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Review

Molecular origin of mitotic aneuploidies in preimplantation embryos ☆

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ABSTRACT

Mitotic errors are common in human preimplantation embryos. The occurrence of mitotic errors is highest during the first three cleavages after fertilization and as a result about three quarters of human preimplantation embryos show aneuploidies and are chromosomally mosaic at day three of development. The origin of these preimplantation mitotic aneuploidies and the molecular mechanisms involved are being discussed in this review.

At later developmental stages the mitotic aneuploidy rate is lower. Mechanisms such as cell arrest, apoptosis, active correction of the aneuploidies and preferential allocation of the aneuploid cells to the extra-embryonic tissues could underlie this lower rate.

Understanding the mechanisms that cause mitotic aneuploidies in human preimplantation embryos and the way human preimplantation embryos deal with these aneuploidies might lead to ways to limit the occurrence of aneuploidies, in order to ultimately increase the quality of embryos and with that the likelihood of a successful pregnancy in IVF/ICSI. This article is part of a Special Issue entitled: Molecular Genetics of Human Reproductive Failure.

1. Introduction

The introduction of human IVF into clinical practice made it possible to study human embryos in the earliest stages after conception and it was rapidly discovered that aneuploidies, i.e. numerical chromosome abnormalities, exist in human preimplantation embryos [1–3]. In 1993, chromosomal mosaicism, the phenomenon that not all cells in an embryo have the same chromosomal content was described in human preimplantation embryos for the first time [1] and since then many studies have been published on this topic. Aneuploidies have been found in embryos from women of different age, in arrested and developing embryos, in fresh and in frozen-thawed embryos, and in fragmented and good morphology embryos [4–11].

Mosaic embryos can consist of both diploid and aneuploid cells (diploid-aneuploid mosaics) or of cells with multiple aneuploidies involving more than one chromosome (aneuploid mosaics). The application of high resolution DNA techniques such as array comparative genomic hybridization (CGH) revealed that structural abnormalities, apart from numerical abnormalities, also occur in cleavage stage human embryos, leading to partial mosaicism of certain chromosomal segments [12–14].

Mitotic aneuploidies have been suggested to affect the developmental potential of human preimplantation embryos, possibly leading to developmental arrest or embryo loss at later stages of development [5,9,15,16]. Mitotic aneuploidies may contribute to implantation failure or when compatible with implantation, may result in fetal or confined placental mosaicism [5]. It might also cause serious fetal complications like intrauterine growth delay, congenital malformations, mental retardation, and uniparental disomy [17].

The goal of this review is to describe the frequency of mitotic errors in human preimplantation embryos and to provide insight into the cause and the fate of these mitotic errors.

2. Frequency of mitotic aneuploidies

The frequency of mitotic aneuploidies in human preimplantation embryos can be deducted from the frequency of mosaic embryos. However, there is considerable heterogeneity in the reported frequency of mosaic embryos in the literature, with frequencies varying from 15% to more than 90% [5,18,19]. To resolve this heterogeneity, a systematic review and meta-analysis of studies on the chromosomal constitution of human preimplantation embryos has recently been performed [20]. This review shows that 73% of all human preimplantation embryos after IVF are mosaic, 22% are diploid and 5% contain other abnormalities [20]. Of the mosaic embryos, diploid-aneuploid

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mosaic embryos are most common (59% of all embryos) followed by aneuploid mosaic embryos (15% of all embryos).

Several factors contribute to heterogeneity in the reported frequency of mosaic embryos in the literature; the definition of mosaicism that is used, the number of chromosomes that have been analyzed, the type of embryos that have been studied, the developmental stage at which the embryos have been analyzed and the method of analysis that was used. One of the main reasons is the definition of mosaicism that is used in a study. While some studies classify embryos as mosaic as soon as a single cell has a different chromosomal content, others classify an embryo as diploid even when a percentage up to 50% of aneuploid cells is present [4,21,22].

The number of chromosomes that is analyzed also plays an important role in the varying rates of mosaicism reported [20]. Since the aneuploidies found in human preimplantation embryos are not limited to specific chromosomes, more mosaic embryos will be detected when more chromosomes are tested. Most of the studies target the sex chromosomes and the smaller autosomes as these chromosomes are more often found in aneuploidies in prenatal samples and miscarriages but they might not necessarily be the most frequent in preimplantation embryos [7]. Several studies try to increase the number of probes used in FISH, with 12 probes being the maximum number of probes used in three consecutive hybridization rounds [23]. Mitotic aneuploidies involve all chromosomes, with some chromosomes being implicated more frequently; sex chromosomes (24.1%) and chromosomes 8 (12.1%), 2 (8.6%), 16 (8.6%), 7 (5.2%), 13 (5.2%), 18 (5.2%), 20 (5.2%) and 21 (5.2%) [24,25]. However, these studies investigate only a few chromosomes. Some information concerning the frequency of mitotic errors for all 24 chromosomes is available but no extensive study comparing the frequency of mitotic errors among all chromosomes in a substantial number of embryos has been performed yet [12–14].

Almost all studies on the frequency of mitotic aneuploidies use spare embryos of compromised quality rather than embryos of good morphology that are transferred or cryopreserved. In one study, a cohort of embryos without selection, transfer or cryopreservation was studied, displaying a rate of diploid-aneuploid mosaic embryos not different from the rate reported for spare embryos (53% and 59% respectively) [20,22].

