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## Review article

# Mosaicism in the human preimplantation embryo

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**Summary** — Recent studies have revealed widespread mosaicism in the human preimplantation embryo at the nuclear and chromosomal level arising at fertilization and preimplantation development. Molecular cytogenetic analysis by fluorescent in situ hybridization (FISH), in particular, has for the first time made it possible to analyse all or almost all nuclei in cleavage stage embryos. The genotype of an individual is therefore not strictly defined at conception by the genotype of the germ cells. Rather it seems (at least in vitro) that the processes of fertilization, syngamy and the postzygotic mitotic divisions can be irregular. In some cases, therefore, the eventual genotype only emerges by a process of natural selection.

### chromosomal mosaicism / human embryo

**Résumé** — **Mosaïcisme dans l'embryon humain préimplantatoire.** *Des études récentes ont révélé un mosaïcisme chromosomique très fréquent de l'embryon humain se produisant au cours de la fécondation et du développement préimplantatoire. La cytogénétique moléculaire par hybridation in situ en microscopie à fluorescence (FISH), en particulier, a permis pour la première fois l'analyse de tous ou presque tous les noyaux d'embryons en segmentation. Le génotype d'un individu n'est pas strictement défini au moment de la conception par le génotype des gamètes. Il semble plutôt (au moins in vitro) que le processus de fécondation, de syngamie et de segmentation postzygotique puisse être irrégulier. En conséquence, dans certains cas, le génotype résultant n'est dû qu'à un processus de sélection naturelle.*

### mosaïcisme chromosomique / embryon humain

## INTRODUCTION

With the development and widespread application of in vitro fertilization (IVF) for the treatment of infertility since the late 1970s, it has been possible to study human

gametes and early preimplantation embryos in vitro. Because of the low pregnancy rates at that time, several early studies concerned attempts to karyotype oocytes and cleavage stage embryos (Angell et al, 1986; Plachot et al, 1986) to examine the possibility that ovarian stimulation for IVF caused an

increase in embryo lethal chromosomal abnormalities. These and similar subsequent studies using conventional cytogenetic techniques did indeed demonstrate chromosomal abnormalities, mainly aneuploidy (Jamieson et al, 1994). However, various problems with spreading chromosomes, arresting cells in metaphase and chromosome banding at these stages, prevented analysis of most preparations, severely limiting any conclusions. Nevertheless, they did demonstrate that many human embryos are chromosomally mosaic and that the incidence of chromosomal abnormalities is higher in morphologically abnormal or arrested embryos.

More recently, the use of IVF for preimplantation genetic diagnosis (PGD) of inherited disease has stimulated the development of methods for rapid single cell genetic analysis. For PGD, molecular cytogenetic analysis by fluorescent *in situ* hybridization (FISH) is fast and has two major advantages. First, it allows analysis of interphase nuclei. Second, multiple chromosome-specific DNA probes labelled with different fluorochromes can be used to analyse several chromosomes simultaneously in the same nuclei. For the first time, it has been possible, for example, to analyse all or almost all nuclei in cleavage stage embryos and to assess the full extent of chromosomal mosaicism. It is these studies together with basic observations of nuclear morphology in cleavage stage embryos which are reviewed briefly here.

### **CHROMOSOMAL MOSAICISM**

Chromosomal abnormalities, mainly aneuploidy, are the most significant cause of early pregnancy loss and miscarriage. Spontaneous abortion occurs in 15–20% of clinically recognized pregnancies and about half of these have abnormal karyotypes (Hassold, 1986; Burgoyne et al, 1991). Most

of these are aneuploidies arising in maternal meiosis, particularly meiosis I, during oogenesis. By extrapolation, the incidence of aneuploidy at conception was expected to be relatively high (about 25%) and this has largely been confirmed by karyotype analysis of oocytes and embryos (Angell, 1989; Pellestor, 1991). In addition to aneuploidies derived from the gametes and affecting the whole conceptus, however, recent work has demonstrated that nuclear and chromosomal abnormalities can arise at both fertilization and during the postzygotic mitotic divisions of preimplantation development, resulting in a high incidence of mosaicism of various types.

