Supplementary Information

Correlated time-lapse imaging and gene expression profiling reveals human embryo fate is established before genome activation

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Supplementary Figure 1 Illustration of cleavage and mitosis.

Out of the parameters we measured, we found three that collectively predicted blastocyst formation: (1) duration of the first cytokinesis, (2) time interval between the end of the first mitosis and the initiation of the second, and (3) synchronicity of the blastomeres in the second and third mitosis. The first 3 mitosis events yield a 4-cell embryo from the 1-cell embryo, as opposed to the first 3 cleavage divisions, which yield an 8-cell embryo. Previous studies in the mouse have suggested that the first cleavage division itself sets the stage for predicting the blastocyst axis and that subsequently all blastomeres are not equivalent.\textsuperscript{1-2} Although these studies have been considered controversial in that they may imply that fate is determined in the first division,\textsuperscript{3} it is more likely that the properties of the first cleavage division alter the probabilities of fates in subsequent divisions.\textsuperscript{4}
Supplementary Figure 2 2D views from Figure 2(d) indicating prediction of embryo viability to blastocyst stage.

The 3D plot in the upper left is taken from Figure 2(d). The other plots are separate 2D views that help show the relationship between the 3 parameters: (1) duration of the first cytokinesis, (2) time interval between the end of the first mitosis and the initiation of the second, and (3) synchronicity of the blastomeres in the second and third mitosis.

Typically in an IVF clinic, embryos deemed to be viable for transfer for reproductive purposes are selected by static morphological and growth criteria that are assessed on Day 3. However, these criteria suffer from both underestimating and overestimating
embryo viability. For example, one report indicated that morphological and growth criteria assessed on Day 3 of development do not accurately predict blastocyst formation.\textsuperscript{5} When embryologists were asked to choose two embryos with optimal potential using traditional criteria in a controlled study, results indicated that in 39\% of cycles neither choice resulted in blastocyst growth, in 38\% of cycles one choice resulted in growth and subsequent transfer, and in just 23\% of cycles both choices were transferred.\textsuperscript{5} These results mirrored those of another study where an equal uncertainty in predicting embryo outcome on Day 3 was observed at Day 5.\textsuperscript{6} Conversely, it is well recognized that even embryos judged to be of poor quality can result in viable pregnancies and furthermore, that more than 20\% of discarded, poor-quality embryos can give rise to human embryonic stem cell lines that can be cultured extensively and form diverse somatic tissues.\textsuperscript{7-9}
Most commonly observed abnormal cytokinesis phenotypes. Abnormal embryos (embryos that arrested prior to blastocyst formation) showed a diverse range of behavior that can be categorized into three aberrant cytokinesis phenotypes. In the less common but milder phenotype (top panel), the morphology and mechanism of cytokinesis appeared normal, but the time required to complete the process was extended from a few additional minutes to an hour. A small fraction of the embryos that underwent a slightly prolonged cytokinesis still developed into a...
blastocyst. In the second phenotype (middle panel), embryos formed a unipolar cleavage furrow and engaged in unusual morphological behavior for several hours before finally cleaving and fragmenting into smaller components. In the third phenotype (lower panel), embryos displayed membrane ruffling and/or multiple cleavage furrows before cleaving and fragmenting into smaller components.
Supplementary Figure 4  Fragmentation reversal for developmentally-competent embryos.
While fragmentation in abnormal embryos was rarely reversed, small amounts of fragmentation in developmentally competent embryos were sometimes reversed at the 2-cell stage and prior to the second mitosis.
**Supplementary Figure 5** Relative gene expression value for individual samples.

The average gene expression values of different ESSPs described in Figure 5a were calculated by averaging the relative gene expression values of individual samples at the same developmental stage shown in this figure.
Supplementary Figure 6 Half-lives of ESSP1 and ESSP4.
The half-lives of ESSP1 and ESSP4 were calculated by fitting the logarithm of average gene expression levels against time of harvest with a linear fit, followed by determining the slopes of the fitted lines that represent their exponential decay rates.
Supplementary Figure 7 Verification of the four ESSPs.

