

Each oocyte was evaluated for signs of fertilization 16–18 h after insemination or injection. Normal fertilization was confirmed by the presence of two distinct pronuclei. At about 40–42 h after insemination or injection, each embryo was evaluated for the number of blastomeres, the presence of multinuclear blastomeres and the extent of fragmentation. Such evaluations were performed at ×200 and ×400 magnification on a heated stage of an inverted microscope equipped with Hofmann modulation contrast optics. Embryos for transfer and cryopreservation were selected at this time. Our transfer policy regarding multinuclear embryos was to transfer those embryos in which less than one-half of the blastomeres were multinuclear only if no other embryos were available. As a general rule, if more than 50% of the blastomeres are clearly multinuclear, they are not considered for freezing or transfer.

Two-cell embryos in which both blastomeres were bi-nuclear (BNB), i.e. with two equally sized nuclei, or both blastomeres were multinuclear (MNB), i.e. with more than two nuclei usually of different sizes, were regarded as abnormal and were not considered for transfer.

In a first approach, 2-cell embryos in which both blastomeres were bi- or multinuclear were cultured further until they reached the 3- or 8-cell stage before fixation. In a second approach, these embryos were fixed on day 2, at the 2-cell stage.

The study of chromosomal constitution in abnormal human embryos, which are unsuitable for replacement, was approved by the ethics committee of the University Hospital, Brussels, Belgium.

**Fixation and FISH procedure**

The embryos were disaggregated into component blastomeres before spreading, as described previously (Staessen and Van Steirteghem, 1997). Briefly, the zona was removed by exposure for 1–2 min to acid Tyrode solution. Separate blastomeres were obtained by transferring a zona-free embryo into sodium- and calcium-free phosphate saline buffer with 0.125% of trypsin. The individual blastomeres were then transferred via a 1–2 µl droplet of 0.01 M HCl/0.1% Tween 20 solution (Coonen et al., 1994) onto a slide. The blastomere was observed constantly during spreading, using an inverted-phase-contrast microscope (Olympus CK2; with ×5, ×10 and ×20 lenses) and the number of nuclei recorded. After spreading, the slides were left to dry, washed in phosphate-buffered saline (PBS) for 5 min and dehydrated by means of an ethanol series.

Triple-target fluorescence in-situ hybridization (FISH) was performed as previously described (Coonen et al., 1994) using directly labelled DNA probes for chromosomes X (Vysis GmbH, Stuttgart-Fasanenfjöhr, Germany; Alpha Satellite DNA probe, spectrum Green), Y (Vysis, Alpha Satellite DNA probe, spectrum Orange), and 18 (Vysis, Alpha Satellite DNA probe, 1:1 mixture Green/Orange spectrum). The slides were mounted in the Vecta Shield (Vector Laboratories, Burlingame, CA, USA) antifade medium containing 1.25 ng/ml 4',6-diamidino-2-phenylindole (DAPI) to counterstain the nuclei. The nuclei were examined using a Zeiss Axioskop fluorescence microscope with the relevant filter sets. All the slides were observed and interpreted by two independent observers.

### Results

In general, of the oocytes showing two distinct pronuclei after standard IVF and intracytoplasmic sperm injection (ICSI), 25% from IVF and 12.8% from ICSI were at the 2-cell stage at the first embryo evaluation, 38 h after insemination and 40 h after injection, respectively. After IVF and ICSI, 5% and 10% respectively of the 2-cell embryos had both blastomeres which were bi- or multinuclear (unpublished results).

A total of 101 2-cell embryos (65 from conventional IVF, 36 from ICSI) in which both blastomeres were bi- or multinuclear were cultured further; 89 embryos developed further and were analysed at the 3- to 8-cell stage on day 2 or day 3; 12 embryos did not cleave further and were analysed at the arrested 2-cell stage on day 3.

Among the 89 embryos that developed further, a total of 492 blastomeres were present, 462 (94%) of which were spread. No nuclear material was observed in 15 blastomeres and these were therefore recorded as anuclear. Interpretable FISH results were obtained for 393 (87.3%) of the 447 spread blastomeres containing nuclear material.

