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Mosaicism in preimplantation human embryos: when chromosomal abnormalities are the norm

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Abstract

Along with errors in meiosis, mitotic errors during post-zygotic cell division contribute to pervasive aneuploidy in human embryos. Relatively little is known, however, about the genesis of these errors or their fitness consequences. Rapid technological advances are helping close this gap, revealing diverse molecular mechanisms contributing to mitotic error. These include altered cell cycle checkpoints, aberrations of the centrosome, and failed chromatid cohesion, mirroring findings from cancer biology. Recent studies are challenging the idea that mitotic error is abnormal, emphasizing that the fitness impacts of mosaicism depend on its scope and severity. In light of these findings, technical and philosophical limitations of various screening approaches are discussed, along with avenues for future research.

Keywords

aneuploidy; mitosis; fertility; preimplantation genetic screening

Aneuploidy arises from both meiotic and mitotic errors

Faithful transmission of genetic information is vital to human development. Aberrant chromosome segregation, either during gametogenesis or somatic division, results in **aneuploidy** (see Glossary), with generally deleterious consequences for cellular and/or organismal fitness [1]. Constitutional aneuploidy arising during egg formation has long been recognized as the primary contributor to female age-related fertility decline, and the molecular mechanisms driving these meiotic errors have been studied in detail [2]. Though their prevalence is occasionally disputed [3, 4], a wide body of evidence indicates that mitotic errors are also common during early embryonic development [5, 6, 7, 8, 9, 10, 11]. These errors produce two or more karyotypically distinct cell lineages within a single embryo—a phenomenon termed chromosomal **mosaicism**. Less is known about the origins of mosaicism or the consequences for embryonic survival, but technological advances in genomics and imaging are shedding new light on these topics. This knowledge is critical for

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improving outcomes of *in vitro* fertilization (IVF) [12], as well as broadening our understanding of natural human fertility.

PGS reveals diverse forms of chromosomal abnormalities

First introduced in the late 1980s, preimplantation genetic screening (PGS) has provided unprecedented insight into the frequency and characteristics of aneuploidy in human embryos. PGS technologies (Box 1) assay the ploidy of DNA from embryo biopsies with the goal of transferring those embryos that test uniformly diploid and thereby improving the likelihood of IVF success [13]. PGS was initially applied to single **blastomeres** biopsied from cleavage-stage embryos, three days after fertilization, but randomized controlled trials failed to show improvements in live birth rates compared to non-PGS controls [14]. More recent PGS protocols recommend testing of 5–10-cell **trophectoderm** (TE) biopsies from day-5 blastocysts, which has proved more effective in part because survival to day 5 indicates developmental competence [15, 16]. In discussing the mechanisms of mitotic error, this review focuses primarily on cleavage-stage embryos, which display the full scope of chromosomal patterns, unbiased by strong selection preceding blastocyst formation [11].

PGS studies have revealed that more than half of *in vitro* cleavage-stage embryos harbor chromosomal abnormalities, ranging from gain or loss of individual chromosomes to complex aberrations affecting many chromosomes simultaneously [5, 6, 7, 8, 9, 10, 11]. Meiotic abnormalities, which overwhelmingly arise in the egg compared to the sperm, increase in frequency from 10–20% to >60% with increasing maternal age (Figure 1) [11]. Our recent PGS study of 28,052 day-3 IVF embryos confirms that in addition to these meiotic abnormalities, more than 25% of cleavage-stage embryos harbor aneuploidies of mitotic origin. Incidence of mitotic aneuploidy thus exceeds meiotic aneuploidy among embryos from women less than 40 years of age (Figure 1) [11]. These data also demonstrate that unlike meiotic errors, mitotic errors are constant with maternal age, show only weak propensities for specific chromosomes, and often affect many chromosomes simultaneously (Figure 1) [11]. It is important to note, however, that the inferred incidence of mosaicism varies substantially across studies [4] (Box 2), and estimates based on error-prone fluorescence *in situ* hybridization (FISH) data may be particularly inflated [3]. Nevertheless, even data based on modern screening platforms demonstrate that mitotic errors are sufficiently frequent that the cleavage-stage genome can be said to exhibit chromosomal instability (CIN), characterized by whole-chromosome and segmental (i.e. sub-chromosomal) gains and losses that are hallmarks of many cancers [17].

Forms of mosaicism can be broadly classified into three groups (Figure 2). The first group, **aneuploid mosaicism**, is defined as a mixture of distinct aneuploid complements (i.e., no diploid cells) and is estimated to affect ~15% of cleavage-stage embryos [10]. Aneuploid mosaicism occurs when a mitotic error arises in an embryo already affected with a meiotic aneuploidy, or alternatively, when a mitotic error in the first embryonic cleavage produces two daughter cells with distinct aneuploidies. This stands in contrast to the more common class of **diploid-aneuploid mosaicism**, which has been estimated to affect approximately half of cleavage-stage embryos [10], though other datasets and approaches imply somewhat lower incidence (Box 2). Diploid-aneuploid mosaics comprise a mixture of normal and

aneuploid cells and generally arise due to mitotic error in a cell descended from a diploid zygote. More rarely, diploid-aneuploid mosaicism may arise when diploidy is rescued by a mitotic error in a cell descended from a diploid zygote [18]. **Ploidy mosaics**, also known as mixoploid embryos, are meanwhile composed of a mixture of cells with different multiples of the haploid chromosome number ($n = 23$, for humans). These may include any combination of haploidy (n), diploidy ($2n$), and polyploidy ($>2n$) within the same embryo. While early PGS studies documented a high frequency of diploid-tetraploid mosaics at the cleavage stage (~15% [19]), some modern screening platforms are incapable of detecting this phenomenon, hindering its quantification.

