Published in final edited form as:

Placenta. 2011 September ; 32(0 3): S264–S267. doi:10.1016/j.placenta.2011.07.005.

Non-invasive imaging of human embryos to predict developmental competence

Renee A. Reijo Pera

Institute for Stem Cell Biology and Regenerative Medicine, Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, CA 94305-5463

Abstract

Although some aspects of human embryo development are conserved with those of other species, including the mouse, many aspects such as the timing of reprogramming and occurrence in the absence of transcription, duration of transcriptional silence and identity of genes with modulated expression in the oocyte to embryo transition, appear to be unique. Yet, frequently, the only data available for understanding the programs of early embryo development is that derived from model or agricultural species. We suggest that a specific understanding of basic aspects of human embryo development can affect a two-fold positive impact: 1) We can improve the health of a substantial subset of patients who seek assisted reproduction by improving diagnostics of viable embryo development in the clinic and, 2) we can use the information we gather to improve derivation and diagnosis of pluripotent stem cell lines (including reference or gold-standard human embryonic stem cell (hESC) lines and closely-related induced pluripotent stem cell (iPSC) lines) and their fates in novel basic and clinical applications.

Keywords

human embryo; gene expression; early transfer; non-invasive diagnosis; time-lapse imaging; maternal inheritance

1. Significance of understanding human embryo development to reproductive health

Our lack of knowledge of human embryo development carries a heavy burden in terms of health and finance [1]. Consider that the success rate of assisted reproductive techniques such as IVF is just 25% and that ca. 30 – 40% of successful cases result in multiple births (cdc.gov/art). Multiples often result in premature birth, low birth weight, lung and heart problems, increased risk of cerebral palsy and chromosomal abnormalities and developmental delay. Why the increase in adverse outcomes? Simply, in the absence of knowledge of human embryo development, and to improve pregnancy rates in clinics, multiple embryos (2–5 frequently) are transferred. The cost of multiples is great in terms of finances, as well, with estimates of 5–50 fold increased costs for twins and triplets, respectively, compared to singleton births.

2. Significance to pluripotent stem cell biology

Our lack of knowledge of human embryo development also has a negative impact on pluripotent stem cell biology. Indeed, we lack certainty. We do not know whether current hESC lines were derived from properly reprogrammed embryos or the extent to which they carry significant developmental errors. We lack data regarding key aspects of the cell cycle and chromosome segregation. Consequently, we also lack a comparator for iPSCs and have little evidence that induced pluripotency results in lines that are stable in some aspects and capable of executing native pathways of human development. Thus, it is beneficial to improve our understanding, diagnostics and predictive capabilities regarding outcomes of pluripotent stem cell biology and regenerative medicine.

3. Human embryo development and gene expression

Human development shares many aspects in common with other species and also is characterized by uniquely-human aspects [2–11]. It begins with fusion of the egg and sperm, migration and fusion of the gametic pronuclei and a rapid genome-wide demethylation that passes over the marks of imprinted loci, and reprograms development from that of the gametes to that of an embryo. By Day 3 of development, eight embryonic cells have formed through a series of cleavage divisions. At this time, the embryonic genome is activated and transcription begins for the first time[2, 5]. The purpose of this period of preimplantation development is to transition from oocyte/sperm to embryo programs. Thus during this transition, messages that function in differentiation and maintenance of the germ cells must be degraded while those required for growth and differentiation of embryonic cells must be expressed for the first time[11, 12]. During this time, unique patterns of human genome methylation and gene expression become established and provide the foundation for developmental regulation that is perpetuated throughout the differentiation of the human cell lineages; indeed, errors in this early development may have long-lasting consequences to the embryo, fetus and adult. In 2004, we described gene expression in single oocytes and embryos (not single cells) through the first 3 days of development[5]. This work established that more than 1800 genes were modulated, up- or down-regulated, during the oocyte to embryo transition. Most were of unknown function.

4. Reprogramming in early human embryo development

Reprogramming of the maternal and paternal pronuclei is an essential part of early human embryo development and begins in transcriptional silence[13]. Clearly human embryo development is remarkable in many ways, including the observation that the oocyte provides all of the required resources to carry out the complex developmental pathways of the first 3 days of development, in the absence of transcription[2]. Consider further that native human reprogramming, which occurs in transcriptional silence, involves one of the most difficult sets of chromosomes to reprogram – those inherited from the sperm. These chromosomes are highly condensed, highly methylated and organized around a protamine rather than histone scaffold. Yet, our studies show that reprogramming in the human oocyte to embryo transition that culminates with embryonic genome activation on Day 3 succeeds in >75% of embryonic blastomeres[11].

