Chapter

Molecular and Cellular Mechanisms Underlying Preimplantation Embryo Development

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Abstract

Preimplantation embryo development refers to the maturation of a fertilized ovum to a blastocyst. This process is highly regulated and required for proper implantation of the blastocyst into the endometrium. During this phase, several tasks must be accomplished. The differentiated zygotic genome must undergo reprogramming back to totipotency in order to generate all of the different types of tissue making up a human. Next, certain cells begin to differentiate to prepare for implantation which occurs at approximately day 7 post-fertilization. This progression is a result of a careful interplay between maternally persistent RNA transcripts and activation of the zygotic genome. After the embryonic genome activation, blastomere differentiation begins to occur. Cellular polarity has been shown to be the signal transduction that initiates this differentiation. Understanding the molecular and cellular mechanisms regulating preimplantation embryo development is of fundamental importance for reproductive science and has numerous applications in fields such as assisted reproductive technology and stem cell therapy.

Keywords: development, implantation, fertilization, embryo, zygote, blastocyst

1. Introduction & overview

Until recently, most analysis of human preimplantation embryo development has focused on morphological changes, and little was known about the cellular and molecular mechanisms regulating the process. What was known was mostly derived from analysis of embryo development in fish, amphibians, mice, and other animals; however, recent research has examined this process in greater detail in humans. Across species, there are many general similarities in embryonic development. A major commonality is the transition of embryonic developmental control from the maternal genome to zygotic genome. This is commonly referred to as the maternal to zygotic transition (MZT). The MZT requires two major steps: the degradation of persistent maternal RNA and the activation of the zygotic genome (ZGA) through transcriptional and epigenetic mechanisms.

To examine the mechanisms of preimplantation development, it will be useful to provide a brief morphological overview of the process starting with fertilization.

All times referenced will use fertilization as the zero point. Sometimes the specific time frame may be referred to using the number of cells present e.g., 8-cell stage (**Figure 1**).

Human-specific differences in embryonic development are exhibited in the timing of the ZGA and epigenetic modifications [1]. The ZGA is not a discrete event but occurs over a continuum with two waves of increased transcriptional activity. In humans, a minor wave occurs around the 4-cell stage (~48 hours), and a major wave occurs around the 8-cell stage (~72 hours), whereas in mice the minor wave occurs at the late zygote (~24 hours) and the major occurs at the 2-cell stage (~36–48 hours) [2, 3]. As embryonic gene transcription increases, maternal factors regulating development decrease. After the ZGA, the embryo continues division and transitions from a loose cluster of cells to a densely packed ball known as a morula (~day 4). This process is known as compaction. After formation of the morula, divisions continue, and the first cellular differentiations begin causing cavitation. The resulting embryonic structure is known as a blastocyst (~day 5). Cells along the outer edges of the embryo differentiate in trophectoderm (TE) and the inner cells become the inner cell mass (ICM), which are the cells that will become the fetus. The blastocyst is what will then implant into the uterine wall for continued embryogenesis. For this to occur properly, the blastocyst must "hatch" from its protective outer coating, the zona pellucida (~day 6). The final step is implantation of the embryo into the uterine wall (~day 7–8). Cells of the trophectoderm begin to differentiate into syncytiotrophoblast and cytotrophoblast. Syncytiotrophoblast