A final class of mosaicism—not mutually exclusive from the groups above—is defined not by the presence or absence of diploid cells, but by its characteristic chromosomal signature. **Chaotic mosaic** embryos display a severe pattern of irregularity with multiple chromosomes affected and each cell possessing a seemingly random chromosome set (Figure 2). This form of mosaicism remains the least understood despite its prevalence at the cleavage stage (~25% [19], though criteria and estimates vary across studies).

Despite their common origins in mitosis, the chromosomal characteristics, molecular etiology, and fitness consequences of each class of mosaicism are distinct, and will be discussed in more detail here.

Molecular factors contributing to mitotic error

Relaxed control of the cell cycle

Cleavage-stage development is characterized by cell division with little cell growth, such that the 16–32-cell morula occupies approximately the same volume as the original zygote. As the embryonic genome is largely inactive until the 4–8-cell stage, these initial cleavage divisions proceed under control of maternal RNAs and proteins provided in the ooplasm [20, 21]. Oscillation of cyclins and cyclin-dependent kinases (Cdks)—essential regulators of cell cycle progression—thus occurs through mechanisms independent of transcription and is significantly dampened [22]. Recent studies have used single-cell expression profiling to identify maternal-effect genes differentially expressed in aneuploid/euploid and arresting/normally-developing embryos [23, 24]. Intriguingly, zygotes destined to arrest based on mechanical properties exhibited downregulation of genes involved in cell cycle regulation and mitotic checkpoint control, including *CDK1*, *CDK2*, *CHEK2*, *BUB1*, *BUB3*, *BRCA1*, and *ATR* [24].

Several previous studies have also indicated that cleavage-stage embryos exhibit weakened mitotic checkpoints, potentially facilitating rapid cleavage divisions [25, 26, 27]. These include the G1 and G2 checkpoints [25], corroborated by the finding that transcripts encoding essential checkpoint proteins RB and WEE1 are not detected in 8-cell embryos [26]. In mature cells, these checkpoints serve to detect damaged or incompletely replicated DNA, signaling to effector proteins that arrest the cell cycle and initiate apoptosis or DNA repair [28]. When checkpoints are weakened, it is possible for the cell cycle to proceed to M phase before chromosomes are fully replicated, aligned on the mitotic spindle, or cleared of DNA damage. Time-lapse imaging studies demonstrate that under these permissive

conditions, mitotic fidelity is threatened by slight deviations in the timing of key cell cycle events [29]. Embryos that diverge in the precise timing of specific parameters (duration of first cytokinesis; time between first, second, and third mitoses) exhibit frequent anaphase lag, whereby cytokinesis occurs before chromosomes are properly aligned and attached to the mitotic spindle such that they are excluded from the reforming nucleus [29]. Based on an observed excess of chromosome loss compared to chromosome gain, anaphase lag has been implicated as the most common mechanism of mitotic error in preimplantation embryos, explaining more than 50% of all cases [30]. This conclusion was derived from FISH data, however, in which failed hybridization which may be falsely attributed to chromosome loss. Nevertheless, our recent study based on SNP microarray data similarly revealed a ~4-fold excess of chromosome loss compared to chromosome gain among samples with putative mitotic errors [11]. Notably, however, this effect was largely driven by a subset of chaotic samples exhibiting loss of multiple chromosomes, including both maternal and paternal homologs [11].

The canonical cell cycle also includes a **spindle assembly checkpoint** (SAC), which monitors chromosome segregation during M phase, stalling progress until all chromosomes are stably attached to microtubules at their kinetochores. Mutational inactivation of SAC components is known to cause CIN and aneuploidy in certain cancers, with affected cells exiting mitosis despite microtubule disruption [31]. This checkpoint appears similarly weakened [25], though still present [32], during cleavage-stage development in humans. Supporting this hypothesis, several key components of the SAC, including *BUB1* and *MAD2*, show significantly greater expression in hatched blastocysts than at earlier cleavage stages [33]. Diminished SAC oversight may permit erroneous kinetochore-microtubule attachments that would not otherwise be tolerated. Such erroneous attachments include monotelomeres—failed attachment of one sister chromatid to the mitotic spindle and syntelomeres—attachment of both sister chromatids to the same spindle pole. Even an operational SAC cannot detect certain attachment defects such as merotelomeres—the binding of a single kinetochore to microtubules emanating from both spindle poles [17]. Prevalent in cancers, erroneous attachments cause delayed migration toward the spindle poles, again increasing the frequency of anaphase lag [34].

Rather than undergoing immediate degradation, lagging chromosomes are frequently encapsulated in micronuclei—small nucleus-like bodies that are separate from the main nucleus [29]. Here, chromosomes are susceptible to severe DNA damage that may compromise their ability to form functional kinetochores and undergo proper segregation [35]. Forms of DNA damage include double-strand breaks, which may be repaired by error-prone mechanisms such as **non-homologous end-joining**, contributing to mosaic patterns of translocation and other segmental rearrangements commonly observed in cleavage-stage embryos [7]. Replication of chromosome segments followed by end-to-end fusion can produce chromosomes possessing two centromeres. Such chromosomes are susceptible to **breakage-fusion-bridge cycles**, generating a cascade of complex rearrangements in descendent cells [36].

