

RESULTS: NSBP1 was uniformly expressed in the embryo from E0.5 until E3.5. However, beginning at E4.5, in the peri-implantation blastocyst, NSBP1 was preferentially expressed in the trophoctoderm and remained predominantly in the extra-embryonic tissues (ectoplacental cone) through E7.5. After siRNA injection, embryo development was significantly delayed at E2.5 and E3.5 in the specific vs. the control siRNA injected groups. When examined, NSBP1 was present in all control siRNA injected embryos. However, downregulation of NSBP1 was confirmed in only a portion of the specific siRNA injected embryos, suggesting that a partial recovery of NSBP1 expression occurred by E4.5.

CONCLUSIONS: The chromatin architectural protein, NSBP1, plays an important role in early embryonic development. Interestingly, the specific timing of NSBP1 downregulation and its differential expression in extraembryonic tissues in the peri-implantation period suggest that NSBP1 may play an important role in embryo implantation.

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P-609

QUANTITATIVE GENE EXPRESSION ANALYSIS OF PLURIPOTENT AND EARLY DIFFERENTIATION GENES IN HUMAN PREIMPLANTATION EMBRYOS AT THE SINGLE-CELL LEVEL.

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OBJECTIVE: Accurate quantitative measurement of gene expression provides an essential step in understanding the properties and state of blastomeres (BLM). To our knowledge, quantitative measurement of the pluripotency genes Oct4 and NANOG, trophoctoderm (Cdx2, HCG), and primitive endoderm (GAT6) in single blastomeres has not been achieved.

DESIGN: Sixty-four individual BLM from 12 cleavage-stage 3PN, 1PN human embryos were analyzed for the quantitative expression of Oct4, NANOG, Cdx2, HCG and GATA6 genes. In addition, 8 housekeeping genes (HKG) were evaluated for stability and consistency in the same samples.

MATERIALS AND METHODS: Seven triploid embryos (3–9 cells) and 5 haploid ICSI embryos (3–10 cells) on days 2 or 3 were used. Total RNA of each individual BLM from disaggregated embryos were extracted using a Picopure RNA extraction kit and retro-transcribed. Pre-amplification of the c-DNAs of the genes of interest were performed and the RT-PCRs were carried out in triplicate for each gene and sample. To determine the best HKG, a simple Ct approach was employed by comparing relative expression of “pairs of HKG” within each sample.

RESULTS: Pre-amplification and RT-PCR efficiencies on single blastomeres for all genes tested were >90%. Of eight HKG tested, the GAPDH and PPIA were the most stable, and the latter was used for analysis. All of the blastomeres, independent of the cell stage expressed at least one of the pluripotency genes (Oct4, Nanog), and over 60% of blastomeres expressed both. Interestingly, the expression of Oct4 was greater in day 2 blastomeres compared to day 3 blastomeres. The levels of expression of the other genes tested were generally low and demonstrated sporadic distribution within BLM, with GATA6 being the most consistently expressed after 6-cell stage in all BLM. Gene expression, in 3PN and 1PN embryos showed similar patterns for the majority of genes tested, with limited or absent expression of trophoctoderm genes (Cdx2 and HCG) in blastomeres of haploid (1PN) embryos.

CONCLUSIONS: We were able to quantitatively assess key developmental genes in single blastomeres from human cleavage embryos. This technique is a reliable method in assessing gene expression from the single cell.

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P-610

Cdx2 AND Oct4 mRNA IN PRE-IMPLANTATION STAGE MOUSE EMBRYOS AND THEIR SINGLE CELLS.

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OBJECTIVE: In blastocysts, Oct4 is expressed in the inner cell mass and Cdx2 in the trophoctoderm. We used duplex RT-LATE-PCR (Reverse Transcription- Linear After the Exponential- Polymerase Chain Reaction) to quantify levels of Cdx2 and Oct4 mRNA in cleavage-stage embryos and single blastomeres to shed light on cell lineages and differentiation.

