Origin, mechanisms, and clinical consequences of embryo mosaicism in humans

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Conflict Statement

Dr. Albertini


Has stock options in OvaScience, Inc.

Is a member of the EMD Serono speaker bureau
Outline

Origins of chromosomal and non-chromosomal mosaicism are gametic and zygotic

Mechanisms of mosaicism involve perturbations in coordination between cell cycle, cytoskeleton, and chromatin remodeling

Clinical consequences of mosaicism bear on both our ability to detect and make best decisions on behalf of our patients
Two Notions About Embryo Quality

That from fertilization to Blast, the embryo dictates its developmental fate by expressing tangible/observable properties or byproducts reflecting implantation and term development potential.

Morphology/Morphokinetics/"omes"/PGD-PGS/Mitochondria.....amongst a cohort, those with potential can be differentiated from those lacking potential.

That a zygote’s developmental potential is determined prior to fertilization, that is inherent to gametes, and in no way manifests during the preimplantation window of development.

High quality gametes are few and far between and require pre-selection.
Mosaicism

**noun** mo·sa·ic \mō-ˈzā-ik\
- a decoration on a surface made by pressing small pieces of colored glass or stone into a soft material that then hardens to make pictures or patterns
- the condition of possessing cells of two or more different genetic constitutions
Origins of chromosomal and non-chromosomal mosaicism are gametic and zygotic.
A mitotic error that occurred in the trophectoderm of the blastocyst. The blastocyst is a mosaic; however, the error is isolated to the trophectoderm, while the inner cell mass remains euploid.
Opitz (again)  

Western medicine is being sensitized to the enormous extent of prenatal death in humans at a time when such deaths, occurring after the first missed period, involve to an ever increasing degree wanted pregnancies conceived by women with rising mean maternal age, decreasing mean fertility, and ever greater desire and intention to assure a good pregnancy outcome. Available data suggest that about two-thirds of human ova, embryos, and fetuses fail to reach birth or the end of the first year of life, with infant mortality of 1.06%, stillbirth rate of 8/1,000, abortion rate of about 15%, and death rate around the time of implantation estimated at 34%. Based on limited data on sperm, ova aspirated from Graafian follicles in infertile women, direct observation of a few implanting ova, the low rate of human fecundity, and the high failure rate of in vitro fertilization, it seems reasonable to suppose that about 30% of human ova perish at the time of fertilization and before implantation. Most of this prenatal death is attributable to chromosome abnormalities (aneuploidy and polyploidy), estimated to be present at the beginning of development in about half of all human ova or embryos.
Mosaicism is a common thing

Metazoans rely on cell-cell variation and cope with

Early Embryonic Chromosome Instability Results in Stable Mosaic Pattern in Human Tissues

Mosaicism is the norm not the exception, as is developmental autonomy.
Chromosomal abnormalities

• The inheritance of the parental pathology
  - true inheritance
    (e.g.: parental translocation)
  
  - Chromosomal nondisjunction
during gametogenesis
    (80-85% of causes relate to oocytes
    10-15% - relate to spermatozoa)

• Mitotic errors in the zygote
Critical events during early mammalian development

Get cell cycles in order- finish meiosis and start mitosis-major source of aneuploidies

Erase maternal mRNAs and stockpile maternal proteins (especially TFs for activating embryonic genome)

Orient cell divisions to generate ICM and trophectoderm

Equally distribute organelles (nucleoli, mitochondria, Golgi, centrosomes)
Gametic Contributions

**OOCYTE**

A haploid genome

Mitochondria

All of the organellar precursors to reconstruct 100 cells in a blastocyst

7 cell cycles worth of cyclins, tubulin (circa 64 mitotic spindles and cleavage furrows)

**SPERM**

A compacted haploid, non-functional genome desperately in need of a makeover

A centrosome

A spark (PLCzeta)
Good Gametes Make Good Embryos

Embryogenesis begins with Oogenesis
Concept emerges that embryogenesis is about carving up the pie!
Blastocyst and inner cell mass

Alessia Deglincerti, Gist Croft, and Ali H. Brivanlou
New look at mammalian embryos (Ellenberg, 2016)

https://www.aab.org/images/reg%20form%2017%20w%20rts_rev_4-12-17.pdf
The tip of an iceberg: genetics

Genetics is changing into a very non-mendelian thing
Retains enormous diagnostic potential
May avail molecular nanosurgery for corrective or eradicating lesions
Is dominated presently by phase 3 technology...gene editing
Invading/reshaping practice of ARTs
As are many other entrepreneurs with vivid imaginations and deep pockets to fill
Mechanisms of mosaicism involve perturbations in coordination between cell cycle, cytoskeleton, and chromatin remodeling.
Genomic instability starts with meiosis I and II in human oocytes
Mechanisms Underlying Genomic Instability