Chromosomes encapsulated in micronuclei are also susceptible to the catastrophic phenomenon of **chromothripsis**, whereby chromosomes suffer extensive segmental

deletions and rearrangements. Studies in cancer cell lines suggest that chromothripsis is triggered by damage occurring during DNA replication and/or by rupture of the micronucleus nuclear envelope [37]. Chromatids are shattered in response to this damage, with fragments randomly segregated to each daughter cell, then stitched back together in random order and orientation [37]. This produces a characteristic pattern of segmental oscillation between two ploidy states (usually disomy and trisomy) [37]. Chromothripsis may also induce translocations when multiple chromosomes are partitioned into a single micronucleus [37]. In subsequent mitoses, micronuclei can be reabsorbed into the main nucleus or fuse with neighboring blastomeres, generating complex patterns of mosaicism [29]. Micronuclei may also be ejected from the blastomere during a process called cellular fragmentation, whereupon their chromosomes are degraded [38].

Relaxed cell cycle checkpoints may also contribute to ploidy mosaicism by occasionally permitting cells to skip M phase. This leads to DNA replication without cell division, producing a tetraploid cell. Mosaic tetraploidy is observed in <1% of molar pregnancies [39], but may be more frequent at earlier embryonic stages. Indeed, some studies have suggested tetraploidy to be a normal feature of blastocyst-stage embryos [19]. Tetraploidy may be transient, however, when accompanied by centrosome amplification, which could induce spindle multipolarity in subsequent mitoses (see following section).

The canonical cell cycle is ultimately reestablished at the **mid-blastula transition** (MBT), concomitant with a major wave of **embryonic genome activation** (EGA) around the 4–8 cell stage [20, 21]. Studies in *Drosophila* and *Xenopus* models suggest that MBT onset is partially regulated by the increasing ratio of nuclei to cytoplasm (N:C ratio), as embryos with less than 75% of the typical N:C ratio require additional cycles before triggering MBT-associated events [40]. EGA revealingly coincides with an increase in mitotic fidelity in human embryos. Screening of all cells from 114 digynic tripronuclear (3PN) embryos—a mitotically-stable model of normal embryos—demonstrated that the frequency of chromosome mis-segregation decreases from >25% at the first mitotic cleavage to <5% after the 8-cell stage [41]. Similar declines have been observed during the initial mitotic cleavages of diploid zygotes [42].

Aberrations of the centrosome and mitotic spindle

The fundamental role of the centrosome in ensuring mitotic fidelity has been recognized since the pioneering work of Theodor Boveri in the late 19th century [43]. As the microtubule organizing center (MTOC) that coordinates bipolar spindle formation, growing evidence suggests that aberrations of the centrosome frequently contribute to catastrophic mitotic errors in cleavage-stage embryos, underlying many cases of chaotic mosaicism. Tightly integrated with the cell cycle, centrosomes undergo an intricate process of duplication, elongation, maturation, disjunction, and movement, such that each daughter cell inherits one centrosome, composed of two **centrioles** and a dense cloud of proteins called the pericentriolar matrix (PCM) [44].

Because most aneuploidies arise during oogenesis, comparisons between centrosomal participation (or lack thereof) in mitosis and meiosis may be instructive. Centrosomes are disabled and degenerated during both male and female meiosis, in a process termed

centrosome reduction [45]. This process is incomplete in sperm, which lose most centrosomal proteins, but retain a pair of centrioles that are transmitted to the zygote during fertilization [45]. Eggs, meanwhile, lose their centrioles completely, but retain centrosomal mRNAs and proteins including γ -tubulin and PLK4. In many species, the role of the centrosome is therefore replaced by a functionally equivalent acentriolar MTOC during female meiosis [46]. Intriguingly, recent work demonstrates that humans are an exception; human oocytes organize the mitotic spindle in the absence of any detectable MTOC, with chromosomes themselves serving as sites of microtubule nucleation [46]. This distinction was recently hypothesized to explain the inherent instability of the oocyte meiotic spindle, with 44% of spindles passing through an apolar stage and 38% passing through a prolonged multipolar stage prior to establishing bipolar orientation [46]. Strikingly, 72% of oocytes with transient multipolar spindles exhibited meiotic anaphase lag caused by a high frequency of merotelic attachments, mirroring known mechanisms of mitotic chromosome mis-segregation [46].

Upon fertilization, the sperm's proximal centriole nucleates microtubules to form the sperm aster, which guides the male and female pronuclei toward one another and recruits centrosomal components from the oocyte-contributed cytoplasm [47]. During this stage, as well as the S phase of all subsequent cell cycles, the centrioles are duplicated, with a single daughter procentriole forming at the base of each mother centriole [47]. The mitotic kinase PLK4 localizes to the mother centriole and serves as the master regulator of the duplication process. The quantity of PLK4 is tightly regulated at this stage, as even modest overexpression can cause multiple procentrioles to assemble in a rosette-like structure [48]. When duplicated faithfully, centriole pairs mature to form centrosomes, then separate to establish the two poles of the mitotic spindle. Excess centrioles, however, cause the formation of extra centrosomes, which in turn may organize multipolar mitotic spindles (e.g., tripolar spindles, in the case of three centrosomes; Figure 3). When mitosis proceeds under these circumstances, chromosomes randomly segregate to each daughter cell, generating chaotic patterns of aneuploidy. Alternatively, experiments in cancer cell lines reveal that supernumerary centrosomes may cluster to form bipolar spindles [34]. Even with a bipolar spindle, however, the presence of extra centrosomes predisposes chromosomes to aberrant kinetochore-microtubule attachments (monotelic, syntelic, merotelic), causing frequent anaphase lag [34] (Figure 3).