Mitotic aneuploidies have been observed at all developmental stages of human preimplantation development, with the first three mitotic divisions appearing more error-prone [15,26].

Comparisons of aneuploidy rates between cleavage stage embryos and blastocysts are conflicting with some studies reporting similar rates [6], some studies reporting a lower rate in blastocysts compared to cleavage stage embryos [5,11,27–29] and some studies reporting a higher rate in blastocysts compared to cleavage stage embryos [30,31]. Meta-analysis shows that mitotic aneuploidies increase from 63% at the cleavage stage to 95% at blastocyst stage [20]. Even though the percentage of mosaic embryos increases at the blastocyst stage, the proportion of aneuploid cells within the embryos decreases so that blastocysts contain relatively more diploid cells (74%) compared to cleavage stage embryos (62%) [5,10,18,20,30,32,33]. The percentage of cells with mitotic aneuploidies decreases further in the fetus and after birth. Mosaicism, if thoroughly analyzed, would most probably be present in all individuals in the general population but in an insignificant, low level that remains undetected and has no apparent phenotypic effect.

Mosaicism has not only been found in human embryos but also in embryos from other species, such as cattle (22% - 42%) and non-human primates (22% - 32.1%) [34–37]. While meiotic aneuploidies are present in mouse embryos albeit at a very low rate, data on mitotic aneuploidies in mice are lacking [38]. In order to study the occurrence and fate of mitotic aneuploidies in mice, mitotic errors are usually induced by for example exposure to acrylamide, a carcinogen present in tobacco that induces chromosomal damage at first cleavage and abnormal preimplantation development, or use of spermatozoa that had been exposed to γ-rays [39,40]. In addition, several mice models have been developed such as the *Syncp3*-null mice or the *BALB/cWt* mice that have an increased rate of non-disjunction events [41,42].

2.1. Technology

The methodology used to analyze human preimplantation embryos also contributes to the variation in the reported frequency of mitotic aneuploidies [20]. Some of the first studies reporting on mitotic aneuploidies in human embryos used karyotype analysis [43–45]. Although karyotyping allows for analysis of all chromosomes, it requires dividing, metaphase-stage cells. Only 24-36% of the embryos analyzed by karyotyping produces metaphases of sufficient quality for accurate chromosome quantification and in less than 25% of the embryos all cells could be analyzed [43,45]. This technique is also biased towards developing cells and embryos as arrested cells cannot be analyzed since these cells do not produce metaphases. Other disadvantages of the karyotyping technique are the difficulty to obtain optimal chromosomal banding making individual chromosomes hard to identify and the possible loss of chromosomes during fixation of the nuclei [43,45]. Karyotyping is no longer used for the analysis of chromosomal aneuploidies in human preimplantation embryos.

The technique that has been used most often for the analysis of chromosomal aneuploidies in human preimplantation embryos is fluorescence in situ hybridization (FISH) [1,7,10,16,18,22,23,46–48]. FISH can be applied to single cells and allows analysis of chromosome number both in metaphase and interphase nuclei. However, the technique is limited by the number of probes that can be used simultaneously as only a limited number of spectrally distinct fluorochromes are available for labeling the probes. To overcome this limitation, multiple sequential FISH rounds can be used. However, during sequential hybridization the efficiency of hybridization decreases with each round so it has been suggested to use no more than three rounds [48]. Also, FISH has an estimated accuracy of 92-99% per probe so there is a substantial risk of misdiagnosis when multiple probes are used [16]. It has been calculated by a mathematical model that the predictive value of FISH for an abnormal test result is 90% when two chromosomes are tested but could be as low as 41% when five chromosomes are investigated [49]. A limitation of FISH is the necessity for fixation and spreading of the nucleus of a single cell on a microscope slide which could lead to chromosome loss or scoring errors that arise from split signals, overlapping signals, damage to the nucleus, loss of micronuclei, hybridization failure and probe inefficiency [16]. In order to improve accuracy, a sequential FISH protocol using additional probes that bind to different loci of the same chromosomes can be used. Subsequent hybridization rounds can also be performed for any non-conclusive result and for confirmation of specific aneuploidies like monosomies [50,51]. The error rate of FISH for aneuploidy screening has been described to lie between 3.7% - 17.3% [50–52]. Moreover, FISH does not allow the detection of partial or segmental aneuploidy since the FISH probes hybridize to a specific locus or the centromere and provide information only about that segment of the chromosome [53].

