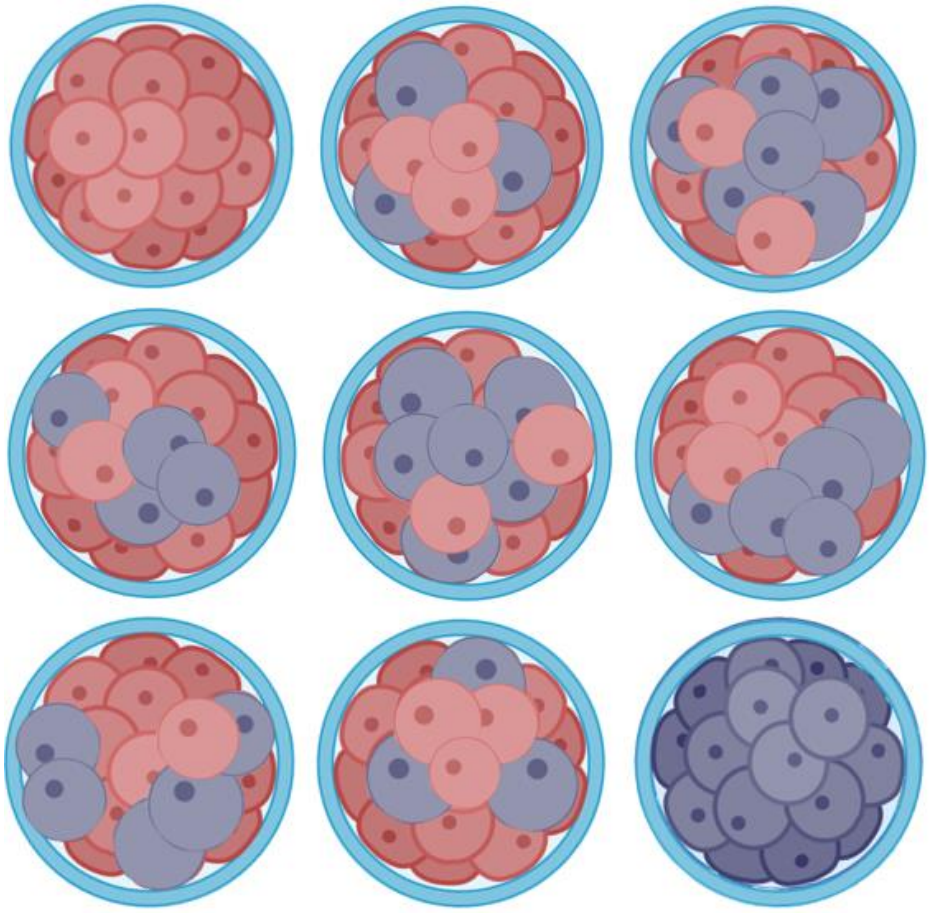
Utrecht University

From Errors during Cell Division to Clinical Outcomes:

A Review about Mosaic Embryos



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Layman's summary:

Every human cell contains structures called chromosomes, which carry our genetic instructions. Think of chromosomes as "blueprints" that tell the body how to grow and function. Most human cells have 46 chromosomes—23 from the egg and 23 from the sperm. When an embryo has the correct number of chromosomes in all its cells, it's called euploid, and if the whole embryo has the incorrect number of chromosomes in all its cells, it's called aneuploid. Mosaicism is right in between, where embryos have some euploid cells, while other cells are aneuploid.

Full aneuploid embryos are often the result of a mistake during meiosis. During meiosis, the egg or sperm are formed. When the egg or sperm is being formed, chromosomes might not divide evenly, resulting in an embryo where all its cells have the wrong number of chromosomes.

Mitosis is the 'normal' cell division, which happens after fertilization. Here, different mistakes can happen, like chromosomes not separating properly, DNA might not be copied quickly enough, or structures in the cells might not work as they should. These mitotic errors can lead to mosaicism. So why is mosaicism important to study? Mosaicism is important in IVF (in vitro fertilization), a process where embryos are created in a lab to help people have babies. Mosaicism can cause problems like the embryo not developing properly, failing to implant in the uterus, miscarrying, or sometimes, leading to complications during pregnancy.

In some countries, like the Netherlands, doctors look at embryos under a microscope to decide which ones to transfer. However, they don't test embryos for aneuploidy in the cells before transfer. If the embryo contains (a lot of) aneuploid cells, this might result in problems later and could end up in a miscarriage. We want to state the importance of mosaicism. We review the different parameters of mosaicism, and how it occurs, and what the clinical outcomes are. This review suggests that better testing methods and clearer guidelines for selecting embryos could help improve IVF success. By spreading the importance of mosaicism, and understanding it better, doctors can make better choices about which embryos to transfer, increasing the chances of healthy pregnancies.

Abstract:

Mosaicism, a condition characterized by the coexistence of chromosomally normal (euploid) and abnormal (aneuploid) cells within an embryo, poses significant challenges in assisted reproductive technologies (ART), including in vitro fertilization (IVF). Euploid cells have the appropriate number of chromosomes, essential for healthy development, whereas aneuploid cells, with either too many or too few chromosomes, can impair embryo viability, increase miscarriage risk, and reduce IVF success rates. This review provides an overview of mosaicism types, errors causing the aneuploidy, and implications for embryonic viability and pregnancy outcomes.

The severity of mosaicism mostly depends on the proportion of aneuploid cells. Low-level mosaicism, involving a smaller fraction of abnormal cells, has a lesser impact on development and pregnancy outcomes, while high-level mosaicism can compromise embryo survival. Additionally, the nature of the chromosomal abnormalities, such as whole chromosome gains or losses, further influences developmental potential.

Chromosomal instabilities may originate either during meiosis, the process of gamete formation, or during post-fertilization mitotic divisions. Meiotic errors, often associated with advanced maternal age, result in uniform aneuploidy across all embryonic cells and do not contribute to mosaicism. Conversely, mitotic errors, including anaphase lagging, spindle checkpoint failures or slow/stalled replication fork, occur during early embryonic divisions and are the primary source of mosaicism. These errors cause the initial identical cells to acquire different chromosomal compositions as the embryo divides, leading to a mosaic state.

Pre-implantation Genetic Testing for Aneuploidy (PGT-A) is a valuable tool for identifying chromosomal abnormalities in embryos prior to transfer. PGT-A is not universally implemented, and variability in testing methodologies across clinics may lead to inconsistent outcomes, including the unnecessary discarding of potentially viable embryos.

To address these concerns, this review states the importance for standardization in testing protocols and embryo assessment criteria. It proposes a hierarchical approach to embryo selection, prioritizing euploid embryos, followed by low level mosaic embryos, and reserving high level mosaic embryos as a last option. Finally, this review discusses the future of embryo selection, emphasizing advancements such as non-invasive genetic testing and artificial intelligence-driven assessment tools. These technologies could improve success rates, minimize ethical dilemmas surrounding embryo disposition, and enhance the safety and effectiveness of reproductive treatments.

Introduction

In the field of assisted reproductive technologies (ART), such as in vitro fertilization (IVF), mosaicism remains a notable and persistent challenge. Mosaicism refers to the coexistence of both euploid and aneuploid cells within an embryo. Whereas euploid cells are chromosomally “normal”, aneuploid cells have an abnormal number of chromosomes, either too many or too few (fig. 1A). This cellular phenomenon has significant implications for reproductive medicine, as it directly impacts embryo viability, increases miscarriage risk, and complicates clinical outcomes (Palmerola et al., 2019).

The presence of aneuploid cells within an embryo greatly increases the risk of pregnancy loss. Embryos with high levels of aneuploid cells are often less viable and more likely to result in miscarriage. In the context of ART, detecting mosaicism early and understanding its implications for embryo selection and transfer decisions can improve the success rates of IVF treatments. Furthermore, in natural conception, undetected mosaicism may lead to spontaneous pregnancy loss or developmental complications later in gestation. Given these significant impacts, investigating the mechanisms and consequences of mosaicism is essential for advancing ART, minimizing chromosomal abnormalities, and improving reproductive outcomes.