- weakened spindle checkpoint signaling
- supernumerary centrosomes
- defects in chromatid cohesion
- abnormal kinetochore-microtubule attachments
- increased spindle microtubule dynamics
Figure 2

interphase production of APC/C-Cdc20 inhibitors

Mad1
Mad2

DNA damage response

Bub1
Bub3
BubR1
BuGZ
Mad2
CENP-A
Mps1
AurB

ciliogenesis
BubR1

insulin receptor endocytosis

Mad2
BubR1

transcription

Bub3
Cdc20

chromosomal rearrangements

Bub1
Bub2
Bub3
Mad2
Mad3 (BubR1 homolog)

interphase progression
Cdc20

integron secretion
Mad1

cell death
Bub1
BubR1

apoptosis activity

cell adhesion and migration
BubR1
Mad1
Strange Cell Cycles

Evidence that human blastomere cleavage is under unique cell cycle control

Human embryos commonly form abnormal nuclei during development: a mechanism of DNA damage, embryonic aneuploidy, and developmental arrest

Courtesy of Shu Hashimoto and Yoshi Morimoto


Multinucleation per se is not always sufficient as a marker of abnormality to decide against transferring human embryos.
Genomic instability is the norm not the exception
Clinical consequences of mosaicism bear on both our ability to detect and make best decisions on behalf of our patients.
Incidence and timing of pregnancy losses: relevance to evaluating safety of early prenatal diagnosis.

Simpson JL.  

Knowing the frequency and timing of pregnancy loss during normal gestation is integral to evaluating the safety of prenatal diagnostic techniques. That preclinical loss rates are high in humans has long been suspected, but in the past decade new data concerning these losses have become available. Cohort studies indicate that many women who show positive beta-HCG assays never show clinical evidence of pregnancy. Cytogenetic abnormalities have also recently been documented in 20% of ostensibly normal in vitro fertilization embryos. All the above are consistent with the sentinel studies of Hertig and Rock, who showed high frequencies of morphological abnormalities in preimplantation embryos.

The “imperfections” of early human development

The shortcomings of our technology
Aneuploidy is a bad thing
Mosaicism

There is a selective advantage of the normal cell line, thus making it possible for a non-mosaic diploid fetus to result from an abnormal conception.

It is possible to form a baby with entirely (or predominantly) normal cells from only a single diploid cell from the inner cell mass of the blastocyst (Lau et al., 1997; Robinson et al., 2002).
Blastomeres are not created equal!
Post-provocateuring

Preimplantation Diagnosis
Preimplantation diagnosis
DNA Microarray Reveals That High Proportions of Human Blastocysts from Women of Advanced Maternal Age Are Aneuploid and Mosaic

- high proportions of aneuploid blastocysts (69.2%)
- including aneuploid TE and euploid ICM, inconsistent anomalies between ICM and TE, or euploid TE cells and aneuploid ICM in the same blastocyst.
- Biopsy from TE in blastocysts does not exactly predict the chromosomal information in ICM if the embryos are aneuploid.
- Some mosaic blastocysts have euploid ICM%
Follow JARG for updates
Early influences

Brief Report

Spatial Separation of Parental Genomes in Preimplantation Mouse Embryos
Wolfgang Mayer, Avril Smith, Reinard Funke, and Thomas Haaf
Max Planck Institut für Molekulare Genetik, 14195 Berlin, Germany

Learning from your mistakes: is aneuploidy so bad, after all?
David F. Alberman
More than morphokinetiics (Prof. Wolfe)
Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts

Original Article

The Effect of Prolonged Culture of Chromosomally Abnormal Human Embryos on the Rate of Diploid Cells

Conclusion: Although mosaicism is frequently observed in blastocysts, the prolonged single culture of blastocysts does not seem to increase the rate of normal cells.

Aneuploidy and early human embryo development

Research:

Zygotes segregate entire parental genomes in distinct blastomere lineages causing cleavage-stage chimerism and mixoploidy

Aspasia Destouni,1,8 Masoud Zamani Esteki,2,8 Maaike Catteeuw,3 Olga Tsuiko,1,4 Efthia Dimitriadou,1 Katrien Smits,5 Anny Kurg,6 Andres Salumets,7,6 Ann Van Soom,3 Thierry Voet,7,9 and Joris R. Vermeesch1,9
“In the absence of any fully euploid biopsies the transfer of mosaics, which may have appeared aneuploid using less sensitive methods, will sometimes result in a viable pregnancy.”

Munne, Grifo and Wells. Mosaicism: “survival of the fittest” versus “no embryo left behind” Fertility and Sterility 2016

http://dx.doi.org/10.1016/j.fertnstert.2016.01.016
Percentages of mosaic, aneuploid and euploid embryos by age groups.
Mosaicism: “survival of the fittest” versus “no embryo left behind”

FIGURE 1

Implantation rates after transfer of euploid embryos are independent of maternal age. * 2,532 cycles of PGD-A by aCGH with known outcome to 8/2015 from Harton et al. (2) and unpublished data; ** 2013 SART data.

