Title:

Genetic Normalization of Differentiating Aneuploid Human Embryos

Text: Summary Paragraph

Early embryogenesis involves a series of dynamic processes, many of which are currently not well described or understood. Aneuploidy and aneuploid mosaicism, a mixture of aneuploid and euploid cells within one embryo, in early embryonic development are principal causes of developmental failure.[1,2] Here we show that human embryos demonstrate a significant rate of genetic correction of aneuploidy, or "genetic normalization" when cultured from the cleavage stage on day 3 (Cleavage) to the blastocyst stage on day 5 (Blastocyst) using routine *in vitro* fertilization (IVF) laboratory conditions. One hundred and twenty-six human Cleavage stage embryos were evaluated for clinically indicated preimplantation genetic screening (PGS). Sixty-four of these embryos were found to be aneuploid following Cleavage stage embryo biopsy and single nucleotide polymorphism (SNP) 23 chromosome molecular karyotype (microarray). Of these, 25 survived to the Blastocyst stage of development and repeat microarray evaluation was performed. The inner cell mass (ICM), containing cells destined to form the fetus, and the trophectoderm (TE), containing cells destined to form the placenta were evaluated. Sixteen of 25 embryos (64%) [95% CI: 44-80%]) possessed diploid karyotypes in both the ICM and TE cell populations. An additional three Blastocyst stage embryos showed genetic correction of the TE but not the ICM and one Blastocyst stage embryo showed the reverse. Mosaicism (exceeding 5%), was not detected in any of the ICM and TE samples analyzed. Recognizing that genetic normalization may occur in developing human embryos has important implications for stem cell biology, preimplantation and developmental genetics, embryology, and reproductive medicine.

Text: Body

Spontaneous miscarriages in human pregnancies have been well documented to be associated with chromosomal aneuploidy. [1, 2] In an attempt to minimize rates of aneuploidy in high risk pregnancies, single cells can be biopsied from early embryos and tested for their chromosome complement prior to uterine transfer. [3] This procedure, termed PGS, is generally performed on polar bodies or 1-2 totipotent blastomere cells biopsied from Cleavage stage embryos. [3] The traditional modality for evaluating the chromosomal makeup of these cells has been by fluorescence *in situ* hybridization (FISH) of 5-12 chromosomes.[3]

Despite initial success, data have failed to demonstrate that PGS by FISH on Cleavage stage embryos improve pregnancy outcomes and the delivery of healthy babies. [4,5] Consequently, this approach has not become the recommended standard of care. [4,5] Potential reasons for the lack of demonstrated clinical benefit from karyotyping Cleavage stage embryos using FISH methodologies could be due to damage caused to the developing embryo during biopsy, testing of only a subset of chromosomes, or the presence of aneuploid mosaicism within the Cleavage stage embryo. Indeed, studies have documented aneuploid mosaicism rates of between 17%-50% in Cleavage stage embryos.[6,7]

Since 2007, comparative genomic hybridization (CGH) of metaphase chromosomes, real-time polymerase chain reaction (PCR), or microarray platforms using single nucleotide polymorphism (SNP) or CGH have been introduced to simultaneously evaluate all 23 pairs of chromosomes. [8,9,10,11] Use of 23 chromosome aneuploidy screening has been used to select embryos for uterine transfer with significant improvement in clinical pregnancy rates when compared to FISH methods that evaluate only between 5 and 12 chromosomes. [3, 11, 12, 13, 14] Recently, the

aforementioned technologies have been employed to evaluate Blastocyst stage TE cells without disturbing the ICM, with additional improvement in clinical pregnancy rates over testing at the Cleavage stage. [3, 11, 12, 13, 14]

PGS using 23 chromosome microarrays shows that aneuploidy is more commonly diagnosed when the biopsy is performed at the Cleavage stage compared to the Blastocyst stage. [15] The observation that cells derived from Blastocyst embryos have lower rates of aneuploidy than cells from Cleavage embryos is intriguing, especially given the well documented rate of confined placental aneuploid mosiacism observed following clinically performed chorionic villous sampling (CVS).[16] These observations taken collectively led us to hypothesize that aneuploid cells within a mosaic Cleavage stage embryo are preferentially allocated to the TE during Blastocyst differentiation, resulting in genomic normalization of the ICM: the future euploid fetus.