The degree of mosaicism, which can range from low to high, reflects the proportion of affected cells within the embryo (fig. 1B). Low-level mosaicism, where only a small percentage of cells display chromosomal abnormalities, is often less impactful on embryo viability. However, high-level mosaicism, in which a larger fraction of cells have genetic inconsistencies, can significantly compromise development due to widespread genetic imbalance. In addition to these quantitative distinctions, the type of chromosomal anomaly can vary in severity and impact. For example, an embryo may show localized changes, such as small segmental duplications or deletions (Zore et al., 2019), or more extensive anomalies like whole chromosome gains or losses (fig. 1C). It is also important to know whether only one chromosome is affected (simple mosaic), or more than one (complex mosaic). If an embryo exhibits both euploid and aneuploid cells, where the aneuploid cells exhibit 4 or more different chromosomes are involved, the embryo is classified as chaotic mosaic (Munné et al., 2017). The variability and different classifications of mosaic embryos complicates the evaluation and prognosis.

Mosaic embryos are often the result of mitotic errors post-fertilization. If errors occur during meiosis - the process by which sperm and oocytes are formed - the whole embryo would consist of only aneuploid cells. Meiosis plays a critical role by halving the chromosome count, ensuring gametes carry the correct 23 chromosomes for a total of 46 after fertilization. A full aneuploid embryo is (in almost all cases) prone to cause miscarriages as the chromosomal implications are non-viable. Only a few cases of chromosomal disorders like Down syndrome (trisomy 21) are viable (Herbert et al., 2015). During meiosis, parental factors could cause these errors which could cause the full aneuploid embryo. Maternal age plays a pivotal role in the incidence of meiotic errors, as aging oocytes are more prone to weakened chromosomal cohesion due to prolonged meiotic arrest (Mihalas et al., 2024). Paternal contributions to aneuploidy, while less common, also play a role under certain conditions. Factors like advanced paternal age or severe male infertility can impact sperm quality, including disruptions in the sperm centrosome, which may affect chromosomal segregation during fertilization.

Post-fertilization, mitotic errors are frequent causes of aneuploidy as the zygote begins dividing, resulting in mosaic embryos consisting of euploid and aneuploid cells. Aneuploidy is commonly observed during the cleavage stage of embryonic development (70% of cases)

but declines by the blastocyst stage (5-15%)(Zore et al., 2019). Errors in the Spindle Assembly Checkpoint (SAC), which play a critical role in ensuring chromosomal integrity during cell division (Fischer et al., 2022), or DNA replication stress caused by slowed or stalled replication forks contribute to chromosomal instability. Abnormalities in the structure of the first mitotic spindle can also lead to severe consequences, such as multinucleation during the 2-cell stage (Ono et al., 2024). Research by Mizobe et al. (2024) indicates that a perpendicular division plane relative to the pronuclear axis correlates with higher rates of aneuploidy, further highlighting the critical role of spindle orientation in maintaining chromosomal integrity.

Another mitotic error that can contribute to aneuploidy involves errors during the separation and incorporation of sister chromatids. Non-disjunction occurs when sister chromatids fail to separate properly during anaphase. This failure can lead to aneuploidy, where one daughter cell inherits both chromatids of a chromosome (resulting in a gain of one chromosome), while the other daughter cell is left without that chromosome (resulting in a loss) (Currie et al., 2022). Anaphase lag occurs when, although sister chromatids are initially separated, one chromatid lags behind during anaphase. Consequently, both chromatids are incorporated into the same daughter cell, leaving the other daughter cell devoid of that chromatid (Coonen, 2004). These types of mitotic errors can lead to chromosomal imbalances in the resulting cells, contributing to mosaicism and affecting the viability of the embryo.

Mosaicism can be detected through Pre-implantation Genetic Testing for Aneuploidy (PGT-A). This tool has been widely adopted many IVF clinics globally, allowing embryologists to detect chromosomal abnormalities before embryo transfer. In its early iterations, PGT-A typically analysed a single cell biopsied from an early-stage embryo (such as a blastomere from a day-3 cleavage-stage embryo), which classified embryos as either euploid or aneuploid, without accounting for mosaicism (Viotti, McCoy, et al., 2021). Nowadays, a trophectoderm (TE) biopsy is taken from blastocyst-stage embryos (day-5 or day-6). Mosaicism can be detected, thus the distinguishment can be made between aneuploid and euploid cells within the same embryo (Tocci, 2020). The technology used for this is next-generation sequencing (NGS). Beyond detecting standard aneuploidies, NGS enables identification of segmental aneuploidies and low-level mosaicism with unprecedented precision, significantly enhancing the embryo selection process (Sachdev et al., 2020). It is even possible to pinpoint the origin of chromosomal errors through PGT-AO (Pre-implantation Genetic Testing for the Origin of Aneuploidy). Because of this, clinicians can further refine selection criteria, potentially avoiding embryos with meiotic errors that are more likely to lead to pregnancy loss (Essers et al., 2024).

While the accuracy of detecting chromosomal abnormalities has improved, the biological nuances of mosaicism present challenges in decision-making. For instance, the high variability of mosaicism between embryos and within different regions of the same embryo complicates the reliability of biopsies. TE biopsies are taken, which is the outer layer of cells destined to form the placenta, as this causes no harm to the inner cell mass (ICM) which ultimately develops into the foetus (fig. 1D). However, this approach still presents challenges; because chromosomal abnormalities might be confined to the inner cell mass or the trophectoderm, a biopsy taken from one may not fully represent the chromosomal composition of the other (Capalbo et al., 2016).

Consequently, embryos with viable, chromosomally normal cells in the inner cell mass may be inaccurately labelled as aneuploid and discarded, underscoring the complexity of assessing mosaic embryos for viability. Further emphasizing the need for cautious interpretation, a double-blinded prospective randomized trial revealed comparable live-birth and miscarriage rates across 484 euploid, 282 low-grade mosaic, and 131 medium-grade

mosaic embryos (Capalbo et al., 2021). This finding suggests that a diagnosis of mosaicism based solely on PGT-A results should be framed as a "pattern consistent with possible mosaicism" rather than a definitive diagnosis and embryos should not be discarded immediately (Capalbo et al., 2016).

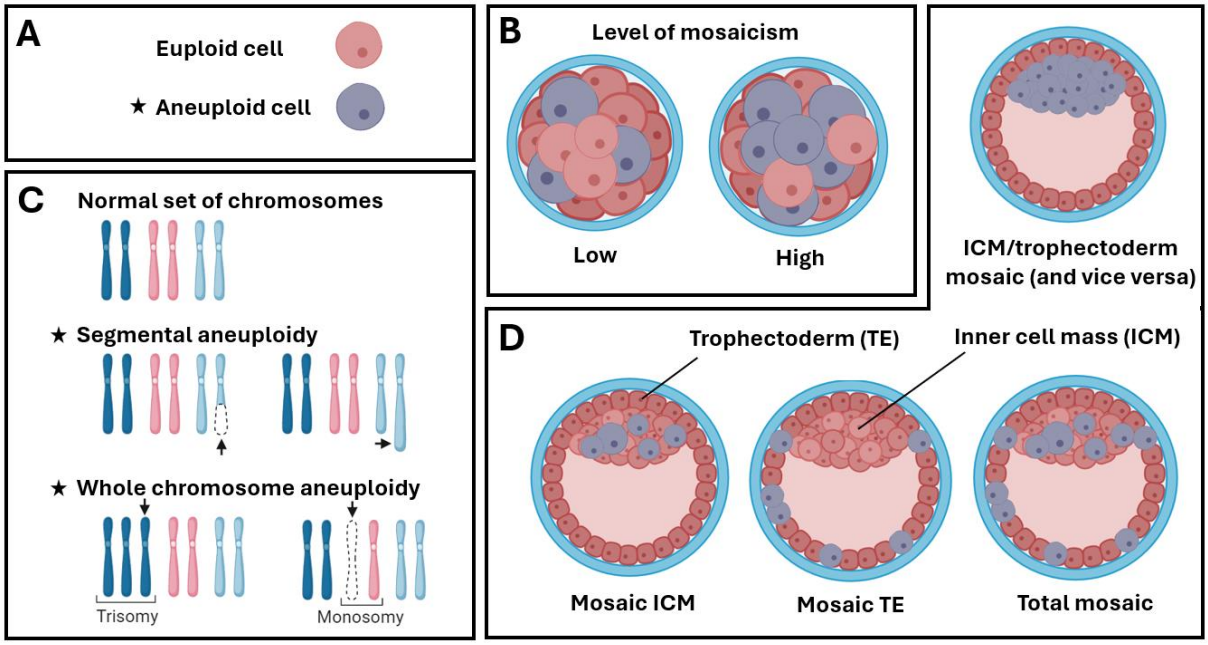

Figure 1: Overview of common mosaic embryo features
This figure schematically shows (A) the different cell types (euploid and aneuploid) involved in mosaic embryos, (B) the different levels of mosaicism, (C) euploidy and types of aneuploidy and (D) the different parts of an embryo (trophectoderm, inner cell mass) and the types of mosaicism involved in these embryos.