### **Fertilization**

IVF involves inseminating mature oocytes in abnormally high concentrations of washed sperm, typically selected for their mobility, to ensure that a high proportion are fertilized. As a consequence, however, some are fertilized abnormally resulting in mosaicism (fig 1). Some oocytes, for example, are fertilized by more than a single sperm. This is monitored by removing the surrounding cumulus cells the following morning and observing the number of pronuclei formed. If fertilization has occurred normally, two pronuclei (male and female) are formed. In about 5–10% of oocytes, however, the oocyte is penetrated by two (or less frequently more) sperm, resulting in tripnucleate zygotes. With some of these tripnucleate zygotes, the three sets of chromosomes assemble on a single bipolar spindle at the first mitotic division, resulting in a uniformly triploid karyotype. In most other cases, however, a tripolar spindle is formed, the three haploid sets of chromosomes assemble in a Y-shaped configuration and are segregated in quasi-diploid sets to three cells. This is because, unlike other mammalian species, in the human the pater-

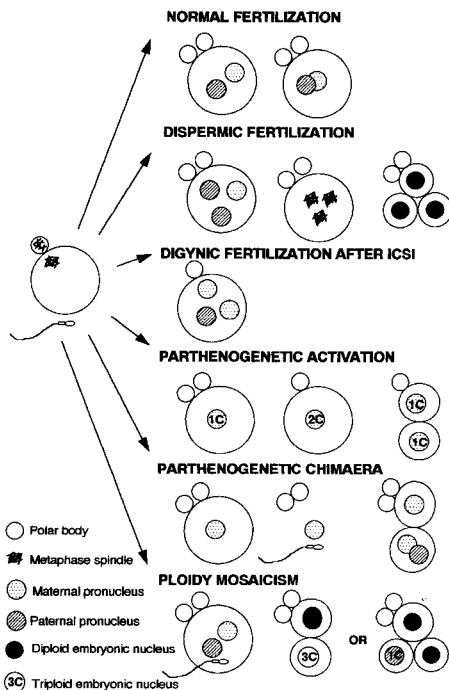
nal centrosome contributes to the first mitotic spindle (Plachot et al, 1989; Sathananthan et al, 1991). This process is highly inaccurate, however, and each of the cells inherits an aneuploid set of chromosomes and is therefore mosaic.

Alternatively, some oocytes (about 1%) become parthenogenetically activated without sperm penetration (fig 1). If the second polar body is extruded in the normal way, the oocyte forms a single haploid pronucleus. In the mouse, haploid parthenotes can develop to early postimplantation stages but there is a tendency for at least some cells to diploidize and these cells may then be at a selective advantage. In the human this would result in haploid/homozygous diploid mosaics. Following activation, however, other pathways have also been

observed. These include the formation of a diploid pronucleus as a result of failure to extrude the second polar body or alternatively 'immediate cleavage' in which the oocyte essentially divides equally instead of unequally resulting in a two-celled zygote with haploid nuclei. As a result, the latter parthenotes are mosaic because of meiotic recombination.

In the human, it is unclear how far these abnormally fertilized zygotes will develop. However, some tripronucleate embryos develop to the blastocyst stage in vitro (Hardy et al, 1989) and a triploid conceptus has been reported to survive to term. Artificially activated parthenotes only develop as far as early cleavage stages (Taylor and Braude, 1994), possibly as a result of the absence of the paternal centrosome (Palermo et al, 1994). Recently, however, a case of a possible human parthenogenetic chimaera was described (Strain et al, 1995). The boy is phenotypically male with hemifacial microsomia. His blood karyotype, however, is 46, XX and further genetic analysis revealed he is a 46, XX <-> 46, XY chimaera in which the female lineage is thought to be parthenogenetic in origin. Interestingly, the two lineages have complete maternal isodisomy for the X chromosome. One possible explanation is that the oocyte was initially parthenogenetically activated and after division was fertilized by a Y-bearing sperm (fig 1).