DESIGN: Two-cell stage mouse embryos were cultured to different preimplantation stages and collected as single embryos. Embryos at the 8-to-16-cell stage were also dissociated into single blastomeres. Cdx2 and Oct4 transcript levels are measured simultaneously in each sample.

MATERIALS AND METHODS: Mouse embryos at the 2-cell stage were purchased frozen and cultured in GEM-PS medium. Whole embryos, as well as single blastomeres collected following laser ablation of the zona pellucida (a), were processed using PurAmp (b), a novel technology developed in our laboratory (Hartshorn et al. 2005, a and b). PurAmp permits cell lysis, reverse transcription, and sequence amplification by sequential dilution in a single tube, without chemical extraction or binding to a matrix. Amplification of Cdx2 and Oct4 sequences was performed using LATE-PCR, also developed in our laboratory (Sanchez et al. 2004; Pierce et al., 2005).

RESULTS: Oct4 mRNA was abundant in embryos at the 8-cell stage but its levels varied among cells. Analysis of all blastomeres recovered from individual 8-cell embryos consistently demonstrated a gradient from low to high levels of Oct4 mRNA per cell. In contrast, Cdx2 mRNA was virtually absent in 8-cell stage embryos, but was observed in mid- to late-stage morulas. Current experiments aim to pin-pointing the exact chronology of Cdx2 and Oct4 mRNA accumulation, as well as to quantify the levels of these two transcripts in individual cells from the late morula stage onward. Since Oct4 protein is restricted to the ICM of blastocyst stage embryos while Cdx2 protein is restricted to the trophoctoderm, the present study may reveal whether there is also an inverse-relationship between the corresponding mRNA levels prior to morphological differentiation.

CONCLUSIONS: We have constructed and validated a duplex RT-LATE-PCR assay for Oct4 and Cdx2. Our findings are consistent with protein evidence that mammalian embryo trophoctoderm differentiation, as measured by expression of Cdx2 mRNA, occurs at the blastocyst stage.

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DOES DAY 1 EARLY CLEAVAGE SCORING PROVIDE MORE INFORMATION FOR EMBRYO SELECTION THAN SEQUENTIAL SELECTION ON DAYS 1, 2 AND 3? S. Walker, S. McLellan, A. Finn, I. Hardy, J. Hill, L. Scott. Fertility Centers of New England, Reading, MA.

OBJECTIVE: Sequential embryo grading (SEG) on Day 1, 2 and 3 increases implantation (IR) by multiple-criteria embryo-elimination. Early-embryo cleavage (EC) on day 1 is an additional criteria but requires scoring twice on D1, which may be detrimental. The benefit of EC with SEG was assessed by correlating EC results with PN; and D2 and 3 scores.

DESIGN: Prospective data collection.

MATERIALS AND METHODS: SEG included: PN/Z-score at 16–18 and EC at 25 hrs (EC = 2 cell; nuclear membrane breakdown (NMBD) or not EC = 2PN); strict D2 (42 h) and D3 (65–66 h) post insemination. D2 scoring: nucleation: 1 nucleus per blastomeres (1n/b), no nuclei visible (nnv), multi nucleated in at least 1 cell (MN); cell number and equality. Embryos were not selected for ET based on EC but sequentially on D1, 2, and 3. EC data were correlated with D1, 2 and 3 scores and the embryos selected for ET. Controls were embryos not scored for EC.

RESULTS: SEG vs. EC are shown in Table 1. There was a correlation between EC and PN score and NPB ratio; highly significant correlation with D2 scoring and 4-cell, 1n/b and even blastomere size. D3 score was significant; more embryos were at the 6–8 cell stage. Of the embryos transferred in the EC series (100), 69% were EC: 85% EC for D3 and 59% for D2 ET. The IR was 24% for D2 and 35% for D3 ET. In the EC series more EC embryos were selected for ET and cryopreservation than non-EC ($P<0.01$). Embryos selected for ET in the previous 3 months (258; 154 D2 and 104 D3) had similar morphology scores to the EC group (Table 2).