Our recent genome-wide association study (GWAS) suggests one factor potentially influencing the frequency of spindle abnormalities. Using PGS data from 20,768 day-3 embryos and genotypes from 2362 IVF patients, we tested for associations between maternal genetic variants and incidence of mitotic-origin aneuploidy. A strong association was detected with common (~30% global minor allele frequency) variants defining a ~600 Kb haplotype spanning the master centrosomal regulator *PLK4*, along with several other genes [49]. Embryos from patients with high-risk genotypes suffer diminished rates of blastocyst formation [49, 50], consistent with lethality of the chaotic aneuploid phenotype. Future work will be necessary to confirm the causal role of *PLK4* and establish the molecular mechanism driving this signal, but the observed patterns of chromosomal chaos (multiple chromosome loss) coupled with the known capacity of *PLK4* to induce aberrant spindle formation are strongly suggestive. Because the associated variant is common, this hypothesis could be

tested by contrasting the frequency of spindle abnormalities in cleavage-stage IVF embryos from mothers possessing different *PLK4* genotypes.

An alternative route to centrosome excess is dispermy (i.e., two sperm) fertilization—the subject of Boveri’s studies of sea urchins [51] (Figure 3). Dispermy is common in conventional IVF, affecting approximately 10% of fertilized oocytes [52], but is virtually eliminated by intracytoplasmic sperm injection (ICSI), which is now used in most IVF cycles in the United States. While also inducing tripolar mitosis (also known as trichotomic mitosis or direct unequal cleavage), dispermy fertilization should be distinguishable from other mechanisms of centrosome amplification based on the patterns of chromosomal chaos that it generates. Namely, dispermy also produces a trippronuclear (3PN) zygote with three sets of chromosomes. In contrast, tripolar mitosis in a 2PN zygote, will produce daughter cells with random hypodiploid karyotypes. Imaging of preimplantation embryos using both fixed- and live-cell microscopy supports a high frequency of multipolar mitosis during the cleavage stages, even for normally-fertilized 2PN zygotes [53, 54, 55]. The largest study to date, for example, observed that 26.1% of 21,261 2PN embryos exhibited tripolar mitosis [56], with previous studies obtaining similar, albeit slightly lower estimates (18% [54]; 17% [57]).

Defects in chromosome cohesion

Both meiosis and mitosis critically depend on chromatid cohesion for accurate chromosome segregation. In both cases, cohesion is mediated by protein complexes called cohesins that form ring structures to link the sister chromatids, facilitating their **amphitelic** attachment to spindle microtubules. Cohesins are composed of two structural maintenance of chromosome subunits (SMC1 α , SMC1 β , SMC3), one stromal antigen subunit (STAG1, STAG2, STAG3), and one kleisin subunit (RAD21, RAD21L, REC8) [58]. While some cohesin subunits are specific to meiosis (SMC1 β , STAG3, RAD21L, REC8), most are shared [58]. Understanding the mechanisms of cohesion dysfunction contributing to meiotic error may thus provide clues about related pathways in mitosis [59].

Cohesion errors frequently impact female meiosis, which begins during fetal development, but enters a decades-long stage of **dictyate arrest** lasting until ovulation [2]. Notably, meiotic cohesin proteins show little to no turnover during the dictyate stage [60]. Upon resumption of meiosis I, homologous chromosomes dissociate triggered by the separase-mediated cleavage of cohesin subunit REC8 along the chromosome arms [2]. This is followed by separation of sister chromatids during meiosis II, initiated by the cleavage of centromeric cohesin. Nondisjunction—once considered the leading cause of meiotic error—refers to the phenomenon whereby homologous chromosomes fail to separate during meiosis I or sister chromatids fail to separate during meiosis II. This may cause either chromosome gain or loss depending on whether homologs/chromatids segregate to the oocyte or the polar body, respectively. More recent studies, however, indicate that **premature separation of sister chromatids** (PSSC) and **reverse segregation** are more common error mechanisms, both of which involve the loss of cohesion between sister chromatids, potentially due to deterioration of cohesin proteins during the dictyate stage [61]. Indeed, studies in mice [62] and humans [63] demonstrate that chromosome cohesion in the oocyte weakens with

increasing maternal age, providing one mechanism to explain the age-associated increase in aneuploidy.

Chromosome dynamics in mitosis closely resemble meiosis II. As such, nondisjunction and PSSC can also afflict mitotic divisions [7, 64]. In mitosis, these errors generate a pattern of reciprocal gain/loss of the affected chromosome in the two daughter cells. Unlike meiosis, mitotic cohesion must be established and disestablished continually, in close coordination with the cell cycle. To respond to these unique demands, even the first mitotic division of the zygote excludes meiosis-specific subunits of cohesin, suggesting a complete and rapid transition to mitotic subunits at the time of fertilization [60].