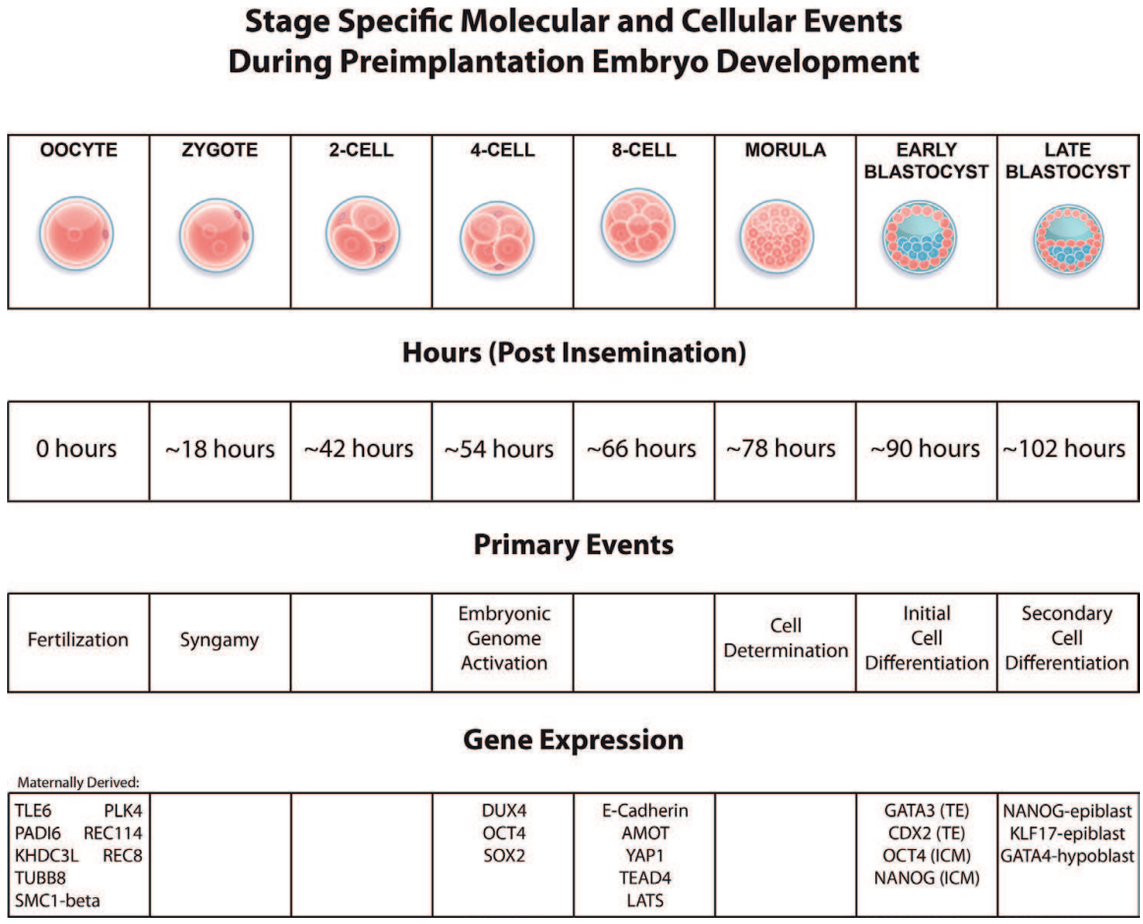

Figure 1.
Stage-specific molecular and cellular events that take place during preimplantation embryo development. Genes listed under each developmental stage are associated with elevated expression at that time of development.

mediates attachment to the uterine wall. Cytotrophoblast can be thought of as stem cells that produce more syncytiotrophoblast or invasive cytotrophoblast, which directly invade the endometrium allowing for implantation.

In summary, the first step of embryo development is fertilization. For this to occur, the ovum and sperm must fuse their nuclear material generating a zygote. After fertilization, the zygote undergoes several divisions, and around the 8-cell stage, heterogeneities in cell types begin to emerge. By approximately day 4–5, the first morphological differences are seen with the formation of a blastocyst. Cells begin to differentiate into the ICM and trophectoderm. After blastocyst formation, a second wave of differentiation occurs forming the epiblast and hypoblast which will form the embryo and primitive endoderm, respectively. Around day 7–8, the embryo will implant into the uterine wall via trophectoderm-derived syncytiotrophoblast and cytotrophoblast which mediate the attachment.

2. Preimplantation development

2.1 Fertilization and zygote genome activation

2.1.1 Fertilization

Fertilization is the first step in embryo development after the entry of the sperm into the oocyte and is generally regarded as the zero point in describing the timeline of development. Following the cortical reaction, the ovum is stimulated to complete its second meiotic division and release the second polar body. This trigger is mediated by the sperm causing an increase in calcium within the ovum via the DAP/IP3 pathway. After the completion of the second meiotic division, the female pronucleus is formed. The male pronucleus rotates 180 degrees positioning the centrosome between itself and the female pronucleus [4]. The centrosome's microtubules function to draw both of the pronuclei toward each other [4]. As the pronuclei approach, they concurrently move toward the center of the cell [4]. Once sufficiently close, each pronuclei membrane ruptures and fusion occurs resulting in the formation of a diploid zygote [4].

2.1.2 Reprogramming zygotic genome

Post-fertilization, the zygote genome must be transformed back into a totipotent state to generate the variety of tissues that will eventually make up the human fetus. Initially, the zygote genome is relatively inactive while maternally derived transcripts and proteins are the major effectors of development and are responsible for reprogramming the zygote genome [5]. Reprogramming is almost entirely dependent on maternal factors. This phenomenon is exhibited by the fact that a terminally differentiated cell nucleus can be reprogrammed into a pluripotent state by maternal factors within an oocyte [6]. Due to the dependence on the maternal genome, certain maternal genes have been implicated in arrest of the early embryo. Specifically, genes involving the subcortical maternal complex (SCMC) have been linked with early embryo failure [7]. The subcortical maternal complex is a recently discovered maternally derived multiprotein complex that has several important functions in sustaining early embryo development. The functions of the SCMC include organelle distribution, translational regulation, and epigenetic reprogramming. **TLE6**, **PADI6,** and **KHDC3** are all genes that code for components of the SCMC, and each has been associated with early embryo arrest [7].

This transition to a totipotent genome and beginning of ZGA is mediated by DNA and histone modifications [5]. Paternally and maternally derived genes are differentially modified due to the asymmetric epigenetic modifications present in the terminally differentiated egg and sperm. During spermatogenesis, sperm histones are replaced with protamines which must be replaced with maternal histones. Maternally derived histone H3.3 replaces the protamines, which yields an increase in the transcriptional accessibility of genes required for pluripotency. Nucleoplasmins are chaperones thought to play a role in replacing protamine with H3.3. In frogs, nucleoplasmin 2 (NPM2) was shown to be required for embryo development [8]. Additionally, knockout of H3.3 in mice causes embryo arrest and shows decreased levels of gene expression associated with pluripotency [9].