5. Subsequent studies of human embryo development distinguish unique developmental programs and prospectively diagnose embryo fate

Recently, we performed experiments in which we tracked the development of 242 embryos from zygote (Day 1, one cell) to blastocyst stage and correlated imaging and molecular characteristics[11]. These studies allowed us to build a model of human embryo development (Fig. 1).

In order to build our model of human embryo development with applications to reproductive stem cell biology and regenerative medicine, our major goal was to identify quantitative morphological markers that predict success in embryo development. We extracted and analyzed several parameters from time-lapse imaging, including blastomere size, thickness of the zona pellucida, degree of fragmentation, length of the first cell cycles, time intervals between the first few mitoses, and duration of the first cytokinesis[11]. We found a set of three parameters that collectively predicted blastocyst formation with 94.1% and 93.1% sensitivity and specificity, respectively: (1) duration of the first cytokinesis, (2) time interval between the end of the first cleavage and the initiation of the second, and (3) synchronicity of the blastomeres in the second cleavage division to yield a 4-cell embryo from a 2-cell embryo. All normal embryos that successfully developed into a blastocyst exhibited similar values in all three parameters, whereas arrested embryos were highly variable. This indicates that embryos that follow strict timing in cytokinesis and mitosis during the first two cleavage divisions are much more likely to successfully develop to blastocyst stage. Considering that EGA does not occur until 1 – 2 days later[2], this data indicated that the fate of an embryo must be predetermined by inherited factors rather than by genes activated in the nascent embryo.

6. Embryo stage-specific gene expression

In order to elucidate molecular mechanisms that may underlie the morphological events that we observed, we performed correlated gene expression profiling for each imaged embryo. Embryos were analyzed as either single intact embryos or were disassociated into single blastomeres followed by gene-specific RNA amplification. Unique to our study, time-lapse microscopy data aided the identification and classification of normal and abnormal embryos as often embryos that developed and behaved abnormally would appear morphologically normal at the time of sample collection. Gene expression analysis revealed several key findings as described[11]. When we analyzed gene expression patterns of 96 genes (chosen from unpublished arrays and mouse data) in 141 embryos with apparently normal development as assessed by imaging, we found that without prior assumption of which genes might fall into similar shared expression patterns, we derived 4 unique embryonic stage specific patterns (ESSPs) of expression (Fig. 2A, Fig. 3). ESSP1 exemplified maternally-inherited mRNAs destined for degradation with a high expression level at the zygote stage and subsequent declined with a half-life of just 21 hours. ESSP2 included EGA genes, first transcribed at ca. the 8-cell stage. ESSP3 was comprised of later-activated genes expressed at the morula-blastocyst stage and ESSP4 contained stable transcripts. In contrast to ESSP1 (with a half-line of 21 hours), the half-life of ESSP4 transcripts was 193 hours, ca.

9 times longer (Fig. 2B). For many genes, embryo expression patterns were first revealed in this study.

Validity of the data set was confirmed via several observations. The gene stability value and coefficient of variation was well within range of a typical heterogeneous sample set [14]. Also, we observed that in single blastomeres, amounts of stable transcripts (RPLPO and GAPDH transcripts) decreased by ca. 1 Ct value per division between 1- and 8-cell stages, congruent with expectations that each cell inherits one half of the pool of mRNA with each cleavage division, during transcriptional silence. Also expression levels of reference genes in single blastomeres and embryos were relatively stable through development.