PGT-A is not universally adopted in IVF clinics, with certain countries, such as the Netherlands, relying primarily on morphological assessment to evaluate embryos. With this method, embryos are assessed on the symmetry and density of cells in first cleavages and blastocyst stage. Although useful, with this approach there is no determination of the presence of aneuploidy rates within the cells of the embryo. The lack of standardization between clinics in embryo assessment makes it difficult to compare clinical outcomes, such as miscarriage rates, and the establishment of uniform criteria for discarding embryos.

A critical question in the field of IVF is whether success rates can be improved by better understanding the different types of mosaicism, the mechanisms behind them, and their impact on clinical outcomes. The ways in which various types of mosaicism arise, and how they influence outcomes such as successful implantation and pregnancy, remain underexplored. Enhancing our understanding on these factors could lead to more informed decisions in embryo selection, improving overall IVF success.

To shed light on this crucial issue, this review aims to answer two key research questions:

1. What are the different types of mosaicism in embryos, and what mechanisms contribute to their origin, particularly in relation to embryo viability?
2. How do the various types of mosaicism impact clinical outcomes, including embryo development, implantation success, and pregnancy viability?

By exploring these questions, this review hopes to offer valuable insights into the mechanisms that lead to mosaicism and how these types of errors might differ in their impact on embryo development and clinical outcomes. The hypothesis driving this research is that some forms of mosaicism are more compatible with embryo viability than others, depending on the type and extent of chromosomal abnormalities. Additionally, it is likely that different types of mosaicism result from different mechanisms. This review aims to fill a significant gap in ART knowledge, providing a clearer understanding of mosaicism and its implications, which could lead to more informed decisions in IVF clinics and, ultimately, improve the success rates of fertility treatments, particularly in regions where genetic testing is not yet widely utilized.

Methodology:

A search was conducted from different sites such as Google Scholar and literature repositories including PubMed and Research Gate. The keywords utilized were “PGT-(A)”, “meiosis (or meiotic errors)”, “mitosis (or mitotic errors)”, “aneuploidy”, “mosaicism” and “(human) embryo / zygote”. Articles found via references from other articles were also included in this review. Excluded from the results were editorial letters and notes, conference papers, short surveys, and articles in the press. All articles used were published between 2014 and 2024, with exceptions of the important key studies (Baart et al., 2007), (Coonen, 2004), (Barbash-Hazan et al., 2009) and (Crasta et al., 2012).

To ensure relevance, this review primarily focuses on studies involving human embryos, both *in vivo* and *in vitro*. However, due to ethical constraints, research frequently relies on alternative model organisms such as bovine embryos, which show significant similarities to human embryos - both contain centrosomes and display similarity in terms of aneuploidy rates and timings of early embryonic divisions - and are thus considered more suitable models compared to mice (Cavazza et al., 2021). Mouse embryos, in contrast, show lower susceptibility to chromosomal abnormalities and lack centrioles, introducing additional complexity when drawing comparisons to human embryonic systems (Bennabi et al., 2016).

Research from cancer studies presents another layer of complexity. While cancer research has provided useful information into chromosomal abnormalities, certain mechanisms, such as centriole duplication, are specific to cancer cells and may not be directly applicable to embryonic cells. Cancer cells often display extensive chromosomal instability due to processes like centrosome amplification and altered mitotic checkpoints, which do not always mirror the mechanisms active during normal embryonic development. Therefore, only a limited number of articles from cancer research were included in this analysis to ensure relevance to the study of embryonic chromosomal abnormalities.

Results

Parameters to define mosaic embryos

Mosaicism can manifest in a variety of forms. Aneuploid cells present in the mosaic embryos might have whole chromosome aneuploidy, or more complex structural changes like segmental aneuploidy. It can also be subdivided in the amount of chromosomes involved and on the variations in frequency of chromosomal abnormalities in blastomeres, such as low and high level.

Type of abnormality

A study by Rechitsky et al. (2020) evaluating trophectoderm biopsies from 14.992 IVF embryos show that 8% and 14% of the embryos show whole chromosome and segmental aneuploidy in some of the cells (mosaicism), respectively. Whole chromosome aneuploidy occurs when one or more entire chromosomes are present with only one copy (monosomy) or three (trisomy) or more copies in some cells of the embryo. While whole chromosome aneuploidy can also arise in oocyte meiosis, chromosome mis segregation occurring during the initial mitotic cell divisions are the main source of whole chromosome aneuploidy, resulting in mosaicism .

Segmental aneuploidy involves partial chromosomal abnormalities, such as deletions or duplications of specific regions of chromosomes. Studies have shown that segmental aneuploidy often is seen in larger chromosomes such as 1, 4, 5, 6 and 9 (Rechitsky et al., 2020). Often, the S phase at 1-cell stage shows stalled or slow replication forks. This process results in DNA damage and incomplete replication, followed by spontaneous chromosome breakage and segmental aneuploidies, as concluded by (Palmerola et al., 2022).

Amount of chromosomes affected

Mosaicism can be classified into distinct categories based on the number of chromosomes involved in the abnormality. Simple mosaicism involves abnormalities affecting only a single chromosome. When multiple chromosomes are affected, the condition is referred to as complex mosaicism (Coonen, 2004). If the abnormalities involve four or more chromosomes, the mosaicism is categorized as chaotic mosaicism, often characterized by extensive and unpredictable chromosomal rearrangements. In the study conducted by Munné et al. (2017), human embryos from various IVF clinics were analysed. The researchers specifically examined cases involving one, two, or more than three chromosomes affected by aneuploidy. Their findings indicated that the likelihood of successful implantation and ongoing pregnancy decreased as the number of chromosomes involved in the aneuploidy increased.

Level of aneuploid cells within the mosaic embryo

Low-level mosaicism refers to situations in which only a small proportion of cells within the embryo is aneuploid, while the majority of cells remain euploid. This type of mosaicism is less likely to have a significant impact on embryo viability, as the chromosomally normal cells can often compensate for the small percentage of abnormal cells. In the study of Starostik et al. (2020) it has actually been found that low-level mosaicism is a common feature of early human development, with 80% (59 out of 74 tested human embryos) of embryos harbouring at least one aneuploid cell. High-level mosaicism, on the other hand, involves a large proportion of aneuploid cells within the embryo. This form of mosaicism is generally associated with a much lower likelihood of embryo viability, as the aneuploid cells can significantly compromise development (Spinella et al., 2018). Often a cut-off value of < 50%

abnormal cells within an embryo is classified as low-level mosaicism and ≥ 50% as high-level mosaicism, however, there is no consensus on the percentage threshold. Table 1 provides an overview of different studies and their respective cut-off level of low and high mosaicism. While the majority adhere to the widely accepted 50% threshold, some studies utilize alternative cutoff values, highlighting variability in classification criteria.

Table 1: Overview of (cut-off) values used to describe low and high level mosaic embryos

This table shows an overview of the cut-off values for low and high level mosaic embryos, with the corresponding article shown and the text in the article where these levels are mentioned.