This study evaluated embryos from clinically indicated PGS cases, using 23 chromosome microarrays, with biopsy performed at the Cleavage stage of development. All embryos were then cultured using standard IVF conditions to allow development to the Blastocyst stage. Blastocyst embryos that had aneuploid PGS results at the Cleavage stage were then dissected into TE and ICM cell populations. Immunocytochemistry using anti-oct3/4 to identify the ICM [17] and anti-cdx2 to identify the TE cells [18] confirmed that the ICM and TE samples contained the appropriate cell type. The TE and ICM DNA samples were then independently amplified and evaluated via microarray. Details are provided in the Methods Section.

Twelve patients were enrolled (average maternal age of 37 years with a range of 21-44) and 126 Cleavage stage embryos were evaluated. Approximately 270,000 to 285,000 SNPs were genotyped per sample analyzed to generate a molecular karyotype. (Fig. 1) The microarray fluorescence hybridization efficiency and the genotype SNP call rate (the ability to detect specific alleles within a chromosome) exceeded 90% for all Cleavage stage embryos and 99% for all Blastocyst (ICM and TE) samples.

Of 62 Cleavage stage embryos with euploid karyotypes, 43 (69.4%) developed to the Blastocyst stage. A euploid karyotype obtained at the Cleavage stage was predictive of progression to the Blastocyst stage with a positive predictive value of 69.4% [95% CI: 57-79%] and a negative predictive value of 60.9% [95% CI: 49-72%]. Embryos that developed to Blastocysts with a corresponding molecular euploid karyotype were clinically considered for transfer or cryopreservation and therefore could not be subjected to further molecular karyotypic evaluation. Only 25 of 64 (39.1%) embryos with an aneuploid karyotype at the Cleavage stage progressed to the Blastocyst stage. This is significantly less than progressed to the Blastocyst stage with euploid karyotypes at Cleavage stage biopsy (p=.0007 by Fisher's exact test). Intriguingly, 16 of the 25 (64%) Blastocyst embryos showed euploid karyotypes in both the ICM and TE. Four (16%) embryos had euploid-aneuploid discordance between the ICM and TE. Only 5 (20%) Blastocyst stage embryos demonstrated aneuploid karyotypes in both the ICM and TE. Of note, for all embryos evaluated, the karyotypes at the Cleavage stage differed from the corresponding karyotypes of the ICM and TE obtained from the Blastocyst stage. (Table 1, Fig. 2)

Aneuploidy for chromosomes 1 through 12 was common at the Cleavage stage, occurring in 17 of the 25 abnormal embryos. However, in only 3 of these 17 embryos, did these abnormalities

persist in the TE or ICM at the Blastocyst stage. Aneuploidy for chromosomes 1-12 are among the least frequently found in routine karyotypes from spontaneous abortion products of conception.

Mosaicism was not observed in any of the ICM or TE samples evaluated [95% CI: 0-16% for each sample]. (Fig. 1) However, our microarray analysis is unable to detect mosaicism at levels below 5% (See Methods Section). Therefore, our results can not completely discount the possibility of low levels of mosaicism below this detection threshold.

There are several plausible explanations that could account for the dramatically different genetic observations seen between Cleavage and Blastocyst stage embryos in this study. Some posit that cytogenetic correction results from the stochastic loss of the extra chromosome, i.e.: trisomy rescue, or the active allocation of aneuploid cells to the TE during early embryogenesis. [19] These mechanisms do not explain our results as many of the corrected aneuploidies involved multiple chromosomes that would be unlikely to have been rectified with simple mechanisms such as single chromosome rescue. Furthermore, mosaicism was not observed in any of the TE samples evaluated.

Plausible explanations for the genetic normalization observed in this study must account for the fate of aneuploid cells within the Cleavage stage embryo. It remains possible that a small number of aneuploid cells are preferentially relegated to the TE resulting in low level mosaicism below our detection threshold of 5% (See Methods Section). However, the proportion of detected aneuploidy in the TE was lower (rather than higher) compared to the ICM cell populations. As mosaicism is known to exist in Cleavage stage embryos,[6,7] it is also possible that the removal of

the only abnormal cell from the mosaic embryo at Cleavage stage leaves only euploid cells behind. However, if only 1 cell in a Cleavage stage embryo were aneuploid, the chance of repeatedly removing only this cell from multiple embryos is exceedingly low. Indeed, our data shows that Cleavage stage embryos, diagnosed via a single cell molecular karyotype, as euploid progressed to the Blastocyst stage at a rate of 69.4% compared with only 39.1% of aneuploid embryos. It is possible that some level of mosaicism is common among developing embryos. One could postulate that low levels of mosaicism translate into a higher rate of development to the blastocyst stage. Our data could be consistent with this hypothesis given that, if mosaicism was present in many of the embryos evaluated, one would be more likely to have an aneuploid cell obtained during biopsy compared to embryos with higher levels of euploidy.