The experiment described in Figure 5a was repeated using 61 samples of single intact embryos and single dissociated blastomeres. Similar results were obtained. (A) Relative gene expression values of individual samples. (B) Average gene expression values of samples at the same developmental stage. In previous studies, we and our collaborators sought to address whether embryo loss is caused by EGA failure (lack of ESSP2 expression). In those studies, we were not able to analyze single cells at that...
time and we never observed failure to execute EGA as long as the first cell division had been completed. In contrast, in this study, at the single cell level we have observed that individual blastomeres within an embryo frequently fail to execute EGA. This indicates that qualitative properties of embryo development may be linked to arrest of individual blastomeres; an embryo may contain fewer cells at blastocyst stage (and have a reduced inner cell mass) if a portion of blastomeres arrest prior to EGA. Moreover, our findings indicate that measurement of gene expression in the whole embryo (a small population of blastomeres) yields an average gene expression that may not be diagnostic of any single cells.
Supplementary Figure 8 Stability of reference genes.

In single blastomeres, both *RPLP0* and *GAPDH*mRNAs decreased by approximately 1 Ct value per division between 1-cell and 8-cell stage, due to the expectation that each cell inherits approximately half of the mRNA with each cleavage division, in the absence of new transcripts prior to EGA during the first 3 days of human development. The expression level of these reference genes in single blastomeres remained stable between 8-cell to morula stage, after EGA began. At the intact embryo level, the Ct values for both *RPLP0* and *GAPDH* remained largely constant throughout development until the morula stage with a slight increase following in the blastocyst stage perhaps due to increased transcript levels in the greater numbers of blastomeres present.
**Supplementary Figure 9** Simulated images of cell membranes.
Left: single cell, middle: symmetric division, right: symmetric division with added perturbation. Occluded cell membranes are added with low probability to generate the dotted lines.
**Supplementary Figure 10** Processing of microscope images.

Left: original grayscale image, middle: principle curvature image, right: thresholded middle image. The image on the right would be used for comparison to the simulated images.
<table>
<thead>
<tr>
<th>Category</th>
<th># of genes</th>
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<tr>
<td>Apoptosis</td>
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<td>Cytokinesis</td>
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<td>Differentiation</td>
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<td>Embryonic gene activation</td>
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<td>Epigenetics</td>
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<td>Germ cell</td>
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<tr>
<td>Housekeeping</td>
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<td>Ligand / receptor</td>
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<td>Maternal effect</td>
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<td>Transcription factor</td>
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**Supplementary Table 1** Genes from different categories used in this study.
Supplementary Table 2 List of genes that followed the 4 different expression patterns in early human embryo development. The “undefined” category includes genes with expression patterns that do not fit into any of the 4 ESSP patterns (i.e., are not defined by any of the 4 ESSP patterns).
Legends for Videos and Datasets

**Supplementary Video 1** Video accompaniment to Figure 2a.
The development of 15 human zygotes was documented with darkfield time-lapse microscopy. Images were taken at 1 second exposure time every 5 minutes for 6 days. Media was changed on Day 3, resulting in the rearrangement of individual embryo’s location. The identity of each embryo was tracked by videotaping the process of sample transfer during media change and sample collection. Among the 15 embryos, 10 developed into a blastocyst and 5 became arrested at different stages of development. Embryo H in this video corresponds to the embryo depicted in Figure 2a.

**Supplementary Video 2** Video accompaniment to Figure 2e (first panel).
A normal embryo typically completed cytokinesis in 13.0 +/- 4.2 min in a smooth and controlled manner.

**Supplementary Video 3** Video accompaniment to Figure 2e (second panel).
Some embryos underwent a slightly delayed but otherwise morphologically normal cytokinesis.