More recently, the examination of the copy number of all 24 chromosomes has become possible with CGH, CGH-microarrays (aCGH) and single-nucleotide polymorphism-based (SNP) microarrays following whole genome amplification (WGA) [12–14,54–57]. With CGH, chromosome copy number analysis is achieved by co-hybridization of differentially labeled DNA samples (a reference, most often diploid sample and a test DNA sample of unknown chromosomal status) to metaphase spreads derived from cells of a chromosomally diploid individual and comparison of the relative levels of hybridization. aCGH is based on the same principle as CGH but uses genomic clones from selected regions of the genome that are spotted on a slide, instead of metaphase cells. These techniques are applicable to cells at any phase

of the cell cycle and avoid the use of fixation and spreading. They also have increased resolution and their analysis can be automated. Another advantage is the possibility to detect segmental aneuploidy [53]. The major challenge in performing genome-wide, single-cell analysis is the possible introduction of bias as a result of failure to amplify one of the parental alleles at random or excess amplification of specific regions [54,57–59]. CGH has proven to work at least equally as well as FISH, having further the ability to test all 24 chromosomes [53,60,61]. The recent advances in the use of array technology for determining the chromosomal status of a single blastomere will lead to important new insights and will more accurately determine the actual preimplantation aneuploidy and mosaicism rates. However, as no test with 100% accuracy is yet available, one should be careful regarding the clinical use of these tests as it could result in discarding viable embryos [61].

3. Origin of mitotic aneuploidies

3.1. Mechanisms leading to mitotic aneuploidy

Anaphase lagging in mitosis describes a delayed movement of one chromatid during anaphase, where the chromatid fails to connect to the spindle apparatus and is not incorporated in either nucleus of the daughter cells. The lagging chromosome is lost, resulting in a monosomy in one of the two daughter cells. Non-disjunction is the failure of the sister chromatids to separate properly during mitosis, resulting in a cell with a loss and a cell with a gain of a chromosome. These two mechanisms are the most common mechanisms leading to mitotic aneuploidies in human embryos [6,7,18,26,30]. Other mechanisms that could cause aneuploidies are chromosome demolition, premature cell division, errors in cytokinesis, cell fusion and chromosome breakage (Fig. 1). A study analyzing 299 blastocysts using FISH for chromosomes X, Y and 18 reports that two-thirds of all complex mosaic blastocysts are abnormal due to anaphase lagging of both an autosome and a sex-chromosome [30]. Cells from the remaining blastocysts contain both non-disjunction and anaphase lagging events with non-disjunction occurring mainly in the sex chromosomes and anaphase lagging in the autosomes [30]. Another study analyzing 47 day 5 embryos using three rounds of FISH reports that chromosome loss is the main mechanism leading to aneuploidy [18]. Even though the authors conclude that the observed chromosome loss is probably the result of anaphase lagging the equally high observed frequency of chromosome gains in this study could also suggest a high rate of mitotic non-disjunction.

The observed monosomies should be interpreted with caution when FISH is used since monosomies could be FISH artifacts either due to overlapping signals or due to hybridization failure. Also, it might be that non-disjunction, resulting in both losses and gains, occurs in a higher rate but the cells with complementary chromosomal content are eliminated or are not analyzable. More recently, a combination of FISH, CGH and aCGH shows no excess of chromosome losses compared to chromosome gains [60].

Chromosome demolition, which involves the destruction and fragmentation of one chromosome, has been proposed as a mechanism for chromosomal rescue of trisomic zygotes [62]. However, if chromosome demolition occurs in a normal diploid cell it will lead to aneuploidy.

Premature cell division without prior duplication could result in haploid cells and polyploid cells may be caused by endoreplication of the chromosomes two or more times. Endoreplication has been observed in human zygotes and in trophoblasts of first trimester human placenta [63,64]. In human embryos, tetraploidy caused by endoreplication in a single two-cell embryo has been observed [65].

Errors in cytokinesis are also likely to contribute to mitotic aneuploidy [10,66–69]. Failed or asymmetric cytokinesis results in the formation of binucleated cells, tetraploidy or spindle pole abnormalities and chromosomal chaos [70]. Analysis of the cells from cleavage stage human embryos at the 2–4 cell stage reveals multinucleated cells in 17% of cases, the incidence of which peaked during the third cleavage