Mutations in cohesin subunits and their regulators have been shown to increase the rate of mitotic error in mature somatic cells. Experimental inactivation of securin, for example, causes whole chromosome losses due to incomplete cohesion dissolution [65]. Overexpression of separase, meanwhile, causes frequent PSSC, contributing to aneuploidies involving both chromosome losses and chromosome gains [66]. Knockout of several cohesion genes (including *SMC3*, *RAD21*, *STAG1*, *NIPBL*, and *PLK1*) causes early embryonic arrest in mouse models [67] while mutation and dysregulation of these genes contributes to Cornelia de Lange syndrome, Roberts syndrome, and several types of cancer [67, 68, 69]. Notably, zygotes predicted to give rise to arrested embryos (based on mechanical parameters) exhibit differential expression of several cohesion genes including *SMC3* and securin, providing preliminary evidence of their importance in maintaining mitotic fidelity during the cleavage stage [24]. Given these observations, the role of cohesion defects in contributing to embryonic mosaicism deserves further attention.

Ovarian stimulation and IVF culture conditions

The finding of widespread mosaicism among IVF embryos raises questions about the relevance of this phenomenon to non-IVF pregnancies in the general population. While preimplantation development *in vivo* remains a “black box”, some studies have indirectly addressed these questions by examining the incidence of aneuploidy across varying ovarian stimulation protocols, IVF culture conditions, and patient populations. One of the first and most extensive studies of this type found significant variation in rates of mosaicism across four different IVF centers (11% to 52%), as well as differences within an IVF center after a change in the hormonal stimulation protocol [70]. Several recent studies, however, seemingly contradict these conclusions, detecting no significant impact of hormonal stimulation on rates of chromosomal abnormalities [71, 72]. While the reason for this discrepancy remains unclear, the recent studies are based on larger patient samples and employ improved PGS methods. A second line of evidence supporting the relevance of IVF data to the broader population stems from the observation that when controlling for maternal age, rates of aneuploidy among embryos derived from fertile egg donors are not significantly different from embryos derived from IVF patients [11]. A third line of evidence derives from the common belief that natural embryonic mortality is high (often quoted as 70% or greater [73]). Given that aneuploidy is the leading cause of pregnancy loss, a substantial proportion of the mortality may be attributable to a high incidence of aneuploidy. A recent review, however, criticized representations of natural embryonic mortality as exaggerations [73],

many tracing to a single flawed study from 1959 [74]. Based on updated data and analysis, natural embryonic mortality may be closer to 40–60% [73]. The application of biological conclusions from IVF to the situation *in vivo* should thus be viewed with skepticism until more data are available from naturally conceived embryos and fertile individuals.

Consequences of mosaicism for embryonic development

Mitotic error and chromosomal mosaicism are increasingly recognized as pervasive features of preimplantation development. The salient question thus becomes: under what circumstances does it matter? While progress on this question has been gradual, recent studies are providing inroads, with implications for embryo selection based on PGS.

The most direct way to investigate the developmental consequences of mosaicism is to contrast its frequency and characteristics across developmental time points. One of the first studies to utilize this approach found that accumulating mitotic errors cause the frequency of mosaicism to increase from the cleavage (49% at the 5–8-cell stage) to blastocyst stage (91%), but that the proportion of aneuploid cells within mosaic embryos decreases over the same timespan (40% at the 5–8-cells stage vs. 22% in the blastocyst) [19]. Several subsequent studies support this conclusion while also revealing a decline in the severity of aneuploid karyotypes [75, 76, 77, 11]. Specifically, the rate of decline from the cleavage to blastocyst stage is directly correlated with the number of chromosomes affected [77, 11]. In line with this observation, cleavage-stage embryos exhibit a much greater incidence of complex (i.e., chaotic) abnormalities (25%) than embryos at the blastocyst stage (10%) [11].

Three models, which are not mutually exclusive, could potentially explain the increasing frequency of diploid cells from the cleavage to blastocyst stage. The first is early mortality selecting against embryos with high-level mosaicism (Figure 4, Key Figure). Indeed, multiple studies support this model by demonstrating that arrested embryos are strongly enriched for chromosomal mosaicism, with most of the mortality occurring prior to blastocyst formation [19, 78, 79, 80]. Meanwhile, mosaic embryos that do not arrest prior to blastocyst formation suffer diminished rates of implantation [81]. Considering these observations, guidelines for embryo selection based on PGS have traditionally recommended against the transfer of mosaic embryos. In practice, mosaic biopsies were often classified as either normal or abnormal based on unstandardized criteria.

These guidelines were recently called into question by high-profile reports of euploid live births after deliberate transfer of mosaic blastocysts [82, 83]. Assuming technical accuracy of the PGS results, these reports highlight the potential of some mosaic embryos to “self-correct”, effectively purging themselves of mosaicism. These observations are consistent with a second model of clonal depletion (Figure 4), whereby aneuploid cells are actively eliminated or fail to propagate effectively within mosaic embryos, mitigating their deleterious impact. A recent study using a mouse model provides empirical support for this hypothesis, demonstrating that mosaic embryos can be rescued by diploid cells if present in sufficient frequency (> 50%) [84]. This experiment also revealed that the fate of aneuploid cells depends on the type of cell affected. Aneuploidies occurring in the blastocyst were better tolerated—though still deleterious—when isolated to the extraembryonic TE tissue

[84]. Mitotic errors affecting the **inner cell mass** of mouse embryos were more consequential and eliminated by **apoptosis** prior to implantation [84]. While enlightening, the conclusions derived from mouse studies should not be assumed to apply directly to humans, as 80% of mouse embryos form blastocysts *in vitro* compared to only 30–50% of human embryos [85]. If applicable to humans, however, lineage-specific clonal depletion could explain the observation of **confined placental mosaicism** (CPM) in chorionic villus samples taken in later developmental stages [86]. Importantly, multiple studies have demonstrated a lack of preferential allocation of aneuploid cells to the TE, indicating that CPM arises through passive mechanisms [87, 88, 89].