Comparison of Lab Results

- Only 2/11 (18.2%) of embryos demonstrated within laboratory congruent results between both laboratory evaluations.
- 4/11 (36.4%) of embryos, on repeat assessment were found to be normal 46, XX or 46, XY embryos.

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### Table 1. Comparison of embryo ploidy between two PGS 2.0 assessments

<table>
<thead>
<tr>
<th>Embryo ID</th>
<th>Biopsy #</th>
<th>Original PGS analysis (all embryos reported as abnormal)</th>
<th>Repeat PGS analysis (multiple biopsies)</th>
</tr>
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<tbody>
<tr>
<td>A1</td>
<td>1</td>
<td>46,XY, -18</td>
<td>Normal 46,XX</td>
</tr>
<tr>
<td>A2</td>
<td>1</td>
<td>Complex aneuploid</td>
<td>XY, +19, -18q</td>
</tr>
<tr>
<td>A3</td>
<td>2</td>
<td></td>
<td>XY, +11, +16, -21</td>
</tr>
<tr>
<td>A4</td>
<td>3</td>
<td></td>
<td>XX, -3</td>
</tr>
<tr>
<td>A5</td>
<td>1</td>
<td>46,XY, +3, -11</td>
<td>XY, -3</td>
</tr>
<tr>
<td>A6</td>
<td>2</td>
<td></td>
<td>Normal 46XX</td>
</tr>
<tr>
<td>A7</td>
<td>3</td>
<td></td>
<td>45,XY, -18</td>
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<tr>
<td>A8</td>
<td>4</td>
<td></td>
<td>Normal 46,XX</td>
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<tr>
<td>B1</td>
<td>1</td>
<td>46,XY, +3, -11</td>
<td>45,XY, -14</td>
</tr>
<tr>
<td>B2</td>
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<td></td>
<td>45,XY, -14</td>
</tr>
<tr>
<td>B3</td>
<td>3</td>
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<td>45,XY, -14</td>
</tr>
<tr>
<td>B4</td>
<td>4</td>
<td></td>
<td>45,XY, -14</td>
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<td>47,XY, +3</td>
</tr>
<tr>
<td>B6</td>
<td>2</td>
<td></td>
<td>47,XY, +3</td>
</tr>
<tr>
<td>B7</td>
<td>3</td>
<td></td>
<td>47,XY, +3</td>
</tr>
<tr>
<td>B8</td>
<td>4</td>
<td></td>
<td>Normal 46,XY</td>
</tr>
<tr>
<td>C1</td>
<td>1</td>
<td>45,XX, -1</td>
<td>Normal 46,XX</td>
</tr>
<tr>
<td>C2</td>
<td>2</td>
<td></td>
<td>Normal 46,XX</td>
</tr>
<tr>
<td>C3</td>
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</tr>
<tr>
<td>C4</td>
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<td>47,XY, +19</td>
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<tr>
<td>C9</td>
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<td>Normal 46,XY</td>
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<td></td>
<td>47, +18</td>
</tr>
<tr>
<td>D3</td>
<td>1</td>
<td>Complex aneuploid</td>
<td>47XY, +10, -15, +16</td>
</tr>
<tr>
<td>D4</td>
<td>2</td>
<td></td>
<td>46,XY, -15, +16</td>
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<tr>
<td>D5</td>
<td>3</td>
<td></td>
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<tr>
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<td>4</td>
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<tr>
<td>D7</td>
<td>5</td>
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<td>46,XY, -15, +16</td>
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<td>46,XX, +14, -15</td>
<td>46,XX, +14, -15</td>
</tr>
<tr>
<td>D9</td>
<td>2</td>
<td></td>
<td>46,XX, +14, -15</td>
</tr>
<tr>
<td>D10</td>
<td>3</td>
<td></td>
<td>46,XX, +14, -15</td>
</tr>
<tr>
<td>D11</td>
<td>4</td>
<td></td>
<td>46,XX, +14, -15</td>
</tr>
</tbody>
</table>

White and shaded areas represent individual embryos.
### Characteristics of Aneuploid embryos which implanted