Cut-off level: Low and High (%)	Publication	Article conclusion on low (medium) and high level mosaicism
20-40 (low) 41-80 (high)	(Armstrong et al., 2023)	We demonstrated that both low- (20-40%) and high-level (41-80%) mosaic results occurred more frequently in younger patients; however, the complexity of mosaic errors increased with age.
50*	(Viotti, McCoy, et al., 2021)	Low level mosaics (<50%) were associated with better outcomes than high level mosaics (≥50%), and within those groups, the type of mosaicism showed different outcomes (sorted from most to least favourable: one chromosome (simple) > two chromosomes (complex) > chaotic
20-30 (low) 30-50 (medium) 50-70 (high)	(Capalbo et al., 2021)	Equivalent live-birth rates and miscarriage rates across low and medium-grade (20-30, 30-50) mosaic embryos, higher rates for high (50-70) mosaic embryos.
50	(Viotti, Victor, et al., 2021)	Whole-chromosome mosaic embryos with level <50% had significantly more favourable outcomes than the ≥50% group
50	(Lin et al., 2020)	The present study demonstrates that high-level (>50%) mosaic embryo transfer resulted in a comparable live birth rates, but higher miscarriage rate compared with low-level (<50%) mosaic embryos.
50	(Lee et al., 2020)	We propose that embryos with low mosaicism level (< 50%) can be considered for embryo transfers in PGT-A cycles and could result in a euploid live birth.
40-50	(Y. X. Zhang et al., 2020)	This research concludes that no significant difference is found when the cut-off value is 40 or 50%
50	(Spinella et al., 2018)	A significantly higher implantation rate, clinical pregnancy rate/ET and live-birth rate were observed in embryos with <50% mosaicism

The percentage values mentioned in the table represent the cut-off level mentioned in the article for categorizing mosaic embryos. Specifically, these percentages indicate the proportion of aneuploid cells within the embryo. As an example, indicated with the asterisk (*) in the table, the article of Viotti, McCoy, et al., 2021 uses a cut-off value of 50% = low-level mosaicism (<50% aneuploid cells) and high-level mosaicism (≥50% aneuploid cells).

Aneuploidy of gametes

Meiotic errors often contribute to a full aneuploid embryo, containing only aneuploid cells. It is important to note that mosaicism is primarily caused by mitotic errors during the early stages of embryonic development, leading to a population of cells within the embryo that have different chromosomal compositions (euploid and aneuploid). However, parental-origin factors can indirectly contribute to mosaicism. For instance, if a gamete (egg or sperm) is aneuploid due to meiotic errors, the resulting zygote can inherit this abnormality. As the embryo undergoes mitotic divisions, it could be that the full aneuploid embryo undergoes mitotic rescue, potentially resulting in a mosaic embryo. Thus, in this review about mosaicism, it is still important to note how aneuploidy can arise from parental origin.

Aneuploidy in germ cells frequently leads to infertility, pregnancy loss, and severe genetic disorders in offspring. In humans, complete aneuploid embryos are often non-viable and only few aneuploidies can give rise to newborns. Full aneuploid is frequently observed in oocytes and embryos, with rates ranging from 30-70% and 30-60%, respectively, but these cases are often non-viable (Mikwar et al., 2020). In contrast, aneuploidy is observed in only 0.3% of the newborns. Examples of viable cases of full aneuploid embryos are trisomy 13, trisomy 18, trisomy 21, and certain sex chromosome aneuploidies (Herbert et al., 2015).

The sperm from the father can be aneuploid, however, this is at significantly low rates (Currie et al., 2022). The centrosome, inherited from the sperm, is essential for initiating the first mitotic divisions in the human embryo (Amargant et al., 2021). Disruptions in the sperm centrosome or delays in sperm aster formation—a phenomenon more common in infertile men—increases the likelihood of chromosomal abnormalities (Avidor-Reiss et al., 2019). It is possible for embryos to become polyploid (triploidy or tetraploidy), possibly caused by multi-sperm fertilization at the time of fertilization (Samura et al., 2023).

Most aneuploidies originate from oocytes. At birth, oocytes become arrested at the diplotene or dictyotene stage of prophase I, a state known as germinal vesicle arrest, where they remain dormant until ovulation, sometimes for several decades. This prolonged meiotic arrest is a key contributor to chromosomal abnormalities in oocytes (Mikwar et al., 2020). During meiosis, meiosis I separates homologous chromosomes, while meiosis II segregates sister chromatids. In mammalian oocytes, the cohesin complexes responsible for maintaining chromatid cohesion are established during fetal development and persist throughout the lengthy prophase I arrest. Evidence from studies in mice indicates that there is no turnover of these cohesin complexes during this period, implying that the cohesin formed in utero must remain functional throughout the female reproductive lifespan, which can extend up to 50 years in humans (Mihalas et al., 2024).

Despite this, female fertility begins to decline much earlier, primarily due to the gradual loss of oocyte euploidy as maternal age increases. As oocytes age, the integrity of the chromatids' cohesion diminishes, leading to a greater likelihood of improper chromosome segregation during cell division (Mihalas et al., 2024). The oocyte's ability to supply mitochondria and mRNA—both critical for proper chromosomal division—also declines with age, exacerbating chromosomal errors (F.-L. Zhang et al., 2023). Thus, it is known maternal age contributes to aneuploidy, however, whether it contributes to mosaicism is still a debate.

A review by Munné & Wells (2017) and studies such as Escudero et al. (2016) prove that mosaicism is independent of maternal age. These researchers state that mosaicism arises in the embryo during mitotic divisions, whereas aneuploidy originates during meiotic divisions in the process of gametogenesis. As a result, aneuploidy is primarily associated with maternal age, while mosaicism is linked to embryonic factors independent of maternal age.

Conversely, other studies, such as the study from Chan et al. (2019) show that there is a slight correlation between mosaicism rate and advanced maternal age. In the supplementary table an overview is presented of different studies investigating maternal age and mosaicism outcomes.

In conclusion, while the majorities of studies show that the highest rate of euploid embryos is found in the youngest age group (18-35), they also report that mosaicism (with in particular cells with segmental aneuploidy) is also most common in the same age group. In contrast the complexity of mosaicism seems to arise with maternal age. A whole (non-mosaic) aneuploid embryo rates are, instead, highest in women with advanced maternal age (women aged 41 and above). These findings suggest that there may be no correlation between mosaicism and maternal age, but there is a clear correlation between embryos consisting of only aneuploid cells and maternal age. It is important to note that these conclusions are based on a limited number of studies, each with different cut-off values and varying rates for mosaicism (different levels and types), aneuploidy, and euploidy.

Mechanisms underlying aneuploidy post-fertilization

Different mechanisms contribute to mosaicism in embryos. As previously explained, meiotic errors mostly contribute to full aneuploid embryos, whereas mitotic errors, which arise post-fertilization, can often result in mosaic embryos. Some of the most common mitotic errors are discussed in this part of the result section. The main findings of the articles cited can be found in table 2 and schematic summary of the different mechanisms is clearly overviewed in figure 2.

Mitotic Non-Disjunction & Anaphase Lagging

Non-disjunction is a critical error in cell division where sister chromatids fail to separate properly during mitosis. This failure results in an unequal distribution of chromosomes, producing one daughter cell with a deficiency of a chromosome (monosomy) and another with an extra chromosome (trisomy). A recent study focusing on human embryos has emphasized the high susceptibility of the first mitotic division to errors. This research highlights that the initial division is marked by an extended prometaphase/metaphase phase and frequently exhibits lagging chromosomes as a characteristic phenotype (Currie et al., 2022).

Anaphase lagging is a specific type of mitotic error that occurs when a chromatid fails to integrate into the newly forming nucleus during cell division. This failure can disrupt chromosome segregation, leading to monosomy in one cell and trisomy in the other. The occurrence of anaphase lagging is thought to be one of the most important mechanisms behind the formation of mosaic embryos (Coonen, 2004).

Interestingly, human embryos with trisomy may possess a unique capacity for self-correction. Barbash-Hazan et al. (2009) proposed a phenomenon known as “trisomic rescue”, whereby a trisomic cell restores a normal chromosomal count (disomy) through the selective loss of a supernumerary chromosome during subsequent mitotic divisions. This process likely involves anaphase lagging, where the additional chromosome fails to be incorporated into one of the daughter nuclei and is effectively excluded. Often, the cell encapsulates the additional chromosome in a micronucleus and degrades this (X. Zhang & Zheng, 2024). Trisomic rescue represents a fascinating compensatory mechanism that could mitigate the adverse effects of chromosomal abnormalities in early development.