A final model potentially contributing to the decreasing frequency of aneuploid cells is that of trisomic/monosomic rescue, whereby an aneuploid cell line (of either meiotic or mitotic origin) is corrected by a mitotic error that rescues diploidy (Figure 4). Trisomic rescue involves mitotic chromosome loss, while monosomic rescue involves mitotic chromosome gain. Though plausible, the incidence of this phenomenon is not well quantified, as many proposed cases (e.g., [18]) do not rule out the possibility that mosaicism, sampling error and/or clonal depletion could explain diploidy in an embryo with a previously-detected aneuploidy. One potential outcome of trisomic or monosomic rescue is uniparental disomy (UPD), in which mitotic error produces a diploid cell containing two maternal or two paternal chromosomes. While often compatible with live birth, UPD is associated with a range of clinical disorders depending on the specific chromosomes affected [90]. Tellingly, however, UPD is only detected in 3% of day-3 embryo biopsies and less than 1% of day-5 TE biopsies [11], consistent with previous reports [88]. Even though trisomic rescue only generates UPD one-third of the time [91], these findings argue that this correction mechanism is relatively rare.

Further work will be necessary to determine the relative contributions of embryonic mortality, clonal depletion, and trisomic/monosomic rescue to patterns observed in humans, as well as the forms of mosaicism that are subject to each phenomenon. This will require extensive monitoring and PGS applied to embryos that are re-biopsied throughout preimplantation development.

In the meantime, PGS-guided embryo selection protocols should be amended to reflect uncertainty in the developmental fate of mosaic embryos. Recent position statements from COGEN (<http://www.ivf-worldwide.com/cogen/general/cogen-statement.html>) and PGDIS (http://www.pgdis.org/docs/newsletter_071816.html) take important steps in this direction by acknowledging the phenomenon of mosaicism and providing suggestions for prioritization based on specific chromosomal characteristics. Somewhat counterintuitively, however, these guidelines recommend preferential transfer of mosaic embryos with inviable aneuploidies to minimize the risk of transferring a viable embryo harboring an aneuploidy known to confer a developmental disorder. While such prioritization schemes make sense in the case of meiotic abnormalities—the consequences of which are well described—their utility may be limited for mosaic embryos given the current state of knowledge. Indeed, mosaic aneuploidies involving nearly every chromosome can be compatible with live birth [92], but are associated with a range of clinical disorders [93].

Concluding remarks

Genetic screening of IVF embryos is one of few examples in which genomic technologies are being directly applied in the clinic. Yet the success rate of “euploid” single embryo transfer is still less than 50%, partially due to undetected mosaicism [94]. Emerging studies are illuminating the karyotypic complexity of mosaic embryos and the diverse molecular pathways that produce them.

While many forms of mosaicism (e.g., chromosomal chaos) cause early embryonic mortality, other forms are viable later into later development, with some mosaic embryos proceeding to healthy live birth. This complicates embryo selection approaches based on PGS. The decision whether to transfer a mosaic blastocyst is particularly relevant when no euploid embryo is available for transfer, as is the case for many patients of advanced maternal age [95]. These questions will only grow as more sensitive screening methods are implemented and more cases of potentially viable low-level mosaicism are detected.

Given the high financial and emotional costs of failed IVF, research to delineate the characteristics of mosaicism that are compatible with healthy live birth is desperately needed. Now underway, the systematic tracking of outcomes (e.g., miscarriage, developmental disorder, healthy live birth) after transfer of aneuploid and mosaic embryos provides an important step in this direction [83]. The ability to resolve these questions has strong implications for the reliability of PGS as a guiding method for embryo selection. More generally, elucidating the molecular pathways contributing to embryonic mosaicism can help reveal risk factors influencing human fertility, paving the way for future therapies.

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Glossary

amphitely

correct orientation of sister kinetochores toward opposite poles of the mitotic spindle

anaphase

the phase of mitosis in which replicated daughter chromosomes are pulled apart, toward the spindle poles

aneuploidy

the presence of an abnormal number of chromosomes in a cell that is not a multiple of the haploid chromosome set (23, in humans) **aneuploid mosaicism**: the presence of two or more aneuploid cell lines and no diploid cells within an embryo

apoptosis

programmed cell death resulting in elimination of the cell from the embryo

blastomere

a single cell of a cleavage-stage embryo

breakage-fusion-bridge cycle

a mitotic error mechanism whereby chromosome segments fuse to create dicentric chromosomes with two centromeres. During cell division, each centromere is pulled in the opposite direction causing chromosome breakage at a new position. After replicating, these chromosome segments can themselves fuse, initiating a new cycle

centriole

a small, cylindrical component of the centrosome that helps organize the mitotic spindle

