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## Chaos in the embryo

David H Ledbetter

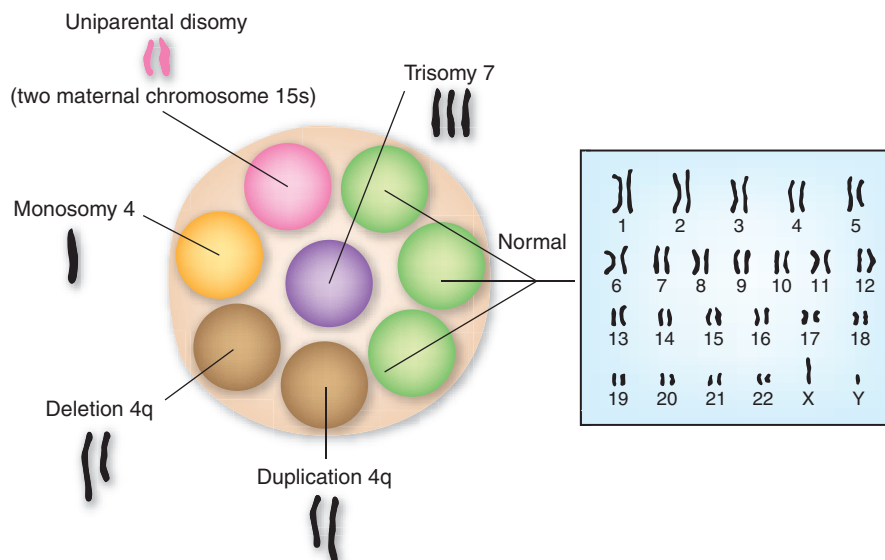
**The chromosomes of human embryos seem to be more unstable than previously thought. An analysis of embryos derived from *in vitro* fertilization reveals high rates of structural abnormalities (pages 577–583).**

It's amazing that any of us has made it this far, let alone that any of our children are healthy. It's well known that high rates of abnormal chromosome segregation (non-disjunction) occur in human female meiosis, leading to chromosomal aneuploidy and early pregnancy loss or offspring with developmental disorders<sup>1</sup>. Studies of early human embryos after *in vitro* fertilization (IVF) have shown even higher rates of aneuploidy than those found in early pregnancy or at birth, a discrepancy that may be accounted for by pregnancies lost before their detection. Such embryos also have high rates of mosaic aneuploidy, meaning that only some early blastomeres have abnormal chromosome number—indicating that mitotic nondisjunction events are also common, at least in this *in vitro* environment<sup>2–6</sup>.

Now, new microarray-based technologies reveal that structural chromosome abnormalities also occur at a shockingly high rate in early embryos. In this issue of *Nature Medicine*, Vanneste *et al.*<sup>7</sup> show that only 9% of IVF embryos have a normal karyotype in all blastomeres. The great majority show abnormalities of chromosome number or structure, such as large-scale duplications or deletions, or uniparental disomy, in which a chromosome pair is derived entirely from one parent. These abnormalities are often in mosaic form (Fig. 1). The normal diploid blastomeres, outnumbered, have to battle for survival to allow normal embryonic development and birth.

This study was made possible by advances in microarray-based technologies that allow genome-wide assessment of copy number (deletions, duplications and aneuploidy) and single nucleotide polymorphisms (SNPs).

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**Figure 1** An eight-cell embryo illustrating the types of mosaicism reported in Vanneste *et al.*<sup>7</sup>. In addition to normal cells, there are mitotically derived aneuploidies (trisomy 7, monosomy 4 and uniparental disomy 15) and mitotic structural imbalances (deletion 4q and duplication 4q, which are reciprocal products of the same structural breakage event).

The authors used whole-genome bacterial artificial chromosome (BAC) arrays as well as improved statistical methods for accurate identification of copy number changes from the DNA of single blastomeres compared to normal reference samples. This technology is changing rapidly, with a host of whole-genome oligonucleotide, SNP and bead-chip formats now available for highly accurate copy number assessment in customizable formats<sup>8–10</sup>.

A key feature of the report by Vanneste *et al.*<sup>7</sup> is that they were able to study embryos from young, healthy couples with normal fertility (the couples were at risk for inherited genetic diseases unrelated to fertility). These embryos are the best representation of normal embryogenesis available for study in humans.

To determine baseline rates of meiotic aneuploidy and structural imbalances before mitotic cell division, the authors examined fertilized oocytes, which showed a low level of aneuploidy<sup>7</sup>. The picture was markedly different for the three- and four-day-old cleavage-stage embryos, most at the eight cell stage: only 2 of 23 embryos showed completely normal results in all blastomeres tested (9%), whereas the majority showed abnormalities. Almost half of the embryos had no normal blastomeres at all!

