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Developmental biology

A stressful start for human embryos

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Analysis of early human embryos reveals that DNA duplication after fertilization is highly inefficient. This causes DNA damage, chromosome breaks and abnormal numbers of chromosomes, impairing embryo development.

Fertilization of human eggs brings a mother's and a father's DNA together into one cell. It is crucial that this parental DNA is transferred accurately and without damage to the trillions of daughter cells that will eventually develop from the fertilized egg. Writing in *Cell*, Palmerola *et al.*[1] show that the accurate transfer of DNA often fails very early in development, directly after fertilization, owing to inefficient and incomplete copying of the parental genomes before the first division of the embryo.

The unification of the parental genomes in a fertilized egg, also called a zygote, involves several steps that are unique to this stage of life. For instance, the maternal and paternal chromosomes are initially enclosed in two separate nuclei, which must come together. After fertilization, the chromosomes need to be extensively modified through the addition and removal of molecular groups, and copied through DNA replication, to ensure that two complete genomes are available when the zygote divides into two cells. All of this happens in the absence of transcription. Whether human zygotes can replicate and transfer parental genomes efficiently and without DNA damage despite these unusual circumstances has been unclear.

Palmerola and colleagues studied parental genomes in human zygotes before and after DNA replication. They noticed that DNA damage was undetectable directly after fertilization, but present in about 50% of zygotes after DNA replication was completed. Further investigation revealed signs of DNA replication stress — a phenomenon involving the slowing or stalling of structures called replication forks, which form when the DNA duplex unwinds to enable replication, and which move along the strand as replication proceeds.

Strikingly, the authors found that replication forks moved along DNA much more slowly in zygotes than in cells at later stages of embryo development. This prompted them to investigate whether some zygotes fail to complete DNA replication before they divide. This would be dangerous, because incompletely replicated chromosomes can break, causing the loss of a piece of a chromosome[2,3] — a condition called segmental aneuploidy[4,5]. This condition affects 15–25% of human embryos, and has been suggested to cause embryonic failure[4].

When the authors blocked DNA replication shortly before zygotes divided, they observed a prominent increase in chromosome breakages, compared with cells in which replication was unperturbed. This indicated that some DNA regions are indeed replicated only shortly before a zygote divides. Might these regions sometimes fail to replicate before division? To test this hypothesis, the authors examined unperturbed embryos, and found that chromosomes often broke in gene-poor regions, which replicate late. The break sites overlapped with those induced by blocking DNA replication. These data strongly suggest that replication stress causes segmental aneuploidy in human embryos.

DNA replication stress can also cause entire chromosomes to distribute incorrectly during cell division[2]. This leads to a condition called whole-chromosome aneuploidy, in which cells have an incorrect number of chromosomes. More than 50% of human embryos contain this type of aneuploid cell, which is considered to be a leading cause of embryonic failure and miscarriage[6]. Palmerola *et al.* therefore investigated whether DNA replication stress in human zygotes contributes to whole-chromosome aneuploidy. They used a similar experimental approach to that used before, but inhibited DNA replication over a longer period. After

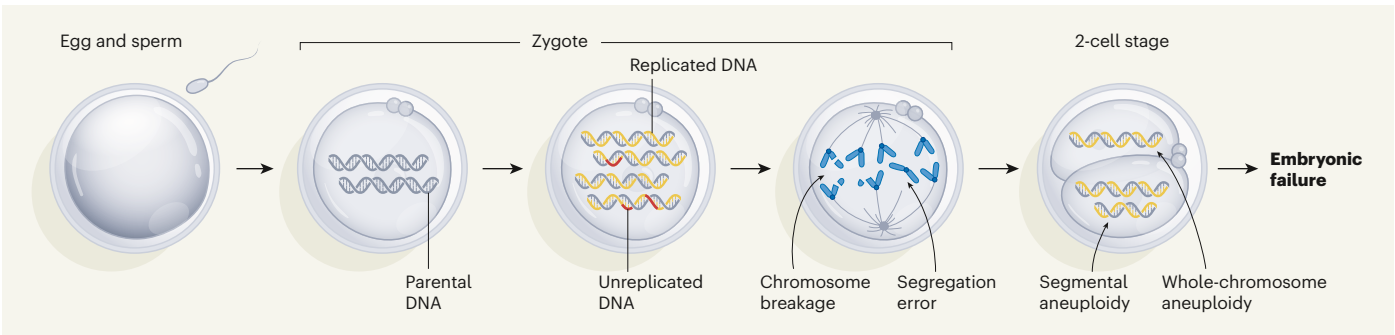

Figure 1 | DNA-replication errors at the beginning of human embryo development. Fertilization of an egg by sperm generates a zygote — the first cell of the embryo. Before the zygote divides into two cells, the parental DNA derived from the egg and sperm must be duplicated through DNA replication. Palmerola *et al.*[1] report that, in human zygotes, DNA replication is slow and erratic (a phenomenon known as replication stress) and is sometimes not completed before cell division. Incomplete DNA replication leads to chromosome breakages, and can also cause errors during segregation of the duplicated chromosomes into daughter cells. These defects respectively cause phenomena called segmental aneuploidy (in which parts of chromosomes are missing from the 2-cell embryo) and whole-chromosome aneuploidy (in which whole chromosomes are missing). The aneuploidies can lead to embryonic failure.

cell division, the treated zygotes were much more likely to exhibit whole-chromosome aneuploidy than were untreated zygotes. DNA replication stress could thus be one of the reasons that whole-chromosome aneuploidy is common in human embryos.

Interestingly, the authors found that DNA also replicated slowly in mouse zygotes. However, these were much less prone to defects than were their human counterparts. The group provide evidence that this might be explained by differences in how mouse and human zygotes process stalled replication forks: mouse zygotes efficiently generate DNA damage foci (sites at which proteins involved in DNA repair cluster around a break), whereas chromosome breaks are more likely in human zygotes. RNA that encodes proteins involved in DNA repair or chromosome breakage were present at different levels in mouse and human zygotes, which might explain this observation.

Together, Palmerola and colleagues' findings establish that DNA replication stress causes segmental aneuploidy in the early embryo. Furthermore, because they find that DNA replication stress can cause mis-segregation of entire chromosomes, the process they have uncovered is also likely to be one of the factors causing whole-chromosome aneuploidy in human embryos[6–8] (Fig. 1).

This work reveals interesting differences between mouse and human embryos, as observed in other studies[8–10]. It is becoming clear that, although mouse embryos are a useful model, studies addressing clinically relevant questions related to human embryo development benefit from experiments performed directly in human embryos or alternative model systems. Research on human embryos, although difficult and ethically controversial, is important for improving assisted-reproduction technologies such as *in vitro* fertilization. Palmerola and colleagues' work is an impressive example of how much information can be obtained using human embryos, in spite of the challenges.

The study points to further avenues for investigation. For instance, an obvious next step is to determine the causes of replication stress in human zygotes. Perhaps this stress is linked to the unique properties of the zygote and the comprehensive changes that parental genomes undergo after fertilization. It will also be interesting to investigate whether replication stress is a problem in later embryonic cell divisions. A final question is why human embryos have not evolved to replicate their DNA and segregate their chromosomes more efficiently, given the severe consequences of errors in these processes.

Palmerola and colleagues' work is also a reminder that any procedures applied to human embryos during the sensitive zygote stage — such as embryo freezing — might have unforeseen consequences. This uncertainty underscores the importance of further investigation of this crucial stage of human development.

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