DNA Replication Fork & Replication Stress

Errors occurring during DNA replication in interphase are particularly significant, as they lay the foundation for chromosomal mosaicism depending on the fidelity of replication and the degree of DNA damage that occurs (Crasta et al., 2012). DNA replication stress describes conditions that can alter the replication fork's progress or increase its instability, leading to incomplete or abnormal DNA replication intermediate structures (X. Zhang & Zheng, 2024). For instance, reduced replication fork speed or complete fork stalling are well-documented contributors to replication stress. Research by Palmerola et al. (2022) in human oocytes and embryos revealed that slow replication speeds, particularly prevalent in zygotes compared to later embryonic stages, can result in incomplete DNA replication. This failure may cause chromosomes to break, leading to the loss of chromosomal fragments (segmental aneuploidy). The stress is especially detrimental in large genomic regions, such as centromeres and telomeres, which require longer replication times and are more vulnerable to damage. It is important to note that DNA replication stress is not only limited to segmental aneuploidy, it can also cause entire chromosomes to distribute incorrectly during cell division (whole chromosome aneuploidy).

Spindle Assembly Checkpoint (SAC)

The Spindle Assembly Checkpoint (SAC) is a crucial cell cycle control system that ensures genome stability by monitoring proper attachment of chromosomes to the spindle apparatus during mitosis and meiosis. SAC prevents chromosome mis-segregation by delaying anaphase until accurate attachment is achieved, primarily by sensing unattached kinetochores and halting cell cycle progression until proper connections form with microtubules (Bennabi et al., 2016). While it is still relevant for understanding the mechanisms behind chromosomal aberrations in embryos, it is good to mention that this is not considered one of the primary causes. SAC dysfunction is more often a major contributor to chromosomal aberrations in cancer cells.

Merotelic attachment occurs when a single kinetochore is connected to microtubules from opposite poles, while syntelic attachment describes both kinetochores being connected to microtubules from the same pole. Merotelic attachments are not detected by the SAC, while syntelic might be detected (Godek & Compton, 2018). Recent work by Vázquez-Diez et al. (2019) demonstrates that failures in SAC activation can permit cells to divide with unresolved chromosome alignments, a condition frequently associated with whole chromosome mosaicism.

Spindle Structure, Division Orientation and Genome Unification

Alterations in the assembly of the first mitotic spindle in human embryos significantly impact chromosomal stability, particularly through their effects on spindle morphology. Spindles with a high aspect ratio (high-AR) exhibit sharp, elongated shapes with well-focused poles, promoting mononuclear embryos at the 2-cell stage, as demonstrated in a study by Ono et al. (2024). In contrast, low-aspect-ratio (low-AR) spindles display defocused poles and dual structures, where chromosomes fail to unify during anaphase, resulting in multinucleation. These findings underscore the critical influence of spindle architecture on nuclear formation in 2-cell-stage embryos.

Proper orientation of the first cell division plane relative to the pronuclear axis is equally essential for chromosomal stability and euploidy. Research by Mizobe et al. (2024) demonstrates when division plane occurs at a right angle to the pronuclear axis, it promotes stable chromosomal segregation and normal embryonic progression. Conversely, when the division plane is misaligned—specifically perpendicular to the pronuclear axis—euploidy rates decrease, and aneuploidy rates increase. This underscores the importance of proper division orientation for accurate chromosomal segregation. Such errors contribute to genetic instability, increased rates of aneuploidy, and reduced fertility (Porokh et al., 2024).

In non-rodent mammals, such as bovine zygotes, dual spindle formation has been observed even in the presence of centrosomes, suggesting a conserved feature among mammals. These centrosomes show a loose association with the dual spindles. This dual spindle pathway provides a possible explanation for the frequent loss of entire parental genomes in blastomeres of human IVF embryos, contributing to chromosomal instability and mosaicism (Schneider et al., 2021).

Following up on bovine research, Cavazza et al. (2021) used high-resolution live-cell imaging to study human zygotes and bovine embryos, aiming to uncover the causes of errors during early mammalian embryogenesis. Their findings revealed that in both species, parental genomes cluster with nucleoli within each pronucleus shortly after fertilization. This clustering accelerates the unification of the parental genomes, improves the efficiency of chromosome capture by the newly forming spindle, and reduces the risk of mitotic chromosome segregation errors and micronuclei formation.

Table 2: Overview of the most common mitotic errors resulting in mosaic embryos

This table shows an overview of the following mitotic errors; non-disjunction, anaphase lagging, DNA replication stress, Spindle Assembly Checkpoint (SAC), spindle morphology and orientation. For each error, the mechanisms, effect/outcome and studies used in this literature review (main findings / model) are clearly stated.

Mechanism	Description	Effect/outcome	Studies in this review	Main findings of the studies	Model used
Mitotic non-disjunction	Failure of sister chromatids to separate during mitosis, Tripolar division	leads to unequal distribution of genetic material among the cells such as monosomy and trisomy	(Currie et al., 2022)	The first mitotic division exhibits phenotypes that can cause nondisjunction or unequal distribution of genetic material among three cells.	Human zygotes
Anaphase lagging	Chromatid fails to incorporate into nucleus	Like non-disjunction, anaphase lagging leads to unequal distribution of genetic material among the cells.	(Coonen, 2004)	Anaphase lagging is how human embryos acquire a mosaic chromosome pattern	Human embryos
			(Barbash-Hazan et al., 2009)	Self-correction of aneuploid and mosaic embryos occurs (trisomic rescue)	Human embryos
DNA replication stress	Errors during DNA replication, particularly during slow replication forks	DNA damage accumulation; hotspots of chromosomal instability in late-replicating, repetitive DNA regions	(Crasta et al., 2012)	Micronuclei can occur or the chromosome can be distributed to daughter nuclei	RPE-1 & U2OS cells
			(Palmerola et al., 2022)	Asymmetric sister fork progression and low replication fork speed in the first cell cycle	Human oocytes - embryos
			(Nakatani et al., 2022)	The low replication fork speed increases replication stress	Mouse embryos and ESC
Spindle Assembly Checkpoint (SAC)	Monitors kinetochore attachment to correct chromosome segregation	Missegregation; linked to age-related declines in SAC activity and aneuploidy in embryos	(Vázquez-Diez et al., 2019)	APC/C inhibition extends mitosis, allowing time for correct chromosome alignment and reducing segregation errors	Mouse embryos
Spindle morphology and orientation	Abnormal spindle morphology and orientation of cell division	Disrupted mitotic processes, multinucleation, and increased aneuploidy	(Ono et al., 2024)	The first mitotic spindles lead to multinucleation at the 2-cell stage. Centrosome mispositioning during the first mitosis is partially	Human zygotes

				corrected after the second mitosis, restoring mononuclearity.	
			(Mizobe et al., 2024) ; (Porokh et al., 2024)	Often aneuploidy is observed when the first plane of division was perpendicular to the pronuclear. Axis. The first division predisposes human embryos to genetic (in)stability and may contribute to aneuploidy and age-related infertility.	Human embryos
			(Schneider et al., 2021)	The dual spindle assembly pathway is conserved in nonrodent mammals	Bovine zygotes
			(Cavazza et al., 2021)	The clustering of nucleoli in human zygotes serves as a key marker of effective chromosome organization, promoting accurate mitotic chromosome segregation and supporting healthy embryonic development.	Human and bovine zygotes

External Factors

Environmental and procedural factors, especially those associated with IVF, could contribute to full aneuploid embryos and aneuploidy post-fertilization, thereby affecting mosaic rates. Numerous studies have been conducted on the choice of culture media, culture conditions and specific laboratory conditions, concluding that this does influence aneuploidy outcomes. As an example, the preliminary study of Holmes et al. (2020) investigated the effect of media exchange methods between sequential and single-step culture media. Although the sample size was small (13 and 26 patients in phase I and phase II trials, respectively), PGT-A results showed a >10% decrease in euploid blastocysts in the single-step medium, showing the importance of how differences in culture media affect human blastocysts.