**Supplementary Video 4** Video accompaniment to Figure 2e (third panel).
In the more severe phenotype, the abnormal embryos often formed a one-sided cytokinesis furrow accompanied by extensive membrane ruffling before finally completing the division, possibly resulting in embryo fragmentation.

**Supplementary Video 5** Video accompaniment to Figure 2e (fourth panel).
Imaging was also performed on a subset of triploid embryos which exhibited a distinct phenotype of dividing into 3-cells in a single event.
Supplementary Video 6 Video accompaniment to Figure 3a. Results of 2D tracking algorithm for a single embryo. Images are acquired every 5 minutes. The movie shows the most probable model, the original image, the Hessian (principle curvature image), the thresholded Hessian, and the simulated image (which corresponds to the most probable model). The plots on the bottom show the particles, with dots placed at the centers of the cells, before and after re-sampling.

Supplementary Video 7 Video accompaniment to Figure 3b. 2D tracking for a set of 14 embryos. One embryo was excluded from image analysis since it was floating and out of focus. Once the algorithm is capable of making a prediction of blastocyst, the embryo is labeled with “viable” for blastocyst or “non-viable” for non-blastocyst. On day-3 there is a media change that allows the embryos to be cultured to the blastocyst stage. This process was videotaped to assist in maintaining embryo identity.

Supplementary Video 8 Video accompaniment to Figure 4a. Abnormal membrane ruffling was observed during the first cytokinesis of this arrested 2-cell embryo.

Supplementary Video 9 Video accompaniment to Figure 4b. This arrested 4-cell embryo underwent a severely abnormal cytokinesis during its first division.

Supplementary Video 10 Video accompaniment to Supplementary Figure 2f. Video microscopy data aided in the identification of abnormal embryos (bottom) from normal embryos (top).

Supplementary Dataset 1 Raw data used to generate Figure 2d.
Supplementary Dataset 2 Complete probe list used for each experiment, as well as the corresponding Unigene ID and RefSeq Accession ID of each ABI assay-on-demand probe, as provided on Applied Biosystems’ website.

Supplementary Dataset 3 Comparison of our qRT-PCR gene expression data in 1-cell and 2-cell embryos to the microarray data in human oocytes as described in Kocabas et al. We note that due to the differences in experimental design and data handling, we would only expect qualitative agreement between these 2 data sets. Expression of two genes, AURKA and CCNA1, was also analyzed in a separate report by Keissling et al (J Assist Reprod Genet (2009) 26:187–195)\textsuperscript{11}; expression of these genes was consistent with our data and that of Kocabas et al. These genes are indicated by an asterisk; overlap between gene sets was minimal due to differences in experimental design.

Supplementary Dataset 4 Taqman probes used for qRT-PCR analysis.

Supplementary Dataset 5 High throughput qRT-PCR data set 1.
This excel file contains the relative expression values of all samples and genes assayed in the first high throughput qRT-PCR experiment. Samples were named using a 3-part nomenclature: part 1 depicted the developmental stage of the embryo, part 2 indicated the order of the sample collected within its category, and part 3 reflected whether the embryo was collected as a whole embryo or single blastomere. For example, the name “2c-7-1” referred to the 1\textsuperscript{st} blastomere of the 7\textsuperscript{th} 2-cell embryo collected, whereas “B-10-W” was the 10\textsuperscript{th} blastocyst collected as a whole embryo.

Supplementary Dataset 6 High throughput qRT-PCR data set 2.
This excel file contains the relative expression values of all samples and genes assayed in the second high throughput qRT-PCR experiment. The sample nomenclature scheme was the same as Supplementary Dataset 5.

Supplementary Dataset 7 High throughput qRT-PCR data set 3.
This excel file contains the relative expression values of all samples and genes assayed in the second high throughput qRT-PCR experiment. The sample nomenclature scheme was the same as Supplementary Dataset 5.

REFERENCES