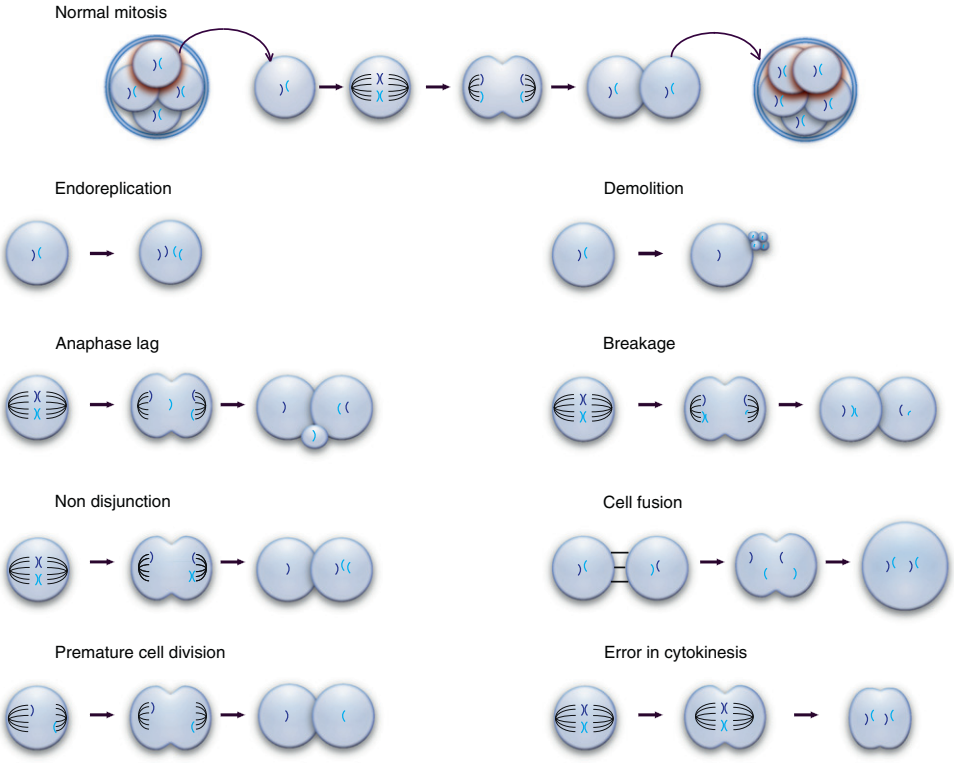

Fig. 1. Mechanisms leading to mitotic aneuploidy. Schematic representation of the mechanisms that could lead to aneuploidy.

division (65%) [68]. The proportion of binucleate blastomeres within normally fertilized embryos increases from 5% in 2–4 cell embryos to 10% in 9–16 cell embryos, whereas in polyspermic or parthenogenetic embryos the proportion is higher during early cleavage stages but decreases at the nine- to 16-cell stage (25 and 6%, respectively), due to arrest of these embryos [68]. Comparison of the size of the multinucleated blastomeres to that of normal mononuclear blastomeres suggests that multinucleated blastomeres arise from failure in cytokinesis. This may result from deficiencies in maternal molecules involved in polarization, cell division and compaction or from defects at the cell surface affecting cell-cell interactions. Cell-cell interaction in cytokinesis has been shown to be important in normal mitosis by the observation of reversible uncoupling of gap junctions between blastomeres in mice [71]. The formation of binucleated blastomeres could also arise from haploid mononucleated blastomeres where endoreplication occurred followed by karyokinesis but not cytokinesis [72].

Cell fusion of blastomeres is another possible mechanism contributing to mitotic aneuploidies. Cell fusion has been demonstrated in frozen-thawed human embryos resulting in polyploid or diploid-polyploid mosaic embryos [73]. It is suggested that the occurrence of blastomere fusion could be associated with changes of the cell membrane, for instance fluidity that might happen normally during embryo development or might occur after freeze/thaw. Also, changes in pH, temperature or osmotic pressure might cause blastomere fusion. However, so far cell fusion has been linked to treatment effects and there is no evidence that it will occur spontaneously in embryos. Even if it occurs spontaneously, cell fusion is a less frequently described mechanism compared to the other proposed mechanisms.

Another mechanism leading to aneuploidy is chromosome breakage causing partial chromosome loss and gain [6,13,14]. The breakage affects almost all chromosomes [6,74], with some chromosomes having defined fragile sites implicated more often [75]. Further, structural aberrations are reported in embryos from women with repeated implantation failure or advanced maternal age; gain/loss of entire chromosome arms is most common but aberrations affecting only part of an arm are also present [53,76–78]. Partial aneuploidy is likely to result in an unstable karyotype due to the formation of acentric and dicentric chromosomes [78]. Despite the importance of the data reported, these studies are limited by the resolution of the CGH which is 40 Mb and so chromosome breakage resulting in smaller deletions or duplications would not have been detected. SNP array and array CGH technologies have a higher resolution [57,79–81]. Using such techniques, it has been shown that seventy percent of top quality embryos from young and fertile patients (couples entering the IVF-program for sex selection) are mosaic for segmental deletions, duplications and amplifications [12]. Based on the aneuploidy patterns observed it is hypothesized that these aneuploidies occur via interstitial or terminal DNA double-stranded breaks and fusions in the zygote or during the first two cleavages [74]. These high levels of partial aneuploidy have yet to be confirmed by other investigations.

3.2. Molecular mechanisms involved in aneuploidy

Preimplantation mitotic aneuploidy could be the result of several interrelated events. The mechanisms contributing to mitotic aneuploidy in human embryos will be discussed in the following sections.

3.2.1. Maternal factors leading to aneuploidy

The activation of the human genome occurs at the 4- to 8- cell stage and many of the proteins regulating correct chromosome segregation during the first divisions are provided by the oocyte [82]. A defective maternal mRNA and protein pool could lead to failure of the mechanisms that guide and control cell division. Free radicals that are accumulating in the oocytes throughout the years until fertilization, exposure to external factors like radiation or chemicals, or poor vascularization of the antral follicle during oocyte maturation might harm the oocyte protein pool. Examples of mechanisms that are affected by maternal proteins involve microtubule kinetics, cell cycle checkpoints, DNA repair proteins, chromosome cohesion, telomere shortening and mitochondrial function [83–90]. Although the overall mosaicism rate in human embryos does not increase with maternal age [26,91], data suggest that mitotic non-disjunction does [76,92,93].

In mice oocytes, the mRNA and proteins are stored in specific compartments in the cytoplasm [94]. The transmission of mRNA and proteins to daughter blastomeres during the first cleavages could be asymmetrical, resulting in differences of the maternal pool among the blastomeres. Evidence for such asymmetrical divisions in human embryos is lacking.