The parental genome undergoes active demethylation through the action of 10–11 translocation (**TET**) methylcytosine dioxygenases prior to cleavage, and this demethylation continues throughout the preimplantation period [10]. During spermatogenesis, the parental genome is highly methylated, so this active demethylation is required for totipotency. Additionally, the maternal pronuclear DNA has also been shown to undergo active demethylation as well [8]. The demethylation process is critical for proper embryo development. In mice, lack of TET3 causes increased methylation of the paternal genome which resulted in increased incidence of embryonic failure [8]. Additionally, Cullin-ring finger ligase-4 (CRL4) ubiquitin ligase has been shown to upregulate TET3 activity and play a role in female fecundity. In mice, deletion of CRL4 component results in embryo lethality [11]. Additionally, increased levels of DNA methylation have been linked with aneuploidy and negative embryo quality [12].

2.1.3 Zygotic genome activation

As the zygote genome is being reprogrammed, there is a concurrent shift in regulatory control over development. There is a decrease in maternal transcript activity and an increase in embryonic transcripts. In addition to their role in establishing totipotency, histone modifications also play a major role in ZGA. In humans, there is an increase in trimethylated histone H3 lysine 4 (**H3K4me3**) which is associated with gene activation, and a decrease in histone H3 lysine 27 (**H3K27me3)** which is associated with gene repression [13]. Several mechanisms exist to ensure proper timing for transcriptional activation of the zygote genome. One mechanism is the titration of maternal repressors. During the first few cellular divisions, the volume of the embryo does not change. As divisions progress, there is an increase in the nucleus to cytoplasm ratio which dilutes maternally persistent repressors [2]. Another method regulating ZGA timing is the synthesis of transcription factors. The embryo lacks key transcription factors, but translation of maternally supplied mRNA leads to their synthesis [2]. These factors are synthesized and eventually, their accumulation will reach a threshold leading to transcriptional activation of the zygote genome. One such transcription factor is **OCT4**. **OCT4** is a transcription factor that is responsible for stem cell self-renewal, and it is used in the induction of pluripotent stem cells. Binding regions for OCT4 are upregulated in accessible regions during the MZT [2]. In mice, OCT4 knockout embryos do not gain totipotency and are non-viable. These embryos develop to the blastocyst stage, but the entirety of the embryo differentiates into trophoblast, and none of the cells maintain pluripotency required for formation of the ICM [14, 15]. In humans, **OCT4** seems to play an earlier role directly associated with zygotic genome activation. Human **OCT4** expression begins as early as the

cleavage stage. Lack of **OCT4** in humans results in failure to form a viable blastocyst [16, 17]. Not surprisingly, upregulation of **OCT4** plays a role in the tumorigenesis of several cancers [16]. **SOX2** is a transcription factor that forms a complex with **OCT4** to co-bind the regulatory region of DNA [18]. Another transcription factor that is vital to human ZGA is **DUX4**. **DUX4** has been shown to have increased expression before the ZGA and bind to promoters of ZGA genes, increasing their transcription. Additionally, knockout of the murine analog for Dux halts embryo progression at the 2-cell stage [19].

The progression from maternal to embryonic gene activation is a major regulator of early embryogenesis. Progression through each stage of development is associated with specific transcriptome activation derived from a combination of maternal and embryonic RNA. In early embryogenesis, this combination is almost entirely maternal RNA, and later it is entirely embryonic. Oocytes up to the 2-cell stage embryo show high levels of maternal mRNA. Two cell stage embryos show low levels of RNA from both maternal and embryonic sources. It is not until the 4–8 cell stage that embryonic RNA levels begin to increase. Maximum zygotic gene expression does not occur until the blastocyst stage. This variation in gene express allows for a stage specific gene expression profile to be created. Examination of gene expression patterns at different stages of development could provide insight into preimplantation embryo viability [20].

2.2 Heterogeneity leads to differentiation and blastocyst formation

2.2.1 Generation of asymmetry between blastomere

Molecular and morphological blastomere asymmetries are what will eventually result in differentiation. The first cleavage introduces variability between blastomeres. Partition error and transcriptional noise (fluctuations in gene expression) are two processes that contribute to blastomere heterogeneity. These chance fluctuations can be enough to determine a cell's lineage specification. Partition error is the unequal distribution of cellular contents (such as maternal mRNAs) during cellular division. Both phenomena increase heterogeneity between cells; however, in the early embryo, partition error likely contributes to this variability the greatest due to low levels of transcription [21]. Once transcription begins to increase, the heterogeneity initially caused by partition error can be amplified leading a cell to differentiated fate. Lineage analysis has shown that cell fate bias may begin as early as the 4-cell stage in mice [5].

2.2.2 First differentiation and the morula to blastocyst transition

In humans, blastomeres begin to undergo compaction followed by the first signs of polarization between days 3 and 4 post-fertilization (8–16 cell stage) [22]. Compaction refers to the transition of the blastomere arrangement from a loose bundle of cells to a tightly packed mass increasing the area of cell-to-cell contacts. Polarization of the blastomeres is