<table>
<thead>
<tr>
<th>Patient</th>
<th>N Embryos transferred</th>
<th>PGS Result</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>43, XY, -13, -15, -18</td>
<td>Normal birth, 46, XY</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>45, XY, -21</td>
<td>Normal birth, 46, XY</td>
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<tr>
<td>3</td>
<td>2</td>
<td>45, XY, -21&lt;br&gt;46, XX</td>
<td>Normal birth, 46, XY</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Partial 47,XX,17p11.2-pter 45, XY, -22</td>
<td>Normal birth, 46, XX</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>47, XY, +22&lt;br&gt;Partial 45,XY,-1plar-p36,12</td>
<td>Normal birth 46, XY</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>45, XY, -21</td>
<td>Chemical pregnancy</td>
</tr>
</tbody>
</table>
Table 1. Clinical Outcomes of Single Mosaic Blastocysts Transferred.*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Chromosomal Constitution</th>
<th>Mosaicism † percent</th>
<th>Karyotype ‡</th>
<th>Clinical Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>arr(4)x1,(10)x1</td>
<td>40</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>2</td>
<td>arr(6)x1,(15)x1</td>
<td>50</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>3</td>
<td>arr(2)x1</td>
<td>40</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>4</td>
<td>arr(2)x1</td>
<td>35</td>
<td>46,XY</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>5</td>
<td>arr(5)x1</td>
<td>50</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>6</td>
<td>arr(5)x1,(7)x1</td>
<td>40</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
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<tr>
<td>7</td>
<td>arr(11)x1,(20)x3,(21)x3</td>
<td>30</td>
<td>NA</td>
<td>No pregnancy</td>
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<tr>
<td>8</td>
<td>arr(1)x1,(6)x3,(10)x3,(12)x3,(13)x3,(14)x3,(21)x3</td>
<td>50</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>9</td>
<td>arr(3)x1,(10)x3,(21)x3</td>
<td>35</td>
<td>NA</td>
<td>No pregnancy</td>
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<tr>
<td>10</td>
<td>arr(1)x3</td>
<td>50</td>
<td>NA</td>
<td>Biochemical pregnancy † §</td>
</tr>
<tr>
<td>11</td>
<td>arr 9p21.2q34.3(25,609,645-140,499,771)x3</td>
<td>45</td>
<td>NA</td>
<td>Biochemical pregnancy † §</td>
</tr>
<tr>
<td>12</td>
<td>arr(15)x3</td>
<td>30</td>
<td>NA</td>
<td>No pregnancy</td>
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<tr>
<td>13</td>
<td>arr(18)x1</td>
<td>50</td>
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<td>14</td>
<td>arr(18)x1</td>
<td>50</td>
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<td>15</td>
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<td>16</td>
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<td>17</td>
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<tr>
<td>18</td>
<td>arr 10q21.3q26.3(67,216,644-134,326,648)x3</td>
<td>50</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
</tbody>
</table>

* NA denotes not available.
† The approximate percentage of aneuploid cells in the transferred blastocyst is listed (see the Supplementary Appendix).
‡ The karyotype was determined by means of chorionic-villus sampling.
§ Biochemical pregnancy was defined by the presence of a low peak in levels of the beta subunit of human chorionic gonadotropin (β-hCG) (<100 mIU per milliliter), a rapid decrease in the urinary or serum β-hCG concentration, and no substantial delay in the onset of the next menstrual period, but with no detection of an identifiable pregnancy by means of ultrasonographic examination.
New look at human embryos

Very different from mouse and macaque


A developmental coordinate of pluripotency among mice, monkeys and humans.

Nakamura T$^{1,2}$, Okamoto I$^{1,2}$, Sasaki K$^{1,2}$, Yabuta Y$^{1,2}$, Iwatani C$^3$, Tsuchiya H$^3$, Seita Y$^3$, Nakamura S$^3$, Yamamoto T$^{4,5,6}$, Saitou M$^{1,2,4,5}$. 
Four cell mouse embryo contributes to different areas of the blastocyst
Effects of pre-implantation chromosome mosaicism on embryo development and survival

Reversine-treated embryos formed blastocysts but failed to develop past implantation. Increasing the proportion of control blastomeres in the embryo rescued the lethal phenotype. Numbers represent the viability of early postimplantation embryos that had successfully implanted.
DNA Microarray Reveals That High Proportions of Human Blastocysts from Women of Advanced Maternal Age Are Aneuploid and Mosaic

- high proportions of aneuploid blastocysts (69.2%)
- including aneuploid TE and euploid ICM, inconsistent anomalies between ICM and TE, or euploid TE cells and aneuploid ICM in the same blastocyst.
- Biopsy from TE in blastocysts does not exactly predict the chromosomal information in ICM if the embryos are aneuploid.
- Some mosaic blastocysts have euploid ICM%
Summary

**Origins** of mosaicism are both gametic and zygotic, influenced by age and ARTs

**Mechanisms** of mosaicism involve perturbations during gametogenesis and early embryogenesis as a result of alterations in cell cycle checkpoints

**Clinical consequences** of mosaicism are rooted in controversies as wide ranging as technicalities in detection and the likelihood that embryos that could have developed to term are instead being discarded
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Ali Brivanlou, PhD, MD

Videos courtesy of
Jean-Philippe Wolf
Shu Hashimoto
Yoshi Morimoto

And our patients