TABLE 1. EC and embryos scores

	2-cell	NMBD	2PN	P
Good PN Score	75	75	61	0.05
NPB Ratio (5–7)	68	52	48	0.05
Day 2, Even	91	76	50	0.01*
Day 2, 1n/b	81	63	27	0.001**
Day 2, MN	3	15	22	0.01*
Day 2, 4-cell	85	72	27	0.01*
Day 2, 2-cell	3	5	41	0.01*
Day 2, 3-cell	0	9	19	0.01*
Day 3, 8-cell	62	54	25	0.01*
Day 3, 7- 8 cell	75	65	39	0.01
Day 3, 6- 8 cell	85	72	59	0.05

Data is the percent of embryos in each scoring category for each EC stage.

TABLE 2. Non-EC Group and Embryos Scores

	PN score	D2 4-cell	Day 2 2-cell	1n/b	Even size	6–8 cell	IR
Day 2 ET	77	54	40	76	71	–	24
Day 3 ET	94	86	14	90	83	93	35

Data is percent of embryos in each scoring category.

CONCLUSIONS: When SEG is employed, EC provides limited additional information, with strong correlates between EC and current morphology parameters. The detrimental effects of scoring may outweigh its benefit. With large cohorts of embryos, EC may be beneficial, providing an additional selection parameter.

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ALIGNMENT OF THE PRONUCLEI AND POLAR BODIES IN DAY 1 PRONUCLEAR ZYGOTES IS HIGHLY PREDICTIVE OF DAY 3 EMBRYO QUALITY AFTER IN VITRO FERTILIZATION (IVF).

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OBJECTIVE: To determine if a parallel, perpendicular or oblique alignment of the pronuclei and polar bodies in day 1 pronuclear zygotes is correlated with day 3 embryo quality.

DESIGN: Retrospective cohort analysis.

MATERIALS AND METHODS: An evaluation of all embryos conceived via controlled ovarian hyperstimulation and IVF in 2006 at the University of Vermont was performed. Cycles using both standard IVF and IVF with intracytoplasmic sperm injection were included. Day 1 pronuclear zygotes were examined and an assessment of the orientation of the long axis of the pronuclei to the polar bodies was recorded. Embryos whose growth arrested prior to day 3 were excluded from the analysis. Embryo quality was assessed on day 3 based on cell number and embryo grade. Day 3 embryo grade was based on percent fragmentation (FG) with grade A embryos having 0–10% FG, grade B having 10–20% FG, grade C having 20–30% FG and grade D having >30% FG. Multiple regression analysis was used to evaluate the relationship between day 1 pronuclei/polar body alignment and day 3 embryo quality. Because there is no gold standard for day 3 embryo quality, 3 different multiple regression models were used. The first model considered only day 3 embryo grade. The second model considered day 3 embryo cell number, with 8 cells representing the highest quality embryos and embryos containing more or fewer than 8 cells representing progressively lower quality. The third model considered qualitative scores, with “high quality” embryos having a score of 7–10A, “medium quality” embryos having a score of 7–10B, >10A or <7A, and “poor quality” embryos having a score of <7B, >10B or any C or D grade embryos.

RESULTS: 82 patient cycles containing 364 embryos were included in the analysis. All 3 multiple regression models demonstrated a statistically significant correlation between pronuclei/polar body alignment and day 3 embryo quality. An oblique alignment was associated with the highest quality, followed by a parallel alignment and a perpendicular alignment was associated with the poorest embryo quality (all $P < 0.001$).

CONCLUSIONS: An oblique alignment of the pronuclei and polar bodies in day 1 pronuclear zygotes is associated with high day 3 embryo quality and a perpendicular alignment is associated with poor embryo quality. The finding that pronuclei and polar body alignment is strongly associated with day 3 embryo quality indicates that it may also be associated with implantation potential.