On the basis of these FISH results, the categories presented in Table I were identified. Results are shown in relation to the nuclear status at the 2-cell stage and separately for embryos obtained after IVF and ICSI. In 27 of the 89 (30.4%) embryos that developed further, all analysed blastomeres contained a single nucleus with XX1818 or XY1818 signals and we therefore concluded that these were uniformly diploid (category A).

In 32 embryos (35.9%) a combination of mononuclear XX1818 or XY1818 blastomeres, together with multinuclear and/or non-diploid blastomeres was observed. A combination of mononuclear XX1818 or XY1818 blastomeres was observed together with: (i) binuclear blastomeres in which both nuclei were XX1818 or XY1818 (category B; n = 11); (ii) bi- or multinuclear blastomeres with a total number of signals equal to XX1818 or XY1818 (category C; n = 6); (iii) mono-, bi- or multinuclear blastomeres with a total number of signals differing from XX1818 or XY1818 and therefore defined as non-diploid and mosaic (category D; n = 13); or (iv) mononuclear triploid blastomeres (category E; n = 2).

Finally, in 30 embryos (33.7%) each blastomere was non-diploid (category F); a complex mosaicism indicative of a chaotic division was observed in 24 of these. Results from the separate fixation of blastomeres indicated that bi- and multinuclear blastomeres were present and contributed to the mosaicism observed. In addition, two completely haploid embryos and two completely triploid embryos were observed after conventional IVF. One embryo after IVF and one after ICSI were composed of mononuclear triploid blastomeres and abnormal mosaic blastomeres.

The results obtained did not differ between IVF and ICSI or between the 2-cell embryos with bi- or multinuclear blastomeres (χ²-test: not significant). Furthermore, the data were grouped according to developmental speed (Table II). No statistically significant difference (χ²-test: not significant) in the distribution among the different categories was observed for either 3- to 4-cell embryos on day 2, for 3- to 4-cell embryos on day 3, or for 5- to 8-cell embryos on day 3.

Moreover, of the 101 embryos 12 did not cleave further and were analysed at the arrested 2-cell stage. Only one embryo
Multinuclear blastomeres analysed by FISH

Table I. FISH analysis of IVF and ICSI 3- to 8-cell embryos derived from two-cell embryos with binuclear (BNB) or multinuclear (MNB) blastomeres

<table>
<thead>
<tr>
<th>Nuclear status and ploidy of blastomeres in 3- to 8-cell embryos</th>
<th>IVF (n = 57)</th>
<th>ICSI (n = 32)</th>
<th>Total (n = 89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNB (n = 29) MNB (n = 28) (%)</td>
<td>BNB (n = 8) MNB (n = 24) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All blastomeres, mononuclear and diploid (A)</td>
<td>9 (31.0)</td>
<td>9 (32.1)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Mixture of mononuclear, diploid blastomeres and:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- binuclear (both diploid)</td>
<td>4 (13.8)</td>
<td>3 (10.7)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>- binuclear and multinuclear</td>
<td>3 (10.3)</td>
<td>1 (3.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>- binuclear and multinuclear diploid blastomeres (C)</td>
<td>4 (13.8)</td>
<td>5 (17.8)</td>
<td>3 (10.8)</td>
</tr>
<tr>
<td>- mono-, bi- or multinuclear non-diploid and mosaic blastomeres (D)</td>
<td>1 (3.4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>- mono- and multinuclear non-diploid blastomeres (E)</td>
<td>6 (20.7)</td>
<td>7 (25.0)</td>
<td>3 (10.8)</td>
</tr>
<tr>
<td>- mosaic 100%</td>
<td>1 (3.4)</td>
<td>1 (3.6)</td>
<td>–</td>
</tr>
<tr>
<td>- haploid 100%</td>
<td>–</td>
<td>2 (7.1)</td>
<td>2 (6.8)</td>
</tr>
<tr>
<td>- triploid 100%</td>
<td>–</td>
<td>1 (3.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>- triploid/mosaic</td>
<td></td>
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</tr>
</tbody>
</table>
| No statistical differences between IVF and ICSI: χ²-test.

was diploid in both blastomeres, the others were completely abnormal and highly polyploid.