Another critical factor is the stimulation protocol used for follicle growth in IVF. This is needed to increase the number of eggs for fertilization. For stimulation, gonadotrophin-releasing hormone (GnRH) agonist is used to prevent a premature luteinizing hormone (LH) rise, ultimately increasing follicle growth. Follicle growth is a complex process, and disturbances in the hormonal signalling pathways that regulate follicular development can interfere with the proper chromosomal segregation during meiotic divisions (He et al., 2021).

A study by Baart et al. (2007) found that 61% of the patients where a mild stimulation protocol was used, a lower aneuploidy number was found in the embryos, compared to patients where a high stimulation protocol was used. The differences between the mild and high stimulation protocol was the type and dosage of the hormones. The mild ovarian stimulation regimen used GnRH antagonist co-treatment, and the conventional protocol used high-dose gonadotrophin regimen and GnRH agonist co-treatment. Summarizing, this study concluded that the mild protocol generated less oocytes, however showed a decrease in full aneuploid and mosaic embryos compared to high stimulation. As differences in mosaic embryos rates were observed, it suggests that the stimulation protocol has an effect on mitotic segregation errors**.**

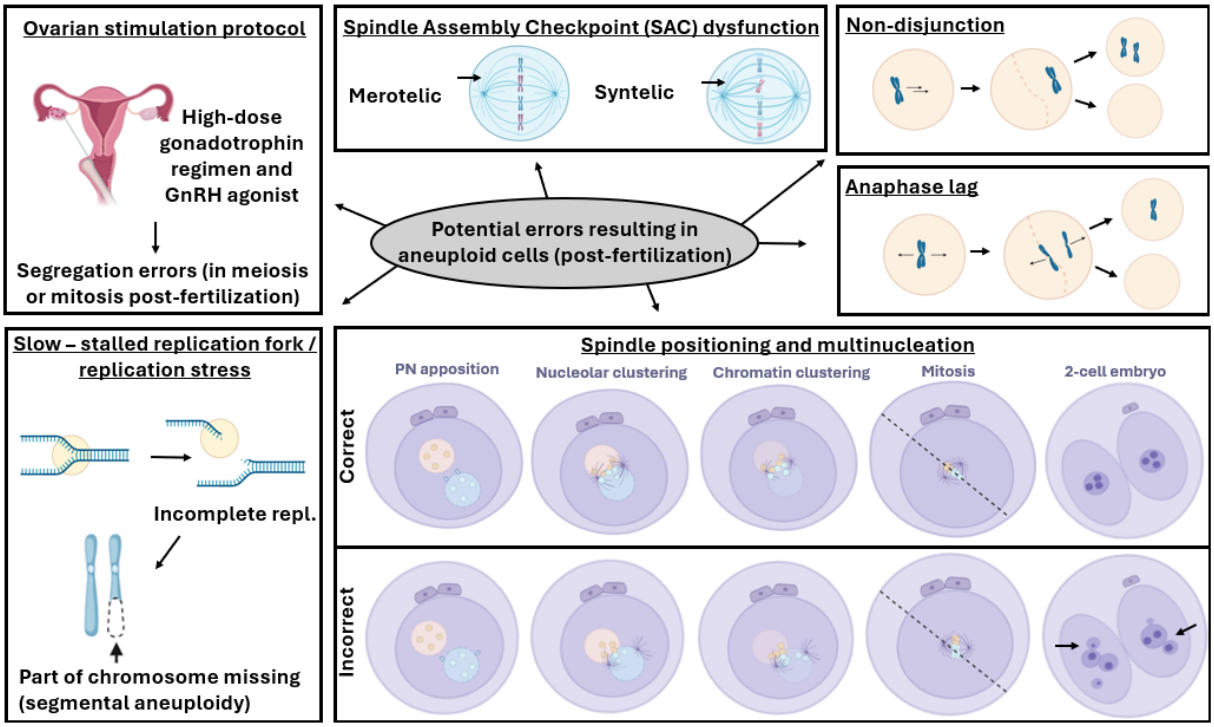

Figure 2: Overview of the most common errors resulting in mosaic embryos

This figure shows an overview of the following errors: mitotic non-disjunction, anaphase lagging, DNA replication stress, Spindle Assembly Checkpoint (SAC) dysfunction, spindle morphology and orientation and external factors. To enhance understanding, schematics were created for each potential contributor to mosaicism, providing a visual representation of the concepts discussed in the text.

Inspiration for the figure of spindle positioning and multinucleation: (Porokh et al., 2024).

Clinical Outcomes

The clinical implications of mosaic embryos are a central focus in IVF due to their impact on embryo viability, implantation success, and potential developmental outcomes.

Embryo transcription is initiated at the one-cell stage, however this is of very low magnitude and thus the zygote mostly relies on maternal RNA and proteins for survival (Asami et al., 2022). Gene expression increases after the 8-cell stage, meaning aneuploidy generally has minimal effect on development until this stage. After the 8-cell stage, chromosomal integrity plays a crucial role in embryo viability, as errors often result in developmental arrest. The transition to embryonic genome activation marks a critical period in which embryos with significant chromosomal abnormalities are likely to cease development, contributing to higher rates of embryonic arrest due to aneuploidy. Screening embryos at later developmental stages is therefore advantageous for selecting those with the highest developmental potential.

For both meiotic and mitotic errors the viability of an embryo depends on the extent and nature of the chromosomal abnormalities. Aneuploidies resulting from meiotic errors are typically non-viable in embryos, with the notable exception of trisomy 13 (47, XX or XY, +13), trisomy 18 (47,XX or XY, +18), trisomy 21 (47,XX or XY, +21) and a few known sex chromosome aneuploidies (Herbert et al., 2015), (Mikwar et al., 2020). If present, these specific aneuploidies result in Patau syndrome, Edward's syndrome, Down syndrome and other conditions. Babies with severe syndromes like Patau and Edwards often have serious brain, heart and spinal cord defects. As a result, many babies born with these syndromes only live a few days.

As indicated earlier, when euploid cells undergo mitotic errors, often daughter cell or cells become aneuploid, resulting in a mosaic embryo. While some embryos contain cells with segmental aneuploidy in non-essential chromosomal components, which may lead to successful pregnancies and healthy offspring, others may suffer from developmental delays depending on the genes affected by the chromosomal alterations (Zore et al., 2019). Whole chromosome aneuploidy is more often associated with a broader impact on clinical outcomes, as the gain or loss of entire chromosomes generally produces more severe genetic imbalances, often resulting in developmental arrest or early pregnancy loss (Spinella et al., 2018). Besides the type of aneuploidy present in certain cells in the embryo, the level (amount) of the cells affected is important. Clinical studies indicate that embryos with high level mosaicism—when the embryo consists of many aneuploid cells—tend to have lower implantation rates, an increased likelihood of miscarriage, and, in rare cases, congenital abnormalities if a mosaic embryo reaches full-term pregnancy (Capalbo et al., 2021) (Viotti, Victor, et al., 2021). An overview of the implantation rates, live birth rates and miscarriage rates, comparing full euploid and mosaic embryos, from different studies (all using euploid embryos as control group) is stated in table 3. From this data it is clear that (on average) implantation and live birth rates are higher in the euploid group compared to the mosaic embryo group. Conversely, the miscarriage rate is higher in the mosaic embryo group.

The detection of chromosomal abnormalities in embryos are commonly detected via PGT-A in clinics. Here, women—who for example have had multiple miscarriages or multiple failed IVF cycles—can potentially identify problems that have caused previous IVF cycles or pregnancies to fail. Such problems can be the previously explained causes such as full aneuploid or mosaic embryos PGT-A identifies chromosomal abnormalities through bulk analysis of a trophectoderm (TE) biopsy based on NGS profiles. However, the findings of Chavli et al. (2024) suggest that this method may underestimate mosaicism, potentially resulting in both false-negative and false-positive outcomes. Therefore, interpreting PGT-A

results requires caution, and patients considering PGT-A should be informed about its technical and biological limitations.

The ethical considerations surrounding the transfer of mosaic embryos in IVF are significant, especially since these embryos are sometimes the only option for patients aiming to conceive. Mosaic embryos, after extensive counselling, may offer a potential path to pregnancy when no euploid embryos are available or when the patient already failed to conceive. Mosaic embryos can be used for implantation when there are no other options possible, although they are associated with significantly poorer outcomes compared to euploid embryos. Current research supports that mosaic embryos can still lead to the birth of healthy, live children and that the selective transfer of mosaic embryos under specific circumstances is accepted (Lin et al., 2020) (Spinella et al., 2021).