In contrast with studies of aneuploidy in pregnancy loss or liveborns, the authors found that mitotic nondisjunction was much more common than meiotic nondisjunction, as evidenced by mosaicism for aneuploid and diploid blastomeres or mosaicism for different

Kim Caesar

aneuploidies<sup>7</sup>. Two of these mitotic events involved uniparental isodisomy (two identical copies of a whole chromosome)—raising questions about the frequency of mosaic uniparental disomy, which could lead to clinical consequences when recessive disease alleles are present or when imprinted genomic regions are involved.

The most surprising result from this study was that large-scale structural chromosomal imbalances occurred in 70% of all embryos tested. Forty percent of these involved loss or gain of a whole chromosome arm, probably due to breakage within centromere regions; most embryos with such abnormalities would be unlikely to survive to term. Fifty-five percent of all embryos showed gains or losses involving the terminal ends of chromosomes (telomeres) that were either simple deletion or duplication events or involved more complex mechanisms of rearrangements. This very high percentage of chromosomal abnormalities involving the centromeric and telomeric regions is similar to that seen in live born children with developmental disabilities, as well as in human tumors.

Only 48% of all embryos contained any normal diploid blastomeres, strongly indicating that 'survival of the fittest' at a cellular level begins very early in human embryonic development—at least in an IVF setting. In most cell culture settings, karyotypically normal cells grow and divide more successfully than abnormal cells and therefore increase their percentage over time, but much less is known about this cellular selection process *in vivo*.

Could this remarkably high rate of chromosomal instability also be the case *in vivo* after natural conception? And, if so, what are the implications for the potential clinical impact of such transient or 'cryptic' mosaicism for karyotypically abnormal cells that may be much more prevalent than imagined?

Except for the special case of tumorigenesis, there is no precedent for high rates of mitotic aneuploidy or structural chromosomal instability in normal human tissues. Hundreds of thousands of chromosome studies are performed each year on peripheral lymphocytes, skin fibroblasts, amniotic fluid cells and chorionic villus tissue, and rates of mosaicism for aneuploidy or structural imbalances are low. This baseline data would suggest either that the high rates observed in IVF embryos are an artifact of *in vitro* manipulation or that early human embryos undergo a unique, transient phase of chromosomal instability. Because it is impossible to study naturally conceived early human embryos, there is no current strategy to resolve these two possibilities.

Studies of mouse embryos, comparing rates of structural abnormality in embryos derived from naturally conceived pregnancies with those in embryos derived from IVF pregnancies, could provide some clues. But mouse embryos in general make a poor model for humans in this area, particularly given that mice have lower rates of aneuploidy compared to people.

Whether high chromosomal instability is a feature of only IVF embryos, or whether it affects all embryos, these data raise questions about the frequency of cryptic mosaicism

that may have important clinical implications. These mosaic abnormalities may survive only in the placenta, with indirect effects on embryonic growth and survival; or the abnormalities may be present in the embryonic lineage and have direct pathogenic consequences.

Rapid advances in microarray technology will allow detection of ever smaller chromosomal imbalances, which may cause neurodevelopmental disorders or predispose an individual to more common diseases. As these microarray technologies make their way into clinical practice in prenatal diagnostic settings, the data from Vanneste *et al.*<sup>7</sup> suggest that caution is warranted regarding cryptic mosaicism for aneuploidy, structural imbalances and uniparental disomy. A careful reassessment of the relative diagnostic accuracies of chorionic villus sampling (derived from extraembryonic tissues) compared to amniocentesis (derived from fetal cells) as a reflection of fetal genetic status may also be in order.

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## Angiogenesis: escape from hypoxia

Mathew L Coleman and Peter J Ratcliffe

**Current attempts to block angiogenesis during cancer and other diseases are limited partly by their effects on normal angiogenic processes. Could a more targeted approach emerge from the identification of a factor required for pathological angiogenesis under conditions of hypoxia (pages 553–558)?**

Hypoxia is a common biological stress. Accordingly, cells have evolved sophisticated signaling pathways that respond to oxygen abundance and direct responses that limit oxygen demand or improve supply. Sometimes these principles are in conflict.

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Whereas severe hypoxia results in cell cycle arrest and apoptosis in most cell types, certain cells are required to proliferate as part of an adaptive or restorative response and need to escape these cytostatic signals. For instance, to improve vascular oxygen supply to hypoxic tissues, the formation of new blood vessels by angiogenesis involves endothelial cell proliferation—a process stimulated by hypoxia-inducible growth factors, such as vascular endothelial growth factor.

How do such cells avoid the cytostatic effects of hypoxia? Findings in this issue of *Nature Medicine* shed new light on these questions<sup>1</sup>. Economopoulou *et al.*<sup>1</sup> show that a molecule involved in DNA repair responses, H2A histone family member X (H2AX, encoded by *H2AFX*), permits the proliferation and survival of endothelial cells under conditions of hypoxia. The findings have the potential to lead to new ways to specifically target pathological angiogenesis in cancer and other diseases.