When the data on the 101 embryos with both blastomeres bi- or multinuclear at the 2-cell stage were expressed, analysed blastomeres contained one diploid nucleus in 28 embryos (27.7%), a combination of mononuclear diploid and non-diploid blastomeres in 32 (31.7%), and all were non-diploid—indicative of a chaotic division—in 41 embryos (41.1%).

In view of results obtained from analysis of 3- to 8-cell embryos, embryos at the 2-cell stage were also analysed (Table III). In total, 59 embryos were spread at the 2-cell stage on day 2 (19 obtained after IVF and 40 after ICSI). From a total of 118 blastomeres, two were lost during spreading, while of the remaining 116, 54 were in metaphase, seven had only a single nucleus and 55 had multiple or fragmented nuclei. One clear point was the high number of nuclei in metaphase at the moment of spreading, especially when multinuclear blastomeres were present. During spreading it was impossible to control loss of chromosomal material; if chromosomes were spread too widely, they were not considered for further analysis. Finally, interpretable FISH results were obtained from 85 blastomeres. Neither blastomere provided interpretable results in 12 embryos while only one blastomere was analysable in nine embryos; these latter embryos were categorized according to results of the single blastomere.

Again, several groups were differentiated. Genetic analysis revealed that in 21 (44.7%) embryos, the total genetic constitution corresponded in both blastomeres with a diploid status (categories A, B, C, D). The diploid genetic constitution was distributed as follows: (i) in a single nucleus (category A; n = 1); (ii) in a diploid metaphase plate in both blastomeres (category B; n = 11); (iii) over several small nuclei (category C; n = 4); and (iv) over two nuclei (category D; n = 5). Furthermore, in two embryos a diploid status was found in one blastomere, the other being tetraploid or triploid (categories E and F).
In the remaining 24 embryos (51.0%), the total genetic constitution in both blastomeres was non-diploid and distributed as follows. In six embryos originating from 2-cell embryos in which both blastomeres were binuclear with equal-sized nuclei, spreading revealed two nuclei in both blastomeres, both being diploid (category G). Moreover, in one blastomere the nuclei were still attached to each other (Figure 1a). After FISH, the different nuclei appeared diploid and in the attached ones the FISH signals were distributed symmetrically (Figure 1d), which may be interpreted as a nucleus in division. In three embryos, a tetraploid status was observed in both blastomeres (category F). In one embryo a combination of a haploid set in one blastomere and a triploid set in the other (category I) was found and in three embryos—all derived from IVF—a triploid chromosomal status was observed (category J). Finally, in 11 of the 47 embryos (23.4%) both blastomeres were abnormal (category K), indicative of an abnormal first division. Most of these chaotic embryos were derived from ICSI.

**Discussion**

A human embryo normally possesses one nucleus in each blastomere, the observation of which is possible only during interphase, when the nuclear membrane is present. The presence of multinuclear blastomeres in human in-vitro-cultured embryos after the first division has been described previously (Tesarik et al., 1987; Winston et al., 1991; Hardy et al., 1993). In this study, we analysed embryos at the 2-cell level in which both blastomeres were diagnosed as being binuclear or multinuclear.

The primary aim was to document cleavage of these 2-cell embryos. An immediate observation concerned the developmental potency of such bin- or multinuclear blastomeres, since most of these embryos (88.1%) cleaved further. In general, 15% of the developing human embryos were blocked at the 2-cell stage (Bolton et al., 1989), indicating that further development of these 2-cell embryos with bi- or multinuclear blastomeres was not impaired.

Other approaches were used to investigate the developmental potency of bi- or multinuclear blastomeres. One approach was to disaggregate the embryos into their component blastomeres and to follow blastomere development separately. Blastomeres from disaggregated human embryos analysed previously by Hardy et al. (1993) showed that either binuclear or abnormal nuclei contribute to cleavage arrest; Pickering et al. (1995) subsequently demonstrated that only 30% of multinuclear blastomeres cleaved further. As such results were obtained from blastomeres under experimental conditions, the question remains as to whether cleavage potency of a blastomere is unaffected by its isolation, especially when acid Tyrode solution has been used to remove the zona before disaggregation of the embryo into its component blastomeres (Van Golde et al., 1996).