Our data suggests that a possible mechanism for the embryo correction seen in this study is the loss of aneuploid cells within developing mosaic Cleavage stage embryos. Indeed, most of the corrections observed would be extremely difficult to rationalize by a mechanism that accounts for the correction of multiple chromosomes. Rather, it appears likely that there may exist in early human embryonic development a drive toward normalization in which aneuploid cells are systematically marginalized. Aneuploid cells within the embryo may undergo apoptosis, thereby leaving euploid cells in the surviving Blastocyst stage embryo. In aneuploid cells, genes such as those coding for the checkpoint proteins *Bub* and *Mad* could initiate apoptotic mechanisms rather than those that lead to mitotic cell division.[20] Another possibility, although unlikely, is that aneuploid cells may replicate less efficiently than euploid cells resulting in their eventual loss within the developing embryo. There could also be as yet unidentified mechanisms that correct aneuploidy during early embryologic development. It is also conceivable that correction of

aneuploidy in a subset of cells within Cleavage stage embryos is a normal event in early embryogenesis.

The concept of embryo normalization has been postulated previously. [21, 22] The results of these previous studies have suggested that embryo normalization may exist, however, evidence for this has been indirect or incomplete, and therefore inconclusive. Previous PGS studies, with FISH or CGH, on cells obtained from embryo biopsy comparing single cells from Cleavage stage embryos versus a few to several TE cells from Blastocyst stage embryos failed to show genetic normalization using standard IVF culture conditions. [13, 23, 24] Other studies had significant experimental limitations including but not limited to the use of FISH technology that evaluated only 9-14 chromosomes, evaluating only a small (less than 10 cells) sample size from developing embryos, failing to differentiate the ICM from the TE in evaluating Blastocyst cells, failing to discriminate between different stages of embryologic development, and using laboratory conditions not representative of standard IVF culture conditions including the use of growth factors and the culture of embryos beyond 6 days of development. [21, 22] Our study is the first demonstration of genetic normalization between the Cleavage stage and Blastocyst differentiation in human embryos by full genomic karyotypic analysis. Our claim is supported by recently described time lapse embryologic videography that documents morphologic normalization of developing embryos. [25]

The genetic normalization observed in this study has significant implications in numerous scientific fields. A current challenge within stem cell biology is the high rate of acquired aneuploidy that is observed with cell colonies in extended culture. [26, 27, 28, 29] Dissecting the mechanism underlying the normalization observed in this study in a stem cell system would be

highly useful and may be applied to cell-based therapeutic approaches using stem cells. An understanding of such *in vitro* reparative mechanisms in developing embryos could potentially add to current strategies for gene repair and stem cell transplant therapy. These mechanisms could also have implications on future therapies in the field of oncology in which aneuploid tumor cells experience unregulated growth. Furthermore, our findings could potentially effect management of patients undergoing infertility care by reassessing the disposition of abnormal Cleavage stage aneuploid embryos. Finally, this data also has important implications for preimplantation and developmental genetics, and embryology.

Methods Summary

IRB approval and patient consent was obtained for couples undergoing IVF with PGS. Cleavage stage embryos were biopsied using laser or acid tyrodes, and underwent clinical microarray analysis.[30] Single cells were subjected to a modified multiple displacement amplification protocol followed by a second round of whole genome amplification. Approximately 200,000 ng of amplified DNA was loaded onto Illumina Infinium high-density HumanCytoSNP-12 DNA beadchips (Illumina, San Diego, CA) and routine microarray analysis and scanning was performed by an Illumina iScan BeadArray reader. Data was analyzed with Illumina KaryoStudio and GenomeStudio software. All final molecular karyotypic analyses were performed with the reader, the laboratory director, blinded to patient names or controls. All interpretations were repeated four times.