Table 3: Overview of different rates between euploid and mosaic embryos

The implantation, live birth and miscarriage rates (%) between mosaic (M) and euploid (E) embryos are stated of different articles, as well as mosaic embryo parameters (if indicated in the article). Some articles do not mention certain rates, such as live birth or implantation (indicated with X).

Implantation rates (%)		Live birth rates (%)		Miscarriage rates (%)		Publication	Mosaic parameters mentioned in the publication?
M	**E**	**M**	**E**	**M**	**E**		
39.0	47.0	28.8	40.7	26.1	12.0	(Yakovlev et al., 2022)	This article shows rates of mosaicism in general – whole chromosome and segmental mosaicism (here mentioned = general mosaicism)
X	X	42.9 / 42.0	43.4	11.0 / 12.7	12.0	(Capalbo et al., 2021)	Mosaic embryos defined as low-grade mosaic (20-30% variation) / medium grade mosaic (30-50% mosaic)
41.8 / 46.5	57.2	X	X	20.4 / 25.0	8.6	(Viotti, Victor, et al., 2021)	Distinguish between mosaicism in general / whole chromosome mosaicism
X	X	27.1	47.0	33.3	20.5	(Y. X. Zhang et al., 2020)	Euploid embryos are compared with mosaic embryos in general, different tables show morphological grading of embryos and levels of mosaicism
X	X	30.0	53.8	40.0	18.2	(Zore et al., 2019)	In this table, the mosaic embryos are segmental mosaic (defined as mosaicism on a portion of a chromosome)
X	X	46.6	59.1	20.3	12.7	(L. Zhang et al., 2019)	In this article euploid embryos are compared to mosaic embryos in general (mosaic defined as >20% aneuploid cells in an embryo)
38.5	54.6	30.8	46.6	7.8	8.0	(Spinella et al., 2018)	In this table, euploid / mosaic embryos in general are shown, in the article comparisons are made between low (<50%) and high (>50%) level mosaicism
26.9	37.2	25.0	X	7.1	18.1	(Lledó et al., 2017)	In this table, euploid / mosaic embryos in general are shown, In the article they do mention that some embryos are aneuploid / missing 2 or more chromosomes
30.1	55.8	27.8	47.0	55.6	17.2	(Fragouli et al., 2017)	No

Discussion:

In recent years, mosaic embryos have garnered increasing attention in the field of assisted reproductive technology (ART), with more research focusing on their implications for embryo development and clinical outcomes. Despite this growing interest, there remains a significant lack of comprehensive understanding, particularly regarding the mechanisms underlying mosaicism and its impact on embryo viability. While mosaicism is increasingly being tested in clinical settings, it is not universally adopted, and when it is assessed, there is a lack of standardization in the interpretation of results. Moreover, the question of whether to implant mosaic embryos continues to be debated, with no clear consensus among clinicians. As research into mosaic embryos expands, it is crucial to address these gaps in knowledge and establish clearer guidelines for clinical practice.

To address these issues, existing literature was reviewed to explore two key questions. The first question is as followed: What are the different types of mosaicism in embryos, and what mechanisms contribute to their origin, particularly in relation to embryo viability? In this review different types of aneuploidy -which can be found in cells of mosaic embryos- were highlighted. These aneuploidies often arise from distinct mechanisms, specifically mitotic errors, each with varying consequences for embryo viability. Whole chromosome aneuploidy, generally originates from errors as a result from mis segregation events (non-disjunction or anaphase lag) or failures in the SAC which where cells proceed through division with misaligned chromosomes. A study by Vázquez-Diez et al. (2019) demonstrated how errors during SAC activation could lead to whole chromosome mosaicism due to chromosome misalignment. Another type of aneuploidy is segmental aneuploidy, which involves partial chromosomal abnormalities, such as deletions, duplications, or inversions of specific chromosomal regions. Potential contributing factors include replicative stress, spindle defects, or chromosomal fragmentation during (mostly early) cell divisions. If replication forks stall or collapse, it might lead to large-scale chromosomal rearrangements as the cell attempts to recover from this stress, leading to chaotic rather than controlled changes. Errors in DNA repair mechanisms may also contribute to segmental mosaicism. The size and location of these aberrations significantly impact developmental outcomes, as larger structural changes or disruptions involving important genes involved in development tend to result in more severe issues.

Additionally, we sought to answer our second research question: How do the various types of mosaicism impact clinical outcomes, including embryo development, implantation success, and pregnancy viability? In this review, it was highlighted (in table 3) that mosaic embryos have a lower implantation and live birth rate compared to euploid embryos. The miscarriage rates is higher when mosaic embryos are used for transfer. Certain studies, like Capalbo et al. (2021), show that the level of aneuploid cells in the mosaic embryos are also involved in these rates. High levels of mosaicism tend to have lower implantation rates, an even larger risk of miscarriage, and are more likely to result in congenital abnormalities if they reach full-term. Low-level mosaicism, where a minor proportion of cells are aneuploid, may still permit successful development, as normal cells can potentially compensate for abnormalities. The type of aneuploidy, which chromosomes are involved, and other factors also play a significant role. This supports our hypothesis that some forms of mosaicism are more compatible with embryo viability than others, depending on the type and extent of chromosomal abnormalities.

There are significant limitations in the field of mosaicism. Different studies use varying definitions and thresholds for classifying mosaic embryos, leading to inconsistency. While some consider an embryo mosaic if it has even one aneuploid cell, others only classify embryos as mosaic if a significant proportion of cells are affected. This lack of standardized

terms makes comparisons difficult. In this review, in table 1, an overview of different studies and their used cut-off values for determination of low- and high level mosaicism is shown. The majority of these studies accept a cut-off value of 50%, however some studies still use alternative values, making the classification standards variable. Besides this limitation, differences in biopsy techniques, the number of cells sampled, and the timing of retrieval can yield differing outcomes. Ethical challenges strongly restrict research on human embryos, as their use in scientific studies is often met with societal and regulatory disapproval. In the Netherlands, the Embryo Law prohibits the fertilization of human oocytes for research purposes. Additionally, the limited availability of fertilized human eggs for research purposes restricts the sample size, reducing the statistical power and reliability of many studies. Furthermore, reliance on animal models, such as bovine and mouse embryos, poses limitations, as these models may not accurately replicate the biological processes of human embryonic development.

To address the complexity of the thresholds for classifying mosaic embryos, and whether these embryos should be transferred, we propose a model for embryo transfer selection: a hierarchical ranking system designed to optimize pregnancy success rates while minimizing associated risks. This hierarchy prioritizes embryos based on chromosomal integrity and viability, beginning with euploid embryos, which possess the correct number of chromosomes. Euploid embryos are favoured due to their high implantation potential and reduced risks of miscarriage or chromosomal disorders, making them the optimal choice for achieving healthy pregnancies.

When no euploid embryos are available, the model shifts focus to embryos with low-level mosaicism, defined as having less than 50% of cells showing chromosomal abnormalities, as summarized in table 1. While these embryos present a slightly increased risk compared to euploid embryos, they have demonstrated potential for successful pregnancies and healthy live births, as studied by Lin et al. (2020) and Spinella et al. (2021). Their selection is often guided by patient-specific factors, with clinicians prioritizing their transfer when more favourable options are unavailable. The proposed hierarchy continues with middle and high-level mosaicism embryos, which exhibit a higher proportion of cells with the incorrect chromosomal number, and carry a reduced likelihood of successful outcomes. Advances in genetic testing and closer monitoring have allowed more informed transfer decisions in certain cases, primarily when no other viable embryos exist.

At the lowest tier of the hierarchy are aneuploid embryos, characterized by full chromosomal abnormalities across all cells. These embryos are generally excluded from transfer due to a low likelihood of healthy development and increased risks of implantation failure or miscarriage. Their use is restricted to rare or experimental scenarios.

To enhance this hierarchical model, we propose a multifactor assessment approach to embryo selection that incorporates a more nuanced understanding of chromosomal mosaicism. This approach relies on the routine use of PGT-A, which is not yet universally adopted in all IVF protocols. Combining both genetic testing and morphological evaluation offers a more comprehensive method for assessing embryos. It is crucial to recognize the occurrence of mosaicism and its varied impact depending on when and where it emerges during embryo development. Rather than automatically discarding embryos with only or partial aneuploid cells, this multifactor approach ranks embryos based on the degree and type of mosaicism, alongside additional viability factors such as chromosomal data, timing, and distribution of abnormal cells.

Looking ahead, advancements over the next two decades are expected to significantly reshape embryo selection and preimplantation genetic testing. A good example of a test

under development is non-invasive PGT-A (niPGT-A). Although still in development, this method offers a less intrusive alternative by analysing cell-free DNA in the culture medium or blastocoel fluid. This method holds promise for reducing embryo damage while maintaining accuracy in chromosomal analysis (Huang et al., 2019). Despite its potential, niPGT-A faces technical hurdles. For instance, Hanson et al. (2021) reported DNA amplification failures in 62 out of 166 tested embryos, indicating that further refinement and validation of this technique in clinical settings are crucial. However, if this is through in the clinical trials, this approach has the potential to become the standard for genetic screening, enhancing safety, accessibility, and minimizing embryo stress. Future clinical practices will likely become more precise and personalized, for example the use of AI to predict pregnancy outcomes. Such technologies will optimize embryo selection and further increase pregnancy success rates. Key research initiatives should focus on optimizing and finding new alternatives for detection of aneuploidy and developing advanced tools for assessing for example epigenetic factors, as this has not been researched thoroughly and functional viability in embryos. Finally, it is essential to explore the developmental implications of mosaicism through longitudinal studies, as there is a lack of research on the long-term outcomes of mosaic embryos in relation to child development.

By integrating these advancements, assisted reproductive technologies can continue to prioritize safety, optimize outcomes, and uphold ethical standards in reproductive genetics. A summary of the current IVF procedure, embryo testing and outcome is clearly overviewed in figure 3, as well as certain new options to explore in this field as mentioned in this text.

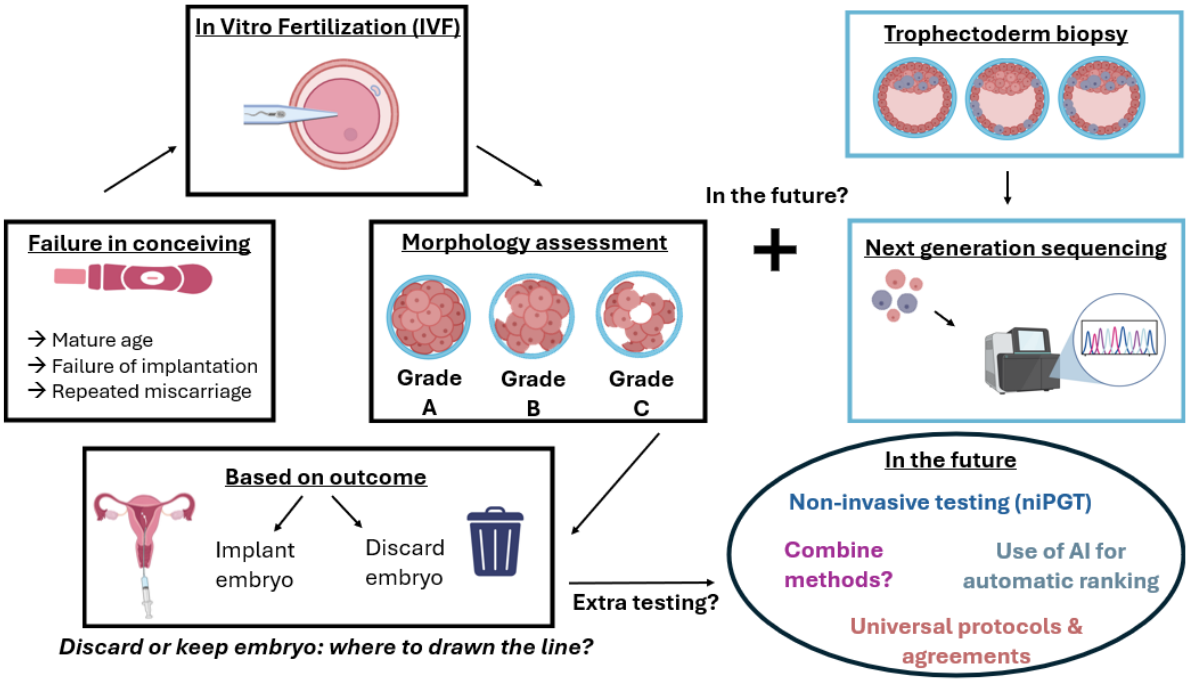

Figure 3: The current IVF cycle in the Netherlands and possible future prospects
This figure shows a schematic overview of the IVF cycle currently utilized in the Netherlands, where embryos are assessed based on morphology. Possibly, in the future PGT-A could be included in this procedure, schematically shown in square boxes with blue border. Future prospects are stated in the circle.

Conclusion:

The main findings of this study indicate that mosaic embryos, the term used to describe the presence of both euploid and aneuploid cells within the same embryo, often is the result of mitotic errors. Meiotic errors can give rise to full aneuploid embryos. Both mosaic embryos and full aneuploid embryos have varying consequences for embryo viability and development. Generally, whole chromosome aneuploidy has a more detrimental outcome compared to segmental aneuploidy, which is also dependent on which chromosome is affected, with higher levels of aneuploid cells reducing viability. This review proposes adopting a hierarchical embryo selection model for assisted reproductive technologies, prioritizing euploid embryos and considering mosaic embryos under specific conditions. The integration of chromosomal analysis, mosaicism extent, and the use of better assessment protocols offers a promising path forward for refining clinical approaches and better outcomes. The proposed model aims to enhance embryo selection in IVF by incorporating routine pre-implantation genetic testing for aneuploidy (PGT-A) as a standard for each country, ultimately boosting IVF success rates and minimizing unnecessary embryo loss.

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Supplementary tables

Overview of studies investigating the correlation between maternal age and (type of) mosaicism or full aneuploidy

These tables all show the outcomes of different studies (publication included), showing the percentage of mosaicism found, the age group, sample size number (amount of embryos biopsied) and methodology. Different colours were used to give an easy overview of the highest percentage (red), middle (orange) and lowest (yellow, green) per row.

Table 1

%	<35	35-37	38-40	41-42	>42	Publication	Methodology:
Single segmental	38%	37%	32%	29%	24%	(Armstrong et al., 2023)	PGT-A NGS
Complex segmental	7%	6%	6%	6%	5%		Total embryos biopsied: 26.745 (only mosaic result)
Single chromosome	30%	31%	34%	36%	37%		
Mosaic complex abnormal	26%	27%	28%	30%	34%		

Table 2

%	<35	35-37	38-40	41-42	>42	Publication	Methodology:
Euploid	58%	51%	41%	27%	16%	(Armstrong et al., 2023)	PGT-A NGS
Low -level mosaic	10%	9%	8%	5%	3%		Total embryos biopsied: 86.208
High-level mosaic	9%	8%	8%	8%	6%		
Aneuploid	17%	23%	32%	42%	42%		

Complex abnormal aneuploid	7%	8%	11%	18%	33%		

Table 3:

%	<35	35-37	38-40	>41	Article DOI	Methodology
Segmental	3	4	4	3	(Reich et al., 2020)	PGT-A NGS
Whole chromosome	15	15	15	15		Total embryos biopsied: 10.545
Other	10	11	12	23		

Table 4:

%	<35	35-37	38-40	>41	Article DOI	Methodology
Mosaic rates	33	30	31	32	(Escudero et al., 2016)	PGT-A NGS
Aneuploidy rates	19	26	36	55		Total embryos biopsied: 8555
Euploid rates	48	44	33	14		

Table 5:

%	18-22	23-30	Article DOI	Methodology
Mosaic rates	17	15	(Villanueva Zúñiga et al., 2022)	PGT-A NGS
Aneuploidy rates	23	26		Total embryos biopsied: 3222
Euploid rates	60	59		