Cell divisions could also result in depletion of critical nutrients, such as nucleoside precursors, required for chromosomal integrity leading to chromosome fragmentation and aneuploidy [95]. For example, folic acid deficiency increases chromosomal instability and aneuploidy in human lymphocytes [96,97]; however such studies have not been performed in human embryos.

3.2.1.1. Cell cycle checkpoints. Cell cycle checkpoints ensure correct cell division by checking whether the processes at every cell cycle stage have been correctly completed before progression to the next stage. When an error occurs the checkpoint sends a signal to halt cell division until repairs are completed, and if repair is not possible then the checkpoint directs the cell towards apoptosis. Cell cycle checkpoints function at the G1, G2 and metaphase (spindle assembly checkpoint –SAC) stages of the cell cycle [98].

The G1 checkpoint ensures that all conditions required for DNA synthesis are present (cell size, environment, quantity of energy, presence of nucleotides and nutrients) and the G2 checkpoint ensures proper duplication of the DNA during the S phase of the cell cycle and progression to mitosis. It has been recently reported that RB, the key protein of the G1 cell cycle checkpoint, and WEE1, the key protein of G2 cell cycle checkpoint, are lacking from normal appearing 8-cell stage human embryos [99]. At the same time, many genes involved in cell division are over-expressed, suggesting that early cleavage stage human embryos depend more on cell divisions rather than functional checkpoints [100].

Further, the SAC protein is essential for normal mitotic progression as it senses failure of kinetochore attachment to microtubules and halts the cell cycle until all chromosomes are attached. Overexpression of SAC components (BUB3, BUBR1 and MAD2) in mice embryos inhibits metaphase-anaphase transition. Depletion of SAC accelerates metaphase-anaphase transition during the first cleavage leading to formation of micronuclei, chromosome misalignment and aneuploidy [101].

Relaxation or absence of cell cycle checkpoints during early human preimplantation development may therefore cause aneuploidy by allowing a blastomere with chromosome errors to enter and proceed through mitosis [29,69]. Also, the presence of extra chromosomes in a blastomere might result in incorrect spindle formation and erroneous chromosome-microtubule attachments leading to genome instability during subsequent divisions.

3.2.1.2. Cohesins. Cohesins are a group of proteins regulating sister chromatid cohesion and ensuring proper chromosome segregation. The cohesins hold the two sister chromatids together to prevent them from premature separation from S-phase till anaphase. Upon entry into anaphase, the connection between the sister chromatids is destroyed to allow their separation. Malfunction of these proteins results in premature chromosome separation while delay in their removal may result in non-disjunction.

The role of cohesins in maintaining diploidy during meiosis is well described [102–104] but only indirect data is available on the effect of cohesins in mitosis during human preimplantation embryo development. Depletion of BUB 1 in mice fibroblasts causes these cells to fail

to align their chromosomes or sustain SAC function resulting in an aberrant mitosis [105]. Further, the centromeres separate prematurely; however, this might be due to SAC disjunction rather than a direct role of BUB1 in protecting chromosome cohesion. *BUB1* expression has been found to be low after fertilization in human embryos up to the 4-cell stage followed by an increase, with the greatest gene expression detected in hatched blastocysts [88]. In combination, these results suggest that the absence of BUB1 and other proteins important for protein cohesion in the cleavage stage human embryo may be in part responsible for mitotic aneuploidy caused by premature chromosome segregation.

3.2.1.3. Other maternal factors contributing to aneuploidy. FILIA is a protein provided by the oocyte that was found to be necessary for successful early embryogenesis in mice. Depletion of FILIA from the mouse oocyte impaired preimplantation embryo development resulting in a high incidence of mitotic aneuploidies from abnormal spindle formation, chromosome misalignment and SAC inactivation [90]. FILIA regulates the proper allocation of key spindle assembly regulators (AURKA, PLK1 and γ-tubulin) to the microtubule-organizing centre. Further, FILIA is required for the placement of MAD2 to kinetochores to enable SAC function [90]. Since this gene is highly conserved in humans, absence of the FILIA protein from the maternal RNA/protein pool might impair proper embryo development but this hypothesis needs to be properly studied.

Another protein present in the oocyte that is required for correct embryo development is the a-thalassemia/mental retardation X-linked protein (ATRX). ATRX has been found in mouse oocytes to bind to pericentric heterochromatin domains at the metaphase II stage where it mediates chromosome alignment at the meiotic spindle. Absence of ATRX from oocytes and 1-cell embryos exhibits chromosome fragments and centromeric DNA-containing micronuclei [83].

STELLA, ZAR1, MATER, PADI6 and FLOPED, maternal proteins which are required for proper early embryo development, have been identified in mouse embryos and most of these proteins are also present in human embryos [85]. None of these proteins has been studied in relation to mitotic aneuploidy in humans but they are, together with FILIA, part of a subcortical complex in mouse embryos [106]. Since FILIA has a role in maintaining diploidy in mouse preimplantation embryos, these proteins could also have a direct or indirect effect on euploidy.

In mouse oocytes, telomere dysfunction leads to disruption of functional meiotic spindles and misalignment of chromosomes during meiotic division [86]. It has been demonstrated that telomere DNA length is also associated with aneuploidy in human preimplantation embryos [87]. Aneuploid human blastomeres display significantly reduced telomere DNA quantity relative to diploid blastomeres from sibling embryos, but the difference in telomere length between aneuploid and diploid blastomeres is lost at the blastocyst stage [87].

