Timing is everything in the human embryo

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A noninvasive imaging method for predicting how human embryos will develop may improve the success and safety of *in vitro* fertilization.

Humans may be the least fertile mammals on earth[1,2]. Although *in vitro* fertilization (IVF) technology has helped many couples—an estimated 3 million IVF babies had been born worldwide by 2002—its success rates are low (~25% per procedure), and it has led to an epidemic of high-risk multiple births[3,4]. In this issue, Wong *et al.*[5] report a noninvasive strategy for evaluating human embryos that could both improve IVF success rates and decrease the likelihood of multiple gestations. Using time-lapse videography, they discovered characteristics of initial cleavages that predict successful development to the blastocyst stage with >93% accuracy.

A new human life depends upon robust, steadily increasing signals from the fertilized egg to the mother. Failure to elaborate sufficient signal (e.g., the pregnancy hormone, human chorionic gonadotropin) results in a menstrual cycle and expulsion of the fertilized egg, thus freeing maternal resources for another attempt with a new egg. How the early human embryo sends the robust, steadily increasing signals is poorly understood, but one mechanism could be rapid duplication of embryonic DNA with a concomitant increase in signaling output to the mother. This suggests a direct correlation between the speed of chromosome duplication, cell division and successful pregnancy, an observation often reported by programs of assisted reproduction[4].

IVF involves hormone stimulation of a woman's ovaries in order to mature multiple eggs, which are removed, fertilized in the laboratory, cultured for 2 to 6 days, and transferred back to her uterus for gestation. Fertilized on day 1, an egg that has duplicated its chromosomes twice and reached the 4-cell stage by early day 2, and reached the 8-cell stage by early day 3, has a higher likelihood of giving rise to an offspring than an egg that duplicated its chromosomes only once and reached the 2-cell stage on day 2 and the 4-cell stage on day 3, but the correlation is imperfect. Faster-cleaving embryos also have a higher likelihood of developing to the blastocyst stage, which marks the first cell-commitment event in early development, but that correlation, too, is imperfect.

Many IVF programs extend embryo culture to day 5 or 6 to transfer a single blastocyst. This practice successfully decreases the risk of multiple gestations while yielding a higher pregnancy rate for women under the age of 36 (ref. 6). But fertilized eggs from many patients do not form blastocysts in culture. Moreover, the well-studied mouse embryo model has taught us that the rapid cleavage rate that occurs *in vivo* between the 4-cell and 16-cell stage is not reproduced *in vitro* under existing culture conditions. Because blastocyst formation begins at a defined interval after fertilization, independent of the number of cell divisions, mouse embryos developed *in vivo* have more than twice as many cells at

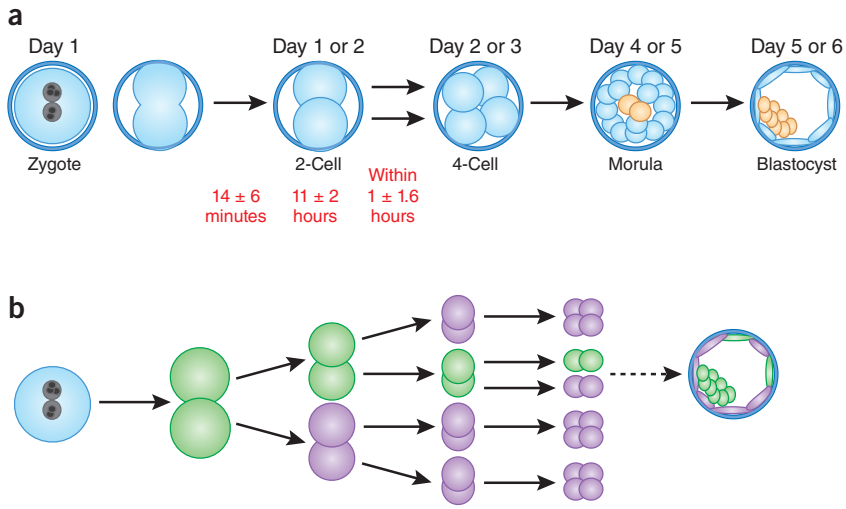

Figure 1 Early human development. (**a**) The zygote possesses one pronucleus containing egg chromosomes and another pronucleus containing sperm chromosomes. Both sets of chromosomes are duplicated before the first cleavage to 2 cells. Wong *et al.*[5] discovered that, for successful development to the blastocyst stage, the first cleavage furrow should be only 14 ± 6 min from the beginning to the appearance of 2 cells; the 2-cell stage should last only 11 ± 2 h; and the cleavage of each of the 2 cells to its daughter cells in the 4-cell stage should occur within 1 ± 1.6 h of each other. The morula forms at the 8- to 16-cell stage, trapping 1 or 2 cells inside that undergo commitment to become the inner cell mass (ICM) within the blastocyst. The ICM gives rise to the fetus. The outer cells of the blastocyst become committed to trophoblast, precursor to the placenta. (**b**) Theoretical aneuploidy in early development. The schematic depicts the highest rate of aneuploidy (purple cells) that could form the ICM from a euploid cell (green) and produce a normal fetus. This theory is supported by several lines of evidence, including chromosomal analyses that reveal both aneuploid and euploid cells in human blastocysts[8].