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GENETIC MODIFICATION OF PREIMPLANTATION EMBRYOS AND EMBRYONIC STEM CELLS (ESC) BY RECOMBINANT LENTIVIRAL VECTORS: EFFICIENT AND STABLE METHOD FOR CREATING TRANSGENIC EMBRYOS AND ESC.

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OBJECTIVE: Viral transgenesis is based on the delivery of transgenes into embryos via recombinant viruses. Lentiviral vectors can transduce preimplantation embryos and ESC without gene silencing. Interestingly, the stage of the embryo development will determine the location of lentiviral integration. We present a novel way of the generation of transgenic blastocysts (BL) and stably transfected ESC via lentiviral vector incubation of early preimplantation embryos.

DESIGN: Mouse and human embryos were transfected by lentiviral vectors; their integration was observed at various developmental stages. In addition, successfully transfected mouse BL were cultured to ESC and their transfection stability was followed during undifferentiated and differentiated ESC development.

MATERIALS AND METHODS: We used a lentiviral reporter vector with the PGK promoter driving green fluorescent protein (PGK-GFP) or monomeric Cherry (PGK-mCherry) to transfect preimplantation mouse and human embryos. After 3 days of incubation, the embryos were observed for the presence of the fluorescent signal (GFP-green, mCherry-red). The positively transfected mouse BL were further cultured to induce ESC development. In parallel, control mouse ESC (mESC) were infected with the same virus to generate transfected mESC. Both groups were cultured under undifferentiated and differentiated stem cell conditions and transfected integration was monitored.

RESULTS: Mouse preimplantation embryos can be successfully transfected with lentiviral vectors; the embryos showed the same developmental speed and morphology as controls. The transfected BL showed full viral integration (trophectodermal and inner cell mass) that resulted in complete transfection of undifferentiated mESC and embryonic bodies upon differentiation. The control mESC showed viral integration after 2–3 days of incubation and the clonal transfection was established only after 2–3 selective passages. The preliminary results on human triploid embryos showed successful viral integration observed at the BL stage in all cells.

CONCLUSIONS: We describe an easy and efficient method for transgenic embryos and mESC generation. This is a superior method for generating stable ESC because transgenes were stable during embryo development, under different stem cell conditions, and do not require selective clonal passaging. We also showed that human preimplantation embryos can be successfully transfected. This method can be used to generate genetically modified ESC for studying human embryogenesis and disease.

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P-614

USING CERTAIN COMMERCIALY AVAILABLE GLASS-BOTTOMED DISH WITH PARAFFIN OIL IMPACTS THE EMBRYONIC DEVELOPMENT OF OOCYTES.

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OBJECTIVE: Birefringent microscopic imaging (Polscope™) has made it possible to visualize the meiotic spindle of an egg without impacting on its viability. Eggs are routinely monitored in media microdrops under an oil overlay in special culture dish that has a glass bottom and plastic sidewalls. We experimentally detected that certain commercially produced glass-bottomed dishes can adversely impact on egg viability. This report examines the impact oil overlays and culture dish composition on embryonic development.

DESIGN: Prospective study using mouse (CB6F1) zygotes created through IVF.

MATERIALS AND METHODS: Mouse pronuclear zygotes produced from IVF were cultured to blastocyst stage in Q1/2 media (Sage) microdrops covered with mineral (Sigma) or paraffin oil (Sage) in glass-bottomed dishes (A: WillCo, GWst-5040, B: World Precision Instrument, FD5040B-100, and C: World Precision Instrument, FD5040-100) or plastic dish (Falcon 351007). Embryonic development was monitored daily to blastocyst stage. Results were tested for statistical significance using χ^2 as appropriate with significance set at $P < 0.05$.

RESULTS: See table.