The secondary aim was to investigate the genetic status of daughter cells derived from these bi- or multinuclear blastomeres. When multinucleation occurs at the first mitotic division and affects both cells of an embryo, the embryo which develops further may be completely abnormal. This reflects the conviction that multinucleation always represents an abnormal phenomenon. For this purpose, the 3- to 8-cell stage embryos developing from these 2-cell embryos in which both blastomeres were bi- or multinuclear were analysed by FISH. Contrary to our expectations, a completely chaotic abnormal genetic constitution was observed in only 33.7% of these further-cleaving embryos, while in 30.4% we found uniformly normal diploid cells, and in 36.8% at least some diploid blastomeres, indicating that the initial cells were not both abnormal.

There were no differences in findings between IVF or ICSI, or for the type of multinucleation. When the data were grouped
Multinuclear blastomeres analysed by FISH

Figure 1. One binuclear blastomere from a 2-cell embryo with both blastomeres binuclear. (a) Isolated blastomere at moment of fixation (phase-contrast microscope: magnification ×100). Bar = 15 µm. (b) Two nuclei attached to each other become visible during spreading (phase-contrast microscope: magnification ×200). Bar = 15 µm. (c) DAPI counterstaining of the nucleus in (b) (fluorescence microscope: magnification ×1000). Bar = 15 µm. (d) Nucleus with FISH signals: two green (chromosome X) and two orange (chromosome 18) signals with symmetrical distribution (fluorescence microscope: magnification ×1000). Bar = 15 µm.

according to developmental speed, no statistically significant difference in the distribution among the different categories was observed between slowly or normally developing embryos. The embryos at 2-cell arrest were highly abnormal and polyploid, but it is not clear whether this abnormal genetic constitution was already present at the initial 2-cell stage or had developed during cytoplasmic arrest when nuclear duplication was taking place.

The results of the genetic analysis of the initial 101 embryos (89 at the 3- to 8-cell stage and 12 at the arrested 2-cell stage) with both blastomeres bi- or multinuclear at the 2-cell stage showed that 27.7% had blastomeres containing one diploid nucleus, 31.7% had a combination of mononucleated diploid and non-diploid blastomeres, and 41.1% had blastomeres that were exclusively non-diploid, indicative of a chaotic division.

To our knowledge, the only comparable study of genetic constitution is that reported by Kligman et al. (1996), who identified 22 embryos at the 2-cell stage on day 2, while binuclear or multinuclear blastomeres were analysed on day 4 after further development. One such embryo developed completely normally and two were diploid but carried an aneuploidy which probably originated from a meiotic error in the gametes. This would indicate a proportion of 3/21 (14.3%) uniformly diploid embryos. In 13 (61.9%) embryos, a proportion of mononucleated diploid cells were observed as well as abnormal nuclei, also suggesting that both initial blastomeres were not defective. Finally, five (23.8%) completely mosaic embryos were found. In our study we found a higher incidence of uniformly diploid embryos than was reported by Kligman et al., though one difference between Kligman’s report and the present study is the time of fixation, this being on day 2 or 3 in our studies, but day 4 in Kligman’s case. In addition, Kligman et al. reported 13 embryos reaching the 6- to 8-cell stage on day 3, though only five continued development and none doubled their cell number. It is possible that the further development of the embryo, which may be compromised by genetic abnormality, is manifested around the time of embryonic gene activation between the 4- and 8-cell stages.

In general, about 15% (Bolton et al., 1989; Winston et al., 1991) of fertilized human oocytes reach the blastocyst stage, indicating that the in-vitro culture conditions for human embryo development are suboptimal. The effect of suboptimal culture conditions on abnormal division (Munné et al., 1997) may therefore be more pronounced in further developmental stages.
Another difference is that we applied only three probes (X, Y, 18), while Kligman and co-workers performed their analysis with five (X, Y, 13, 18, 21), thus permitting the detection of more mosaicsisms.