At a chromosomal level, multicolour FISH studies at cleavage stages confirm the expected characteristics of these abnormally fertilized zygotes but have also provided new information. First, several FISH analyses using sex chromosome and/or autosomal probes have demonstrated that most zygotes with no or only one pronucleus (unipronuclear), and therefore assumed to be parthenogenetically activated, are probably normally fertilized being diploid (or mosaic diploid) and in many cases having a Y chromosome (Coonen et al, 1994; Sultan



**Fig 1.** Abnormalities of fertilization and their genetic consequences in human preimplantation development. For detailed explanation, see text.

et al, 1995). In addition, Munné's group have also shown that although this is the case after IVF, the situation is different following intracytoplasmic sperm injection (ICSI) (Sultan et al, 1995). Following ICSI, only a minority of unipronuclear zygotes were diploid and consequently most of these are likely to have been activated. With tripronuclear zygotes, only a minority are uniformly triploid (Coonen et al, 1994; Munné et al, 1994b). Others are either uniformly diploid and may therefore have been mistakenly classified. Pseudopolypronuclear zygotes have been described resulting from the presence of one or more vesicles with the appearance of pronuclei (Van Blerkom et al, 1987). Many are diploid mosaic as would be expected following chromosome segregation on a tripolar spindle. Some tripronuclear embryos not initially dividing into three cells, however, were nevertheless mosaic (Munné et al, 1994b).

### ***Preimplantation development***

The detection of precise chromosomal anomalies in the preimplantation human embryo is extremely difficult by conventional karyotyping. The usual approach has been to incubate the whole embryo overnight with colchicine to arrest dividing blastomeres at metaphase, followed by attempts to spread the intact embryo. Not surprisingly, this produces poor quality chromosomes, either contracted and difficult to group, or more elongated but overlapping. In these circumstances, complete analysis of a single metaphase is counted as a success and there are few studies with analysis of several cells from individual embryos. Most studies are carried out on 'spare' embryos that are surplus to requirements after those suitable for transfer to the mother have been chosen. A few studies have utilized donated oocytes that are then fertilized specifically for research. In a review of four studies in which

a reasonable number of embryos were analysed (30–50) abnormality rates ranging between 23 and 40% were found (Zenzes and Casper, 1992). Where sufficient detail was given, mosaicism with normal and aneuploid or polyploid cell lines appeared to be the most common abnormality. In an interesting comparison of the chromosome status of untransferred embryos between two groups of women undergoing IVF treatment, those who became pregnant and those who did not, Zenzes and co-workers analysed one to four mitoses per embryo; overall, 13% of embryos only were normal diploid, 28% aneuploid and 36% were mosaic (Zenzes et al, 1992). They concluded that the proportion of spare embryos that are chromosomally normal is significantly greater in pregnant than in age-matched IVF patients who did not become pregnant and also that detection of chromosomally normal embryos for transfer should improve the success rate in IVF.

Munné et al (1994a,b) used multicolour FISH to analyse chromosome abnormalities in normally developing and arrested cleavage stage embryos. With probes to X, Y and chromosome 18, over half of a series of 131 embryos arrested at cleavage stages had nuclei with numerical aberrations and 6.1% were aneuploid (Munné et al, 1994a). In another study, comparing arrested embryos with those not transferred following preimplantation diagnosis, 28.8 and 17.1%, respectively, were chromosomally mosaic (Munné et al, 1994a, b). More recently, 64 normally fertilized embryos were examined with directly labelled probes to the X, Y, 18 and 16 chromosomes (Munné et al, 1995). With the four chromosomes tested, 42 and 23% of arrested and normal embryos, respectively, had numerical aberrations. In addition, there were four aneuploid embryos: two monosomies for chromosome 16, one for 18 and a trisomy for 16. We have also observed mosaicism of autosomes and sex chromosomes in morphologically normal

monospermic cleavage stage embryos. With dual analysis of chromosomes 1 and 17, 16 of 35 (46%) had abnormal nuclei (Harper et al, 1995). One embryo was triploid, one was monosomic for chromosome 1 and ten were diploid mosaics (three diploid/aneuploid and four diploid/haploid). Four other embryos had different 'chaotic' numbers of the analysed chromosomes in most of the nuclei.