Mitochondrial activity in developing human embryos could also correlate with chromosome abnormalities [89,107]. The mitochondrial activity of chaotic human mosaic embryos was found to be significantly less than that of diploid or aneuploid mosaic embryos [89]. Also, a significant difference in the mitochondrial activity was observed when embryos that contained only diploid blastomeres were compared to embryos that contained only aneuploid blastomeres [107].

Absence of DNA repair proteins can also cause mitotic aneuploidy. DNA repair proteins are responsible for detecting and repairing different DNA lesions and errors during replication. DNA repair genes are expressed at high rates in human oocytes and blastocysts, but at low rates after fertilization up to the 4-cell stage [84,88].

3.2.2. Paternal factors leading to aneuploidy

The centrosome, the organizing centre of the mitotic spindle, is paternally inherited [108]. The centrosome consists of two centrioles from which the spindle microtubules are generated. Too few or too many centrioles may result in abnormal spindle formation and chromosome malsegregation. Indeed, a higher frequency of mosaicism, originated from an abnormal spindle organization, has been observed in dispermic human embryos compared to monospermic or digynic embryos [109]. Severe sperm defects may also result in a higher percentage of mitotic abnormalities and chaotic mosaic embryos. Embryos deriving from patients with nonobstructive azoospermia (NOA) undergoing testicular sperm extraction (TESE) have an increased rate of mosaic embryos compared embryos generated with ejaculated sperm (53% and 26.5% respectively) [110]. The incidence of chaotic mosaicism is also significantly higher in embryos from NOA patients compared to embryos from all other sperm categories confirming the hypothesis that testicular spermatozoa might have difficulties in organizing the first mitotic spindle [111].

3.2.3. Effect of in vitro procedures on the occurrence of mitotic aneuploidy

The high percentage of mitotic errors found in preimplantation embryos could be different from the *in vivo* situation and it might be induced by components of IVF/ICSI procedures. Indeed, different mosaicism rates have been observed among embryos obtained from different IVF centers at different chronologies and subjected to different culture protocols [112]. Temperature fluctuation, oxygen concentration, culture medium and hormonal stimulation regimes could affect spindle assembly and chromosome segregation.

Data from several studies confirm that ovarian stimulation affects embryo quality and chromosomal constitution, including postzygotic errors [26,113]. An increase in the proportion of chromosomally abnormal embryos has been described with a conventional high dose exogenous gonadotropin regimen and GnRH-agonists co-treatment compared to a mild stimulation regimen using GnRH-antagonists co-treatment [113]. The increase in chromosomal abnormalities observed is due to an increased incidence of chromosomal mosaicism [113]. Furthermore, a higher daily FSH dose has been linked to an increase in mitotic division errors of chromosome 21 in mosaic embryos [26]. Chromosomal abnormalities are not solely the result of ovarian hyperstimulation as they are also observed in embryos from unstimulated cycles in young women (average age of 31 years-old) [114].

Increased oxygen concentration has been shown to increase non-disjunction events in the early divisions of non-disjunction-prone mice embryos suggesting also that subtle changes in the IVF setting can significantly influence chromosome segregation [115].

Despite the possible influences of IVF procedures in the frequency of aneuploidies, the high incidence of mosaicism found in early human abortions [116,117] suggests that a high incidence of mosaicism is present in *in-vivo* conceptuses as well. Strikingly, the mosaicism rate in chorionic villus samples (CVS) in the late first trimester from IVF/ICSI pregnancies and spontaneous pregnancies shows no difference in the frequency of mosaicism [24].

4. Fate of mitotic aneuploidies

In contrast with the high frequency of mitotic aneuploidies in early preimplantation embryos, a lower percentage of blastomeres with mitotic aneuploidies is observed in later stages of human preimplantation development and subsequently in established pregnancies and live births, suggesting a natural selection against aneuploid blastomeres or embryos [20].

The chromosomes involved in mitotic aneuploidies in human preimplantation embryos are different from those found later in embryonic development indicating that not all types of errors have the same fate [118,119]. It might be that errors concerning specific chromosomes are detrimental for further embryo development causing embryos with such errors to be absent at later stages. Some preliminary data suggested that aneuploidy of chromosomes 1–12 is very common in cleavage stage human embryos but in the majority of the cases these

abnormalities do not persist to the blastocyst stage [118]. Aneuploidy of chromosome 13, 18, or 21 is able to persist throughout development and is even compatible with life.

It is unclear whether there is a difference in this respect between aneuploidies of meiotic origin and aneuploidies of mitotic origin, although the difference in the frequency of chromosome 21 errors originating from mitosis between early embryos and clinically recognized trisomy 21 pregnancies suggests that the majority of embryos or blastomeres with mitotic non-disjunction involving chromosome 21 are not viable [15].

Selection against or correction of aneuploidies is already present in the preimplantation stage, which is demonstrated by the relatively decreasing number of aneuploid blastomeres from the cleavage to the blastocyst and implantation stage [20]. Three underlying mechanisms have been suggested: (i) cell arrest or apoptosis of aneuploid blastomeres and or embryos, (ii) active correction via anaphase lag, non-disjunction or chromosome demolition and (iii) preferential allocation of diploid/aneuploid blastomeres to embryonic or extra-embryonic tissues (Fig. 2). These hypotheses for the fate of mitotic aneuploidies will be discussed in the following paragraphs.