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the blastocyst stage than embryos developed in culture[7]. Should the situation be the same for human embryos, extended culture would lead to blastocysts with fewer cells available to form the fetus—a possible explanation for the low birth weight reported for some IVF babies[3,4].

The obvious way to improve IVF rates of pregnancy and of singleton births is to choose one healthy embryo, as soon after fertilization as possible, for transfer at the ideal time into the uterus of the prospective mother. Herein lies the value of the new work by Wong *et al.*[5]. By stunning time-lapse photography of 100 fertilized human eggs cultured to day 5 or 6, they discovered three characteristics that could predict progression to the blastocyst stage with a sensitivity and a specificity of 93% and 94%, respectively: (i) duration of the first cleavage furrow leading to 2 cells of 14 ± 6 min, (ii) a 2-cell stage lasting only 11 ± 2 h and (iii) the 2-cell blastomeres cleaving to 4 cells within 1.0 ± 1.6 h of each other (**Fig. 1a**). These non-invasive, early-cleavage parameters should be easily adaptable by IVF programs to help select the best embryo for transfer.

In addition to establishing cell-division guidelines that predict blastocyst development, Wong *et al.*[5] correlated patterns of cleavage with gene expression in an additional 142 embryos. Errors in early cleavages that give rise to chromosomal aneuploidy have been cited as leading to embryonic failure[4,8]. But genome-wide analyses of gene expression in normal-appearing, 8-cell human embryos[9] suggested that aneuploidy may be common in the early cleavage stages of apparently normal embryos. These studies revealed a lack of cell cycle checkpoints, such as Rb and Wee1, and overexpression of cell cycle drivers, such as Cyclins A, B and E, and Myc, which allows for the rapid rates of gene amplification needed for maternal signaling.

In fact, aneuploidy in early-cleaving embryos may not be lethal to fetal development because most of the early cells will form trophoblast (**Fig. 1b**). An important feature of early mammalian development is the enormous size of the egg; thus, DNA duplication and cell division to approximately the 64-cell stage occurs without the need for cell growth or, perhaps, for growth factor stimulation. Geometrically, at the 16-cell stage, 1 or 2 cells trapped in the middle of the cell mass initiate commitment to inner cell mass (ICM) cells (precursor fetal cells), while the outer cells form the trophoblast lineage (precursor placental cells). Cultured ICM cells can also give rise to embryonic stem cells, which do express Rb and Wee1 (ref. 9), suggesting that the ICM has active cell cycle checkpoints to help maintain the chromosome integrity of the developing embryo.

Wong *et al.*[5] describe several aberrant embryo phenotypes, as well as gene expression analyses of individual blastomeres, that reveal asynchrony in cell division, in gene expression and in the degradation of maternal messages. These results suggest that the current view of the early-cleaving embryo—that all cells are equivalent and chromosomally balanced—should be revised to recognize that aneuploidy after the 2-cell stage may be common[8], that aneuploid trophoblast cells may be able to give rise to a fully functioning placenta and that only ICM cells (as few as 12% at the 16-cell stage) must be chromosomally balanced to give rise to a normal fetus.

Balancing rapid cell divisions with accurate chromosome allocation is the yin and yang of early human development. A new human life arises from fewer than 30% of fertilized human eggs[1–4]. By careful comparison of cell division characteristics with the emerging lists of cell cycle gene elements[10], we may be able not only to predict which embryos have the greatest potential for life, but also to develop therapies for early embryos that will balance their yin and yang in favor of development to offspring.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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Taking the measure of the methylome

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Two comparative studies from the International Human Epigenome Project find high concordance between different methods for measuring genomic methylation.

With the rapid development of new methods for epigenomic analysis, the need for a systematic assessment of available technologies has become acute. In this issue, Harris *et al.*[1] and Bock *et al.*[2] compare the performance of commonly used techniques for DNA methylation analysis in terms of cost, resolution, genome coverage and accuracy. The findings provide a first benchmark of which method works best for which part of the methylome.

In humans, DNA methylation occurs predominantly at cytosine bases in the form of methyl cytosines (mCs), methyl cytosine guanine dinucleotides (mCGs), hydroxymethyl cytosines (hmCs) and, possibly, in other, yet unknown forms. Collectively, these modifications define the DNA methylome of a cell. Together with the study of other epigenetic marks, methylome analysis forms an integral part of ongoing efforts to elucidate the epigenomes of healthy and diseased cell types.

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Such methylome maps will allow the identification of genomic regions involved in cell differentiation and disease.

Following years of planning by the Epigenome Taskforce[3] and other initiatives, the studies of Harris *et al.*[1] and Bock *et al.*[2] mark another milestone for the International Human Epigenome Project[4]. Together with recent papers by Li *et al.*[5] and Robinson *et al.*[6] (not discussed here), they compare the performance of the main technologies for mCG methylome analysis. Until now, we did not know how well these methods work, what their particular strengths and weaknesses are, or the extent to which the resulting methylation maps overlap. Understanding these issues is especially important when choosing a method for generating so-called reference methylomes, which will be used as definitive resources in future research and must therefore be as accurate and comprehensive as possible.

In all, Harris *et al.*[1] and Bock *et al.*[2] tested six methods, of which five are sequencing-based and one is array-based. Three of the methods—MethylC-seq (data from ref. 7),