Of the 47 embryos we analysed at the 2-cell stage on day 2, 44.7% were found to have both blastomeres diploid, 4.3% were composed of a diploid and a non-diploid blastomere, and 51% had two abnormal blastomeres. Such results indicate: (i) that the total genetic content of a bi- or multinuclear blastomere is not always abnormal; and (ii) that abnormal and normal daughter cells can develop from bi- and multinuclear blastomeres.

Possible explanations for the diploid blastomeres observed at the 2-cell stage and the development of normal daughter cells include misdiagnosis of the presence of bi- or multinuclear blastomeres by light microscopy. However, Pickering et al. (1995) demonstrated a >90% reliability between light microscopic observations of nuclear morphology compared with Hoechst staining; thus, misdiagnosis should not be a major problem. The multinuclear aspect of the blastomere frequently corresponds to chromosome structures at spreading, which may indicate that these multinuclear aspects are in fact observations of non-pathological processes, such as precursor steps of nuclear membrane breakdown during cell division. Another plausible explanation is the presence of pseudonuclei as described previously (Tesarik et al., 1987; Van Blerkom et al., 1987). A possible fusion of the multiple nuclei, resulting in a normal diploid nucleus or the presence of lobular nuclei giving the impression of multiple nuclei cannot be excluded. During mitotic division, we expect that telophase is followed by cytokinesis. In the BNB where two equal-sized diploid nuclei were observed, we expect that an asynchrony between karyokinesis and cytokinesis can occur. Since normal daughter cells are developing from BNB this may reflect a normal phenomenon or a temporary abnormal phenomenon which is restored. A reversible mechanism interacting at the microtubular level of organization may be involved.

Explanations for the diploid blastomeres observed at the 2-cell stage and the occurrence of abnormal daughter cells developing from them can also be found. The observation of multinuclear blastomeres may indicate the presence of non-integrated chromosomes becoming enclosed in their own membrane and being detected by the FISH analysis, resulting in a "false-diploid" status. The daughter cells developing from these blastomeres would be expected to be abnormal, with the loss of the non-integrated chromosomes or non-random segregation leading to chaotic mosaic embryos.

The non-diploid blastomeres and the binuclear blastomeres with non-diploid nuclei observed at the 2-cell stage probably lead to abnormal daughter cells.

In embryos analysed by FISH at the 2-cell stage as well as the 3- to 8-cell stage, triploid nuclei were observed. Although two pronuclei had been observed, the third pronucleus may have been missed or the nuclear material of the polar body may not have been extruded. Some of the tetraploid nuclei could be explained as the observation of a nucleus in division.

Microscopic evaluation or fixation of an embryo provides information at a given moment of the dynamic process of embryo development. Further analysis, using staining methods for the nucleus and/or nuclear membrane without interfering with the vitality of the embryo, will allow further investigation of the dynamics of nuclear formation. Further research must determine if the observations made here can be extrapolated for multinuclear blastomeres in later developmental embryos or are related only to the first division.

In a recent publication, Balakier and Cadesky (1997) reported—also in contrast to the general assumption that embryos with multinuclear blastomeres are developmentally incompetent—that in certain cases these may also retain full developmental capacity and give rise to healthy babies. This is also in agreement with our observation (Joris et al., 1997) of the birth of a healthy child after transfer of an embryo that had been multinuclear in both blastomeres at the 2-cell stage.

On the other hand, our findings support the pathological aspect of multinucleation, since in about 30% of such embryos all the cells are genetically abnormal, implying that such embryos should be rejected for transfer. The genetically normal embryos are, however, morphologically indistinguishable from the genetically abnormal ones. For the consequences at the clinical level, in cases where no normal embryos without bi- or multinuclear blastomeres are present, the following strategy might be followed: always wait until development on day 3. If the embryos progress to the third division, two cells may be biopsied to detect the presence of a diploid chromosome status before transfer. Another approach would be to transfer without screening, but after informing the patients and obtaining their consent to prenatal screening in the case of an ensuing pregnancy.

Daughter cells dividing from MNB observed at the 2-cell stage do not always imply an abnormality. The results here indicate that the total genetic content is frequently diploid and that multinucleation, at least in some cases, may be a temporary and reversible phenomenon.

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