4.1. Cell arrest

Within aneuploid embryos, arrest and apoptosis has been observed more in embryos containing blastomeres containing the same aneuploidy when compared to embryos containing both aneuploid and diploid blastomeres and these mosaic embryos reach blastocyst stage more easily than aneuploid embryos [29]. Cell arrest occurs more likely at the time of compaction and cavitation [11,120–123].

This coincides with the appearance of the first embryonic transcription proteins. [82]. The initiation of cell arrest around the time of embryonic genome activation presumably works as one of the mechanisms to prevent further development of chromosomally abnormal blastomeres.

4.2. Apoptosis

Apoptosis, the process of programmed cell death, might also be responsible for the selection against aneuploid blastomeres. Apoptotic nuclei, fragmented and TUNEL labeled, are significantly increased at morula stage and TUNEL labeled nuclei are not seen until the morula stage, suggesting lack of apoptosis in early cleavage embryos [124]. The onset of the appearance of apoptotic markers seems to increase closely after activation of the embryonic genome [124].

The lack of apoptosis in early cleavage stage embryos could be either due to the ability of the embryos to suppress apoptosis or from the absence of (components of) the apoptotic pathway. In order to elucidate this, several studies have examined the expression of proteins of the apoptotic pathways in human oocytes and embryos. mRNA and proteins for the *BAX* and *BCL* genes that are involved in the regulation of apoptosis are expressed from fertilization to the blastocyst stage [125,126]. Other apoptotic markers, like PDCD5, BAD, caspases and Harakiri are down-regulated or present in relatively small amounts at cleavage stage and increase in blastocysts during human preimplantation development [125–127]. A study in mice embryos suggested that all components of the apoptotic machinery are present even in the early 2–4 cell embryo and when these embryos are chemically induced for apoptosis some caspase activity and DNA fragmentation is indeed observed [128]. Induction of apoptosis after chemical treatment in the early developmental stages has also been observed in bovine embryos [129]. These few studies confirm that early cleavage embryos possess the complete apoptotic machinery throughout early cleavage stage but this machinery is not active.

Apoptosis is controlled by extracellular or intracellular signals that can both induce or suppress apoptosis. Unlike most types of mammalian cells, data suggest that blastomeres do not require external signals to avoid apoptosis [130–132]. Mice embryos, from the 1-cell to 16-cell stage can survive and divide in the absence of exogenous signal even when cultured as isolated single cells [130]. Further, in

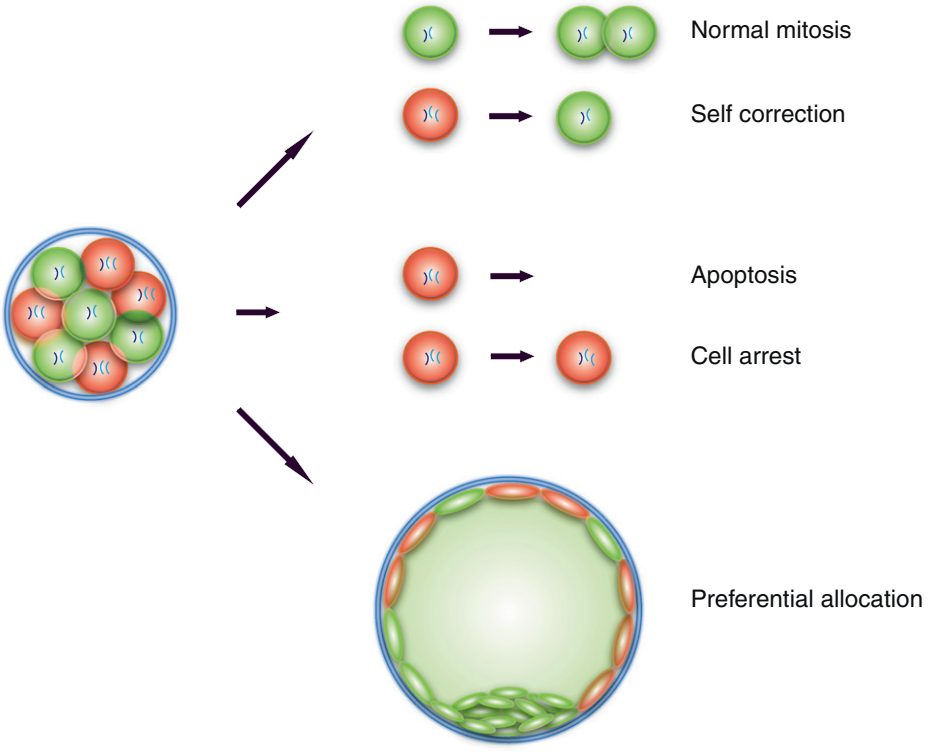

Fig. 2. Fate of mitotic aneuploidy. Schematic representation of the proposed mechanisms regarding the fate of aneuploid (red) and diploid (green) cells within human preimplantation embryos.

cleavage stage human embryos there is limited cell-cell communication via gap junctions, that are necessary to propagate apoptotic signals between cells and gap junctions are expressed extensively only after compaction [131,132]. It is still not clear though whether activation of apoptosis in later developmental preimplantation stages is a result of the establishment of cell-cell communication, the activation of cell cycle checkpoints that identify defective cells or other factors.