centrosome

the organelle that organizes mitotic cell division and chromosome segregation

chaotic mosaicism

a common form of mosaicism in cleavage-stage IVF embryos characterized by seemingly random chromosome complements in each cell

chromosomal instability

a phenomenon in which cells mis-segregate chromosomes at an increased rate, often associated with cancer. This can include gains or losses of whole or partial chromosomes, as well as chromosome rearrangements

chromothripsis

a process in which chromosomes are shattered then stitched back together in random order and orientation generating complex patterns of segmental deletion and rearrangement

confined placental mosaicism

discrepancy between the karyotype of a placental biopsy and the karyotype of the fetus. This form of mosaicism is generally compatible with healthy live birth, when aneuploidy is confined to trophoblast cells

diploid-aneuploid mosaicism

the presence of both aneuploid and diploid cells within the same embryo

embryonic genome activation

the onset of expression of genes encoded by the embryonic genome. In humans, this process occurs at the 4–8 cell stage (day 3, post-fertilization)

***in vitro* fertilization**

an assisted reproductive technology in which the egg and sperm are combined in the laboratory. After laboratory culture, the resulting embryo is then transferred to the uterus, usually on day 5 or 6. **inner cell mass**: the cluster of pluripotent cells of the blastocyst that will give rise to the embryonic tissues.

metaphase

the phase of mitosis in which chromosomes align in a plane at the cell equator and attach to spindle fibers

mid-blastula transition

the stage of embryonic development during which the embryonic genome is activated, the cell cycle lengthens, and cell division becomes asynchronous

mosaicism

the presence of two or more cell lineages with distinct numbers of chromosomes within a single embryo

non-homologous end-joining

a DNA repair pathway whereby broken ends of chromosomes are ligated without the use of homologous sequence as template

ploidy mosaicism

the presence of any combination of haploid, diploid, and polyploid cells within the same embryo

premature separation of sister chromatids

an error mechanism in which sister chromatids dissociate before anaphase, such that they segregate independently, potentially into the same daughter cell

trophectoderm

the outer cell layer of the blastocyst, from which PGS biopsies are often obtained. These cells are essential for interacting with the uterus during implantation and will go on to form the placenta

reverse segregation

a newly described meiotic segregation pattern by which sister chromatids prematurely segregate at meiosis I, followed by either correct or errant segregation of non-sister chromatids during meiosis II

spindle assembly checkpoint

the cell cycle checkpoint that prevents the onset of anaphase until all chromosomes are accurately attached to the mitotic spindle

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Box 1: PGS platforms for detection of aneuploidy

Early implementations of PGS utilized fluorescence *in situ* hybridization (FISH) to screen blastomeres from day-3 cleavage-stage embryos [96]. FISH uses probes labeled with multicolored dyes to hybridize to DNA of chromosomes in interphase nuclei. Ploidy is then assessed by counting chromosomes under a microscope that excites the dyes and causes them to fluoresce. Due to its low throughput, low specificity, and inability to screen many chromosomes simultaneously (maximum of 12), this approach has largely been supplanted by superior platforms that screen all chromosomes simultaneously (comprehensive chromosome screening; CCS).

Current leading CCS technologies include array comparative genomic hybridization (aCGH), single nucleotide polymorphism (SNP) microarray, quantitative real-time polymerase chain reaction (qPCR), and next-generation sequencing (NGS) [97]. aCGH and SNP microarray approaches depend on whole genome amplification (WGA) of DNA extracted from embryo biopsies, followed by hybridization of the DNA to thousands of genome-wide probes. For aCGH (and some SNP microarray approaches), aneuploidies are then detected by comparing quantitative hybridization signals to those observed for a euploid control sample. Alternatively, when parental samples are available, SNP microarrays facilitate the detection of aneuploidy based on inferred transmission of individual parental haplotypes [98, 99]. Detection of aneuploidy using NGS, meanwhile, relies on imbalances in mapped read depth across chromosomes or in comparison to a euploid reference sample [100]. Unlike other contemporary approaches, qPCR does not require WGA and thereby avoids some associated technical artifacts such as allelic dropout [101]. Quantitation is achieved through the hybridization of fluorescent dye and determination of the number of PCR cycles required to achieve a threshold value of fluorescence.

Different screening platforms have specific advantages and disadvantages related to cost, turnaround time, and resolution for various forms of aneuploidy (reviewed in [102, 103]). NGS, for example, has high sensitivity for detecting low-level mosaicism, but is comparatively expensive [102, 103]. Dense SNP arrays are also relatively expensive, but provide the advantage of detecting a wide spectrum of aneuploidies, including UPD and segmental errors, as well as potentially inferring the parental origin of each chromosome copy [102, 103]. aCGH has achieved widespread clinical use, but has limited capacity to detect low-level mosaicism [103]. qPCR, meanwhile, provides rapid turnaround (~4 hours), but cannot detect most sub-chromosomal abnormalities due to the use of sparse genomic markers [102, 103].

Box 2: Approaches and limitations of detecting and quantifying mosaicism

While initial reports of widespread mosaicism were scrutinized based on the unreliability of the FISH platform [3], later studies using improved screening methods (Box 1) generally confirmed high rates of mosaicism. Controversy remains decades later, however, as current estimates of the preimplantation incidence of mosaicism range from 4–90% [4].