More recently, these observations have been extended by analysing a large series of normal embryos which were not transferred following PGD of sex in X-linked disease (Delhanty et al, 1996). In this series triple-colour FISH with probes to X, Y and chromosome 1 were used. Again a high proportion of embryos in which most nuclei were analysed (around 30%) were diploid mosaics. These were mainly ploidy mosaics, especially diploid/tetraploid mosaics, but also included several embryos with haploid nuclei. For PGD which is dependent on analysis of single cells biopsied from cleavage stages, it will be important to determine how these haploid nuclei arise since they could cause a misdiagnosis. One possibility, for example, is that they are supernumerary sperm which did not combine at syngamy. Most strikingly, however, was the recognition that some patients have a high incidence of embryos with chaotic chromosome complements and this was a consistent feature in later cycles. Since these embryos are unlikely to develop much beyond preimplantation stages, it will be important to understand how they arise in these patients.

## NUCLEAR ABNORMALITIES

Finally, abnormal nuclei also arise during cleavage (Winston et al, 1991; Hardy et al, 1993). In particular, although most blastomeres have a single nucleus, binucleate blastomeres containing two equal-sized nuclei are common and other blastomeres

have fragmented nuclei or are anucleate (Hardy et al, 1993). Anucleate blastomeres are more frequent in embryos of poor morphology. Careful analysis of the size of binucleate and mononucleate blastomeres at different cleavage stages between days 2 and 4 suggests that they arise by failure of cytokinesis. This could be the result of cleavage arrest *in vitro*, or alternatively, may represent an intermediate stage in the formation of tetraploid and later polyploid cells in the trophoctoderm lineage.

The relationship between these nuclear abnormalities and the chromosomal abnormalities observed in FISH studies may be complex. For example, with several binucleate blastomeres analysed by FISH, each nucleus was haploid (Delhanty et al, 1996). In addition, some fragmenting nuclei may be the result of apoptosis. Indeed it has been demonstrated that some cells with clusters of micronuclei similar to those occurring in apoptotic cells label with procedures that detect double DNA strand breaks (Juriscova et al, 1996). They also propose that the cytoplasmic fragmentation frequently observed with human embryos *in vitro* could also be a consequence of apoptosis possibly to eliminate chromosomally abnormal cells.

## CONCLUSION

Recent studies have revealed widespread mosaicism in the human preimplantation embryo at the nuclear and chromosomal level. The genotype of an individual is therefore not strictly defined at conception by the genotype of the germ cells. Rather, it seems (at least *in vitro*) that the processes of fertilization, syngamy and the postzygotic mitotic divisions can be irregular. In some cases, therefore, the eventual genotype only emerges by a process of natural selection. The viability of aneuploid, haploid or polyploid cells in these mosaic embryos is uncer-

tain. A few trisomies are compatible with development to term, though most end in miscarriage. Only monosomy X, Turner's syndrome, survives to term; other monosomies are assumed to be embryo lethal at early stages as is the case in the mouse. Polyploid cells may be viable but may segregate into the trophoctoderm lineage at the blastocyst stage and contribute mainly to the placenta (James and West, 1994; James et al, 1995). The same may be true for trisomic cells and may explain the incidence of confined placental mosaicism in chorion villus samples taken early in pregnancy.

The implications of nuclear abnormalities and chromosomal mosaicism for PGD remains to be established. Careful selection of normally fertilized (two pronucleate) embryos and biopsied cells with normal interphase nuclei clearly significantly reduces the possibility of abnormalities. Nevertheless, it is possible that aneuploid, haploid, polyploid and chaotic nuclei could be selected for analysis. Even in these cases, however, a serious misdiagnosis will only rarely result with diagnosis of autosomal dominant conditions being most at risk. For this reason, two cells can be biopsied from embryos at the 8-cell stage without adversely affecting their preimplantation development (Hardy et al, 1990), significantly reducing the risk of serious errors.

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