Individual blastomeres of an early human embryo are commonly assumed to be at the same developmental stage with the view that the embryo *per se* will succeed or die. However, we observed that approximately 25% of embryos contained blastomeres of different stages (Fig. 2C). Further, we observed that maternal transcripts were not degraded in some blastomeres suggesting two properties of human embryo development: First, degradation of maternal transcripts is not a spontaneous process that simply occurs through time. Instead, maternal degradation of RNA in human embryonic blastomeres must be an active process (that likely requires specific RNA degradation mechanisms) to target a specific subset of RNAs with a half-life of ca. 21 hours. Second, since we did not find any embryos or blastomeres that concurrently expressed high levels of maternal transcripts and embryonic transcripts, proper degradation of maternal transcripts may be a prerequisite for EGA. We also observed that gene expression profiles of embryos that arrested in development were as diverse and variable as their aberrant morphological phenotypes. Genes that were expressed at significantly-different levels in normal vs abnormal embryos included cytokinesis components, genes involved in miRNA biogenesis and mRNA storage and processing. Specific genes that showed significantly-reduced expression in abnormal embryos relative to normal counterparts included DGCR8, Dicer, TARBP2, CPEB1 and Symplekin. These data indicate that the defects that we observed in the dynamic morphology of normal embryonic development reflect the intrinsic health of the embryo; dynamic morphological defects were strongly associated with significant differences in intrinsic programs and pathways that regulate mRNA processing and packaging. Housekeeping genes were not different between the two groups.

7. New methods of data analysis

Finally, we note that gene and pathway identification has been enhanced greatly in recent years. Research by D Sahoo and colleagues reported development of a novel set of tools (termed MiDReG for mining developmentally regulated genes) to first examine Boolean distributions of gene expression and conserved patterns and then to predict intermediate, developmental genes and gene sets that function specifically to determine fate[15, 16]. This method was recently validated by Sahoo, Weissman and colleagues with application to B-cell development. The algorithm predicted 62 genes that are expressed after the KIT progenitor cell stage and remain expressed through CD19 and AICDA germinal center B cells. Both qRT-PCR and published literature of knockout mice revealed that the predicted genes have defects in B-cell differentiation and function. Novel genes are under further

investigation. Data demonstrate the power of MiDReG in predicting functionally important intermediate genes in a given developmental pathway that is defined by a mutually exclusive gene expression pattern. Previous studies of RNAseq and epigenetic studies of human embryo development will benefit from use of this methodology to validate data and capture data from other species and allow direct comparisons.

References

1. Racowsky C. High rates of embryonic loss, yet high incidence of multiple births in human ART: Is this paradoxical? *Theriogenology*. 2002; 57:87–96. [PubMed: 11775983]
2. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature*. 1988; 332(6163):459–461. [PubMed: 3352746]
3. Memili E, First NL. Zygotic and embryonic gene expression in cow: a review of timing and mechanisms of early gene expression as compared with other species. *Zygote*. 2000; 8(1):87–96. [PubMed: 10840878]
4. Beaujean N, Taylor J, Gardner J, Wilmut I, Meehan R, Young L. Effect of limited DNA methylation reprogramming in the normal sheep embryo on somatic cell nuclear transfer. *Biology of Reproduction*. 2004; 71(1):185–193. [PubMed: 14998909]
5. Dobson AT, Raja R, Abeyta MJ, Taylor T, Shen S, Haqq C, Reijo Pera RA. The unique transcriptome through day 3 of human preimplantation development. *Human Molecular Genetics*. 2004; 13(14):1461–1470. [PubMed: 15150160]
6. Fulka H, Mrazek M, Tepla O, Fulka J Jr. DNA methylation pattern in human zygotes and developing embryos. *Reproduction*. 2004; 128(6):703–708. [PubMed: 15579587]
7. Duranthon V, Watson AJ, Lonergan P. Preimplantation embryo programming: transcription, epigenetics, and culture environment. *Reproduction*. 2008; 135(2):141–150. [PubMed: 18239045]
8. Wang Q, Piotrowska K, Ciemerych MA, Milenkovic L, Scott MP, Davis RW, Zernicka-Goetz M. A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. *Developmental Cell*. 2004; 6:133–144. [PubMed: 14723853]
9. Zeng F, Schultz R. RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo. *Developmental Biology*. 2005; 283(1):40–57. [PubMed: 15975430]
10. Vanneste E, Voet T, Caignec CL, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F, Frys J-P, Verbeke G, D'Hooghe T, Moreau Y, Vermeesch J. Chromosome instability is common in human cleavage-stage embryos. *Nature Medicine*. 2009; 15:577–583.
11. Wong C, Loewke K, Bossert N, Behr B, DeJonge C, Baer T, Pera RR. Noninvasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. *Nature Biotechnology*. 2010; 28:1115–1121.
12. Schultz RM. The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. *Human Reproduction Update*. 2002; 8:323–331. [PubMed: 12206467]
13. Moore, KL.; Persaud, TVN. Human reproduction. Philadelphia, PA: WB, Saunders Company; 1998. p. 13-35.
14. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology*. 2007; 8(2):R19. [PubMed: 17291332]
15. Sahoo D, Seita J, Bhattacharya D, Inlay M, Weissman I, Plevritis S, Dill D. MiDReG: a method of mining developmentally regulated genes using Boolean implications. *Proceedings of the National Academy of Sciences (U.S.A.)*. 2010; 107:5732–5737.
16. Sahoo D, Dill D, Gentles A, Tibshirani R, Plevritis S. Boolean implication networks derived from large scale, whole genome microarray datasets. *Genome Biology*. 2008; 9:R157. [PubMed: 18973690]