In addition to changes across embryonic stages (discussed in the main text), many of these discrepancies can be attributed to the conflation of two different frequencies 1) the frequency of mosaic embryos (i.e. those with at least one aneuploid cell) and 2) the frequency of aneuploid cells within mosaic embryos (sometimes referred to as the “level” or “grade” of mosaicism). The former frequency increases through preimplantation development, while the latter frequency decreases [10].

Discrepancies may also stem from the three different approaches used to detect mosaicism. The first approach contrasts PGS results obtained from repeated biopsies of the same embryo. While discordant biopsies provide direct evidence of mosaicism, this approach has been criticized based on the argument that even a low rate of technical false positives can erroneously inflate estimates of mosaicism when comparing many biopsies [4, though see 104]. A second approach depends on the presence of mosaicism within a multi-cell embryo biopsy. Mosaicism is detected by identifying PGS signals that are intermediate between those expected for uniform euploidy or aneuploidy (Figure I) [105, 106, 107]. Limits of detection may vary across platforms, however, and high-coverage NGS is likely to have superior sensitivity for detecting low-level mosaicism due to its broad dynamic range [107, though see 108]. A final approach infers the existence of mosaicism based on detection of mitotic-origin aneuploidy. Meiotic trisomies can be distinguished by the presence of both parental homologs of a given chromosome [98, 99]. Extra or missing paternal homologs, meanwhile, are highly suggestive of mitotic error [11], as meiotic are rare in sperm cells [109]. Certain aneuploidies (e.g., chaotic mosaics) simultaneously involve both maternal and paternal homologs, providing increased confidence of mitotic origin [11].

By depending on a single biopsy, the latter two approaches described above may systematically underestimate the incidence of mosaicism due to sampling error. As blastomere biopsies comprise only one cell and TE biopsies comprise ~5–10 cells, uniform diploid or aneuploid cell populations will often be sampled from mosaic blastocysts—especially low-level mosaics. This likelihood is compounded by the fact that cells from the same lineage are not evenly mixed throughout the embryo, but are grouped in spatial proximity to one another, especially during preimplantation stages [110]. A strong role of sampling error is supported by recent studies documenting high levels of discordance in repeated biopsies from the same embryos [111].

Outstanding Questions

- What are the rates of mosaicism and how do they vary throughout early development?
- What are the predominant molecular mechanisms contributing to mitotic error?
- What genetic and environmental factors predispose embryos to mitotic error?
- How does the incidence of mosaicism in IVF embryos compare to the situation *in vivo* for naturally conceived pregnancies?
- Which forms of mosaicism are compatible with healthy live birth? Which are associated with mortality, birth defects, or later disease?

Trends

- Along with meiotic errors, frequent mitotic errors occurring after fertilization contribute to prevalent aneuploidy in human embryos. New technological innovations are enabling sensitive detection and characterization of the complex patterns of mosaicism that ensue.
- Recent studies are revealing a surprising variety of molecular mechanisms that may contribute to chromosomal mosaicism in human embryos. Many of these mechanisms are known to contribute to cancer formation, including cell cycle dysregulation, defective chromatid cohesion, and centrosome overduplication.
- The fitness consequences of mosaicism are not as clear as those of meiotic-origin aneuploidy. While associated with negative pregnancy outcomes, some mosaic embryos are viable, and low-level mosaicism may be a normal feature of human development.
- Future research should focus on understanding the risks associated with various forms of mosaicism to guide implementation of genetic screening approaches.

Figure 1. Characteristics of day-3 blastomeres with putative meiotic or mitotic errors

Data from [11]. Trisomies including both maternal or paternal homologs are classified as maternal or paternal meiotic errors, respectively, and arise through non-disjunction or PSSC. Mitotic errors, meanwhile, are identified as those non-meiotic errors involving paternal homologs (including those that also involve maternal homologs). Note that some forms of aneuploidy (e.g., maternal monosomy) are ambiguous in origin and thus cannot be classified as meiotic or mitotic based on these data. (a) Incidence of various forms of aneuploidy with

respect to maternal age. (b) Per-chromosome incidences of meiotic- and mitotic-origin aneuploidies.

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Figure 2. Forms of mosaic aneuploidy affecting preimplantation human embryos

For each example, deviations from diploidy are indicated with the number of extra or missing maternal or paternal homologs.

Figure 3. Centrosomal defects can induce mitotic aneuploidy via multiple routes

Oocytes fertilized by two sperm or cells that experience centriole amplification will possess more than two centrosomes. When cell cycle checkpoints are relaxed, as is the case in cleavage-stage embryos, cells with extra centrosomes may undergo multipolar mitosis, resulting in severe aneuploidy. Alternatively, centrosomes may cluster, allowing for bipolar spindle formation, but predisposing chromosomes to aberrant kinetochore-microtubule attachments. Such attachments may lead to anaphase lag, whereby the chromosome fails to be incorporated into the daughter nucleus. These chromosomes may be sequestered in micronuclei, incur DNA damage, and induce cellular fragmentation.

Figure 4, Key Figure. Models to explain the declining incidence of mosaicism from the cleavage to blastocyst stages of preimplantation development

The embryonic mortality model invokes selection against embryos based on the proportion of aneuploid cells. The clonal depletion model describes apoptosis or reduced propagation of aneuploid cells within mosaic embryos. Monosomic and trisomic rescue are proposed mechanisms by which aneuploid cells can give rise to diploid cells through mitotic chromosome gain or loss, respectively.