During embryo growth and development between Cleavage stage and Blastocyst stage, all embryos remained in a standard commercially available media. All Blastocyst stage embryos with euploid Cleavage stage results either underwent uterine transfer or were cryopreserved for later clinical use. Blastocyst stage embryos grown from embryos with aneuploid Cleavage stage results underwent surgery to separate the ICM from the TE. Each cell type was confirmed by immunocytochemistry using anti-oct3/4 for the ICM and anti-cdx2 for the TE. An average of 100 TE cells and a range of 40 ICM cells to the entire ICM cell population were obtained from each embryo for microarray analysis. These samples then underwent separate DNA amplification, microarray analyses, and scanning as described above. Our laboratory identifies mosaic cell populations at a level of 5% (See Methods Section).

All coded and de-identified samples were kept by an individual not responsible for molecular karyotype interpretation. Molecular data was analyzed in a blinded manner as described above. The samples were then de-identified and data tabulated. Binomial confidence intervals for proportions were calculated by the modified Wald method.

Text: Methods Section

Details Materials and Methods for the Data Described in this Paper All patients underwent standard in vitro fertilization (IVF) and microarray PGS secondary to repeat pregnancy loss (RPL) or unexplained infertility. IRB approval was obtained and all couples were consented. All agreed to donate their genetically abnormal embryos to research with signed informed consent.

All Cleavage stage embryos were biopsied and underwent clinical microarray analysis. To accomplish this, an embryo biopsy was performed either by ZILOS-tk™ laser (Hamilton Thorne Biosciences Inc., Beverly, MA) or by acid tyrodes and one cell was removed from each embryo for genetic testing. Each single blastomere was placed into an eppendorf tube of 5 ul 0.2N potassium hydroxide (KOH) DNA stabilizing buffer.

All single cells were first subjected to a modified multiple displacement amplification protocol using *phi* 29 DNA polymerase followed by a second round of whole genome amplification. Approximately 200,000 ng of amplified DNA was loaded onto Illumina Infinium high-density HumanCytoSNP-12 DNA beadchips (Illumina, San Diego, CA) containing 301,232 genetic markers and routine microarray analysis and scanning was performed by an Illumina iScan BeadArray reader.

Data interpretation included the comparison of raw blastomere DNA genotypes to an established embryonic cell normalized DNA data set. Data was analyzed with Illumina GenomeStudio and KaryoStudio software. All final molecular karyotypic analysis was performed with the reader, the laboratory director, blinded to patient names or controls. All interpretations were repeated

four times. Clinical reports were generated and transmitted to the appropriate clinicians and embryologists.

During embryo growth and development between Cleavage stage and Blastocyst stage following oocyte fertilization, all embryos remained in a standard commercially available media. All Blastocyst stage embryos with euploid Cleavage stage results either underwent uterine transfer or were cryopreserved. Blastocyst stage embryos grown from aneuploid Cleavage stage embryos underwent surgery to separate the ICM from the TE. An average of 100 TE cells and a range of 40 ICM cells to the entire ICM cell population were obtained from each embryo and placed in separate eppendorf tubes containing DNA stabilizing buffer (as described above). Immunocytochemistry was performed on an aliquot of cells from each tube using anti-oct3/4 to confirm the identity of the ICM and anti-cdx2 to confirm the TE cells. DNA amplification, microarray analyses and scanning was then performed as described above. Embryos that failed to develop to the Blastocyst stage were unable to be analyzed due to high levels of cellular fragmentation and embryo degeneration.

All coded and de-identified samples were kept by an individual not responsible for molecular karyotype interpretation. The Blastocyst stage ICM and TE microarray interpretations were performed blinded to all corresponding patient or embryo information. These interpretations were repeated by the same blinded reader four separate times, confirming the integrity of the results with all data analyzed. The samples were then de-identified and data tabulated. Binomial confidence intervals for proportions were calculated by the modified Wald method.

Detailed Materials and Methods Describing Single Cell Microarray Validation and Clinical PGS Results Amplification of DNA from small cell populations, particularly single cells, is potentially problematic. Potential sources of laboratory error may be derived though imperfections in the amplification process, the microarray experimental protocol, and visual interpretation of molecular karyotypic data. Before launching our clinical PGS microarray program, our methodology was validated by performing a blinded evaluation that compared 10-probe FISH results with our 23 chromosome SNP microarray protocol. Following this successful validation, we launched our clinical PGS program. We have performed clinical microarray PGS on approximately three thousand embryos from over 300 IVF PGS cycles with highly successful results.