Fig. 1.

Proposed model for human embryo development based on correlated imaging and molecular analysis[11]. Shown is timeline of development from zygote to blastocyst including critical brief times for prediction of successful development to blastocyst and a diagram of embryo development. Key molecular data indicates that human embryos begin life with a distinct set of oocyte RNAs. Following fertilization, degradation of a subset of maternal RNAs specific to the egg (ESSP1; Embryonic Stage Specific Pattern 1) must be degraded as the transition from oocyte to embryo begins. In parallel, other RNAs are ideally partitioned equally to each blastomere as development continues (ESSP4). The successful degradation and partitioning of RNAs culminates with embryonic genome activation, with transcription of ESSP2 genes in a cell autonomous manner. During the cleavage divisions, blastomeres may arrest or progress independently. The outcome of cell autonomous development is that individual blastomeres may arrest or progress; as the embryo progresses to morula stage and beyond, blastocyst quality will be impacted by the number of cells that arrested or progressed beyond 8 cells. Imaging data demonstrates that critical periods of development predict success or failure: cytokinesis, second cleavage division and synchronicity of

appearance of 3rd and 4th blastomeres. Data suggests improved ability to diagnose embryo outcome with key imaging predictors.

Fig. 2. Gene expression analysis of single human embryos and blastomeres. (A) Four distinct embryo stage specific gene expression patterns (ESSPs) exist in human preimplantation embryos throughout development. (B) The half-lives of ESSP1 and ESSP4. (C; left) The ratio of maternal to embryonic genes in embryos changes during preimplantation development. (C; right) Some embryos contained blastomeres of different developmental ages[11].

Pattern 1	Pattern 2	Pattern 4
Anillin	CCNA1	CTK7
ATG5	CCR4	FABP5
ATP2C1	DNM2	FGFR2
Aurora A	EIF1AX	GAPDH
Bclaf1	EIF4A3	HMOX2
Cofilin	EOMES	Hsp70
CPEB1	GDF3	IGF2R
DAZL	GTF2A1	LSM3
Dgcr8	H2AFZ	PABPC1
DIAPH1	JARID1B	PRMT5
DIAPH2	Nanog	RPLPO
Dicer	NES	YY1
DNAJA4	Prdm14	
DNMT3B	PTMA	Undefined
Drosha	RUNX2	ATF4
ECT2	SERPINA1	ATF7IP
EHMT2	SERTAD1	ATF7IP2
FGFR1	SOX2	B2M
FYN	STELLAR	BNC2
GABPB2	YY1	BTF3
GDF9		CDX2
HMOX2	Pattern 3	CTNNB1
HPRT1	B-actin	Dby
HSF1	Bcl2	Hsp70.1
IGF1R	GCM1	PRDM1
IGF2R	HDAC7A	Symplekin
LAMB1	IFITM1	TARBP2
MKLP2	POU5F1	Xist
MYLC2	SALF	
NLRP5	TGFB2	
PARN		
PDCD5		
PVR		
RhoA		
Sycp3		
TACC3		
TAF4		
TBP		
TERT		
VASA		
XPO5		
YBX2		
Zar1		
ZP1		

Fig. 3.

Component genes of ESSP (Embryonic Stage-Specific Pattern) groups in human embryo development. These genes characterize the 4 basic patterns of gene expression in the preimplantation human embryo.