Upon activation of apoptosis, some aneuploid blastomeres among blastocysts could undergo apoptosis leaving the blastocyst with a higher proportion of diploid blastomeres [118]. Weaker mitotic checkpoints could also initiate apoptosis of aneuploid blastomeres as shown by deletion of genes coding for checkpoint proteins like BUB and MAD, in mice [118,133].

According to one hypothesis, early cell death would be naturally prevented during the early cleavage stages to maintain adequate cell numbers that are needed in order for the embryo to proceed through development [129]. After the onset of apoptosis, and if apoptosis would result in excessive cell death then implantation failure or embryonic loss might occur. However, there are no experimental data to prove or disprove this hypothesis.

4.3. Self-correction

Uniparental disomy (UPD) where both copies of a certain chromosome in an individual originated from the same parent has been suggested as proof for the occurrence of trisomic zygote rescue by loss or removal of one of the three chromosomes or monosomic zygote rescue by replication of the missing chromosome [25,26,134,135].

The exact mechanism for such self-correction is not known but the same mechanisms that cause mitotic errors, i.e. anaphase lagging and non-disjunction might also be able to correct them [33,62]. Whether the correction of the abnormal blastomeres and embryos is an active mechanism or an accidental event needs further investigation.

4.4. Preferential allocation

Human preimplantation embryos might deal with aneuploid blastomeres by preferential allocation of these blastomeres to the trophectoderm where chromosome aneuploidy might be better tolerated, and displacing diploid blastomeres to the inner cell mass or even the embryo proper [136]. The finding of confined placental mosaicism, i.e. the presence of aneuploid cells only in the placenta and not in the fetus, is the primary experimental data to support such a preferential allocation [137]. Confined placental mosaicism can be diagnosed by finding aneuploid cells by chorionic villus sampling without confirmation in a follow up amniocentesis, and has been reported in 1%–2% of all chorionic villus samples [138]. The degree of aneuploid cells in Confined placental mosaicism seems to correlate with pregnancy outcome like intra-uterine growth retardation [139,140]. On the other hand, in cases of an abnormal embryo (trisomy 13 or 18), the presence of diploid cells in the placenta could facilitate complete embryonic and fetal development [141].

Studies in human blastocysts showed a similar degree of mosaicism in the inner cell mass compared to the trophectoderm or the overall blastocyst and could thus not confirm the theory of preferential allocation [27,28,31,95,121]. Preferential allocation of diploid cells to the embryo proper has not been studied yet [27,121].

In mice, injection of only a small percentage of diploid donor ES cells (20% diploid cells combined with 80% cells with chromosomal abnormalities) in tetraploid blastocysts resulted in fully diploid normal adults [142]. Since tetraploid cells are excluded from the embryo proper, offspring resulting from these injected blastocysts must have originated from the injected ES cells, possibly indicating preferential allocation of diploid cells to the embryo proper.

Nonetheless, it remains to be seen whether this preferential allocation is indeed an active process or that for example in cases of confined placental mosaicism it was simply a matter of chance that diploid cells could form the embryo.

4.5. Threshold for the percentage of aneuploid blastomeres

Similar to the specific chromosome involved in the aneuploidy, the percentage of diploid blastomeres in a preimplantation embryo could be related to the ability of that embryo to develop into a child. The ratio of diploid blastomeres and aneuploid blastomeres may need to be above a certain threshold for development into a normal fetus [27]. The minimum ration or number of diploid blastomeres needed for proper development can only be speculated on, and could be dependent on other factors such as culture environment and uterine receptivity. But, limited indirect evidence supports this hypothesis. Human frozen-thawed embryos that have lost nearly half of their blastomeres due to the cryopreservation procedure are still able to result in live births, implying that not all blastomeres of human preimplantation embryos are necessary for proper development into a child [21,143]. The latter can also be concluded from the experiment mentioned above, where the injection of a small percentage of diploid donor ES cells in tetraploid mice blastocysts resulted in fully diploid normal adults [142]. This experiment could not demonstrate a correlation between chance of offspring and the percentage of diploid cells due to its design.

5. Conclusions

This review shows that mitotic errors are common in human preimplantation embryos and that these errors could be caused by several interrelated mechanisms. During early human development important mechanisms that are required for the regulation of genomic integrity, like cell cycle checkpoints, cell arrest or apoptosis are relaxed or absent leading to an increased rate of aneuploidies. Upon embryonic genomic activation these mechanisms are re-established and aneuploid blastomeres might be removed either by cell arrest, apoptosis, active self-correction or allocation to the trophectoderm. Whereas the high prevalence of mitotic errors might be an intrinsic phenomenon of human development, aspects of the IVF/ICSI treatment such as the ovarian stimulation, the use of sub-optimal sperm, or culture conditions might also contribute to the occurrence of mitotic aneuploidies. A better insight in the cause and fate of aneuploidy in human preimplantation embryos could help in limiting these preimplantation aneuploidies, with the ultimate goal of increasing overall embryo quality and with that treatment success in ART.

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