Development and Validation of Single Cell Microarray Protocol In our development of single cell microarray analysis, a DNA amplification protocol was optimized that does not include polymerase chain reaction (PCR) technology as this introduces an unacceptable level of artifact and compromises diagnostic integrity. We developed our 23 chromosome SNP microarray protocol to include a modified multiple displacement amplification using *phi 29* polymerase followed by a whole genome amplification protocol on a single cell. Additionally, extensive bioinformatic analysis was used to create an embryonic SNP genotype normalized data set. This data set was then compared against clinical samples to identify normal and abnormal SNP genotypes in individual embryonic cells.

Prior to launching our clinical PGS program, after which time the experiments described in this manuscript were performed, our laboratory underwent an extensive validation process on 802

cells from 110 cleavage stage embryos that were evaluated clinically by 10-probe FISH for PGS. [30] Aneuploid embryos were then donated to research under informed consent and the remaining single cells within the embryos evaluated by 23 chromosome SNP microarray analysis. Analyses of blastomeres and cell line controls showed, in many cases, a genomic coverage > 98%, a heterozygous allele detection rate > 90% and a microarray detection rate and genotype call rate > 90%. A 23 chromosome molecular karyotype was obtained from over 99% of all blastomeres and all 31 cell lines. The results from this validation data show that in this cohort of 110 abnormal embryos by Cleavage stage FISH, the vast majority contained some level of mosaicism. This may not be representative of studies conducted with much larger sampling size. Details from this validation data set are available in the supplemental information (SI) section of this paper.

Clinical 23-Chromosome PGS Microarray Data Following this validation study, we launched our 23 chromosome SNP microarray clinical PGS program in December of 2009. To date, we have performed clinical PGS, generally in patients diagnosed with recurrent pregnancy loss, using 23 chromosome SNP microarrays on 2,976 embryos from 317 clinical IVF cycles. 2,704 (90.8%) of these embryos, derived from 249 (78.5%) IVF cycles, were at Cleavage stage of development when biopsy was performed. Conversely, 272 (9.2%) of embryos, derived from 68 (21.5%) IVF cycles, were at the Blastocyst stage when biopsy was performed. The maternal ages were age <35 (20%), age 35-37 (29%), age 38-40 (39%), and age >40 (12%). The clinical pregnancy rate per transfer in the Cleavage stage embryo group was 65%. The clinical pregnancy rate in the Blastocyst stage embryo group (86%) was significantly higher than the Cleavage stage embryo group ($p < .05$). However, the miscarriage rates were not statistically different between the Cleavage stage embryo (9%) and Blastocyst stage embryo (6%) groups.

Mosaicism Detection The approximate percentage of monosomic or trisomic cells within a mosaic cell population can be determined by evaluating the raw genotype data for each chromosome analyzed. Mosaic monosomy is confirmed when the log *R* ratio shows an intermediate reading along the entire length of a chromosome as compared to a cell population with all cells missing the tested chromosome. Additionally, the B allele frequency will shift depending upon the percent of the genotype of the remaining allele. For mosaic trisomies, the log *R* ratio will show an increase in copy number and the B allele frequency will shift depending upon the genotype of the remaining allele. If shifts are observed in the b allele frequency but without alterations in the smooth log *R* ratio, this indicates mosaicism and uniparental disomy. Our lab has tested $\geq$ 40 aneuploid mosaic cell populations and we detect monosomic and / or trisomic mosaicism at the level of 5%.

Figure Legends

Figure 1

Title: 23 Chromosome SNP Microarray

This figure shows a normal and an abnormal molecular karyotypic sample reading using 23 chromosome SNP microarrays.

1A shows the normal diploid diagnostic reading obtained from an ICM cell population for chromosome #1. Normal AA, AB and BB alleles and a 0 reading for the smooth log *R* ratio is observed.

1B represents the normal diploid reading of chromosome 12, from a TE cell population. Normal AA, AB and BB alleles and a 0 reading for the smooth log *R* ratio is observed.

For both 1A and 1B, no shifts are observed in the smooth log *R* ratio or B allele frequency, therefore no mosaicism is identified by our laboratory threshold of 5% of cells analyzed (see detailed methods section for a description of mosaicism).

1C demonstrates X/XX chromosome mosaicism and uniparental disomy from an amniocentesis case not included in this study. Evident are shifts in the b allele frequency. In this case, no significant shift is observed in the smooth log *R* ratio, indicating uniparental disomy. The diagnosis of this cell population is approximately 90% X bearing cells and approximately 10% XX bearing cells. This image has been included to illustrate our ability to identify low level mosaicism within a cell population.

1D demonstrates a monosomy reading of chromosome 18, from a Cleavage stage embryo. A and B alleles are observed without AB alleles represented. A significant shift in the smooth log *R* ratio is observed, consistent with the monosomy karyotype.

Figure 2

Title: Development of Evaluated Embryos

This figure accounts for fate of all of the embryos included in the study. Molecular karyotypes were obtained at the Cleavage stage for all embryos. Cleavage stage embryos with a euploid diagnosis that progressed to the Blastocyst stage were not subjected to further biopsy or intervention and were available for uterine transfer or cryopreservation. Cleavage stage embryos with an aneuploid diagnosis that progressed to the Blastocyst stage underwent embryo surgery at which point repeated molecular karyotypes were obtained from the ICM and TE cell populations.

REFERENCES

1) Hassold, T. *et al.* A cytogenetic study of 1000 spontaneous abortions. *Ann Hum Genet*. **44**, 151-78 (1980).

2) Menasha, J., Levy, B., Hirschhorn, K., & Kardon, N.B. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. *Genet Med*. **7,** 251-63 (2005).

3) Schoolcraft WB, *et al.* Clinical application of comprehensive chromosomal screening at the blastocyst stage. *Fertil Steril*. **94**, 1700-6 (2010).

4) Practice Committee of Society for Assisted Reproductive Technology; Practice Committee of American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. *Fertil Steril*. **90**, S136-43 (2008).

5) Harper, J. *et al.* What next for preimplantation genetic screening (PGS)? A position statement from the ESHRE PGD Consortium steering committee. *Hum Reprod*. **25**, 821-823 (2010).

6) Munné, S., Weier, H.U., Grifo, J., & Cohen, J. Chromosome mosaicism in human embryos. *Biol Reprod.* **51**, 373-9 (1994).

7) Baart, E.B. *et al.* Preimplantation genetic screening reveals a high incidence of aneuploidy and mosaicism in embryos from young women undergoing IVF. *Hum Reprod*. **21**, 223-33 (2006).

8) Treff, N.R. *et al.* Accurate 23 chromosome aneuploidy screening in human blastomeres using single nucleotide polymorphism (SNP) microarrays. *Fertil Steril.* **88**, S1 (2007).

9) Kearns, W.G., Pen, R., Benner, A., Wildra, E., & Leach, R. Comprehensive genetic analyses using a modified whole genome amplification protocol and micro-arrays to identify genetic disorders and determine embryo implantation from single cells. *Fertil Steril*. **88**, S237 (2007).

10) Munné, S. *et al.* Preimplantation genetic diagnosis using array CGH significantly increases ongoing pregnancy rates per transfer. *Fertil Steril*. **94**, S81 (2010).

11) Treff, N.R., Tai, X., Taylor, D., Ferry, K.M., & Scott, R.T. First pregnancies after blastocyst biopsy and rapid 24 chromosome aneuploidy screening allowing a fresh transfer within four hours of biopsy. *Fertil Steril.* **94**, S126 (2010).

12) Benner, A., Chipko, C., Pen, R., & Kearns, W.G. Clinical results on single cells from 470 embryos using 23-chromosome single nucleotide polymorphism (SNP) microarray preimplantation genetic screening (PGS) from 45 patients. *Fertil Steril*. **94**, S123 (2010).

13) Fragouli, E. *et al.* Comprehensive chromosome screening of polar bodies and blastocysts from couples experiencing repeated implantation failure. *Fertil Steril*. **94**, 875-87 (2010).

14) Scott, R.T. Jr. *et al.* Microarray based 24 chromosome preimplantation genetic diagnosis (mPGD) is highly predictive of the reproductive potential of human embryos: a prospective blinded non-selection trial. *Fertil Steril.* **90**, S22 (2008).

15) Fragouli, E. *et al.* Comprehensive molecular cytogenetic analysis of the human blastocyst stage. *Hum Reprod*. **23**, 2596-608 (2008).

16) Wang, B.B., Rubin, C.H., & Williams, J. 3rd. Mosaicism in chorionic villus sampling: an analysis of incidence and chromosomes involved in 2612 consecutive cases. *Prenat Diagn*. **13**, 179-90 (1993).

17) Nichols, J. *et al.* Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell*. **95**, 379-91 (1998).

18) Kuijk, E.W. *et al.* Differences in early lineage segregation between mammals. *Dev Dyn.* **237**, 918-27 (2008).

19) Kalousek, D.K. Pathogenesis of chromosomal mosaicism and its effect on early human development. *Am J Med Genet*. **91**, 39-45 (2000).

20) Kops, G.J., Foltz, D.R., & Cleveland, D.W. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proc Natl Acad Sci U S A.* **101**, 8699-704 (2004).

21) Barbash-Hazan, S., *et al.* Preimplantation aneuploid embryos undergo self-correction in correlation with their developmental potential. *Fertil Steril*. **92**, 890-6 (2009).

22) Munné, S. *et al.* Self-correction of chromosomally abnormal embryos in culture and implications for stem cell production. *Fertil Steril.* **84**, 1328-34 (2005).

23) Northrop, L.E., Treff, N.R., Levy, B., & Scott, R.T. Jr. SNP microarray-based 24 chromosome aneuploidy screening demonstrates that cleavage-stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts. *Mol Hum Reprod.* **16**, 590-600 (2010).

24) Daphnis, D.D. *et al.* Analysis of the evolution of chromosome abnormalities in human embryos from Day 3 to 5 using CGH and FISH. *Mol Hum Reprod.* **14**, 117-25 (2008).

25) Mochida, T., Miura, Y., Iba, Y., & Mio, Y. Analysis of compaction initiation in human embryos using time-lapse cinematography. *Fertil Steril*. **94**, S88 (2010).

26) Mitalipova, M.M. *et al.* Preserving the genetic integrity of human embryonic stem cells. *Nat Biotechnol*. **23**, 19-20 (2005).

27) Enver, T. *et al.* Cellular differentiation hierarchies in normal and culture-adapted human embryonic stem cells. *Hum Mol Genet*. **14**, 3129-3140 (2005).

28) Baker, D.E. *et al.* Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. *Nat Biotechnol*. **25**, 207-215 (2007).

29) Rugg-Gunn, P., Ferguson-Smith, A., & Pedersen, R. Status of genomic imprinting in human embryonic stem cells as revealed by a large cohort of independently derived and maintained lines. *Hum. Mol. Genet.* **16**, R243-R251 (2007).

30) Kearns, W.G. *et al.* Validation of single nucleotide polymorphism (SNP) microarray PGD on single cell(s) from embryos. *Fertil Steril*. **94**, S124 (2010).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Authors

Paul R. Brezina, MD
Johns Hopkins University School of Medicine
Department of Gynecology and Obstetrics
Baltimore, MD

Khanh-Ha D. Nguyen, MD, MPH
National Human Genome Research Institute
National Institutes of Health, Bethesda, MD
Currently at Beth Israel Deaconess Medical Center / Boston IVF
Harvard University, Cambridge, MA

Andrew T. Benner, MS
Center for Preimplantation Genetics, LabCorp
Rockville. MD

Ric Ross, MS
La Jolla IVF, La Jolla, Ca

Andrew Barker, MS
Arizona Center for Fertility Studies, Phoenix AZ

Raymond M. Anchan, MD, PhD
Brigham and Women's Hospital
Harvard University, Boston MA

Kevin S Richter, PhD
Shady Grove Fertility Reproductive Science Center
Rockville, Maryland

Garry R Cutting, MD
Johns Hopkins University School of Medicine
Institute of Genetic Medicine
Baltimore, MD

William G Kearns, PhD (Corresponding author)
Johns Hopkins University School of Medicine
Department of Gynecology and Obstetrics
Baltimore, MD
Center for Preimplantation Genetics, LabCorp
Rockville. MD 20850
Office: 301-251-2804 / Fax: 301-251-2857
Email: wkearns1@jhmi.edu

Pertinent Disclosures and Conflicts of Interest:

Paul R. Brezina, MD, MBA
-None
Khanh-Ha D. Nguyen, MD, MPH
-None
Andrew T. Benner, MS
-None
Ric Ross, MS
-None
Andrew Barker, MS
-None
Raymond M. Anchan, MD, PhD
-None
Kevin S. Richter, PhD
-None
Garry R Cutting, MD
-None
William G. Kearns, PhD
-None

Table 1: Human Embryo Correction from Cleavage to Blastocyst Stage

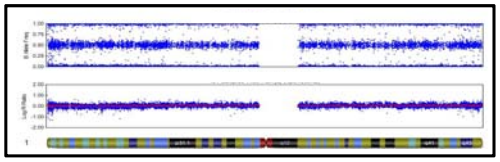
Embryo #	Cleavage Stage	ICM from Blastocyst	TE from Blastocyst
1	48, XX, +9, +16	46,XX	46,XX
2	69, XXY	46, XY	46, XY
3	47, XY, +22	46, XY	46, XY
4	45, XX, -9	69, XXX	46, XX
5	49, XX, +4, +9, +17	46, XX	46, XX
6	45, XX, -15	46, XX	46, XX
7	36, XX, -1, -4, -7, -8, -10, -11, -15, -16, -18, -19	46, XX	46, XX
8	48, XX, +16, +20	47, XX,+16	47, XX,+16
9	49, X, +1, +2, +3, +4, +5, +6, +8, -9, +10, +12, +15, -16, -19, -20, -21, -22	46, XX	46, XX
10	49, XXY, +1, +3, +4, -6, -9, +10, +11, -13, -15, +21	47, XY,+22	47, XY,+22
11	52, XXX, +4, +7, +8, +12, +14	57, XXX,+1,+2,+3,+5, +6, +8, +10, +11, +12, +16	46, XX
12	48, XX, +8, +9	46, XX	46, XX
13	48, XY, +15, +17	47, XY, +15	47, XY, +15
14	48, XX, +1, -8, +21, +22	46, XX	46, XX
15	49, XX, +3, +9, +18	45, XX, -22	45, XX, -22
16	48, XX, +9, +22	46, XX	46, XX
17	50, XY, +1, +8, +16, +18	46, XY	46, XY
18	63, XXY, +1, +2, +3, +4, +6, +7, +8, +10, +11, +12, +13, +14, +16, +17, +18, +20	62, XY, +1, +2, +3, +4, +6, +7, +9, +10, +11, +12, +13, +14, +15, +17, +18, +20	46, XY
19	48, XY, +16, +22	45, XY, -16	45, XY, -16
20	47, XX, +17	46, XX	46, XX
21	48, XX, +1, +2	46, XX	46, XX
22	50, XX, +1, +2. +5, +22	46, XX	46, XX
23	48, XY, +3, +19	46, XY	46, XY
24	47, XX, +13	46, XX	46, XX
25	47, XY, +21	46, XY	45, XY, -21

This table shows the karyotypes of all cleavage stage blastomere cells and their corresponding blastocyst stage TE and ICM. The euploid karyotypes are shaded in Grey. The aneuploid karyotypes are unshaded.

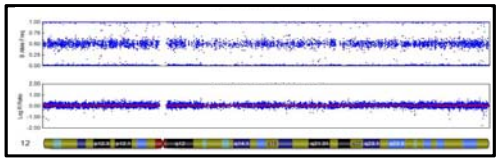
Figure 1

23-Chromosome SNP Microarray

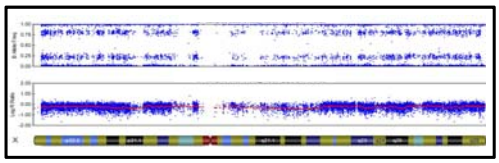
A) Euploid Chromosome 1
[Blastocyst Stage: ICM]



B) Euploid Chromosome 12
[Blastocyst Stage: TE]



C) X/XX Mosaicism
[Taken from Amniocytes-
Not from this Data Set]



D) Monosomy Chromosome 18
[Cleavage Stage]

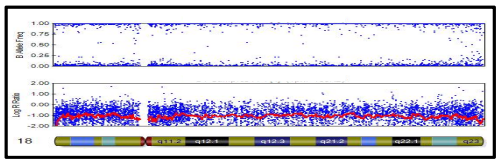


Figure 2

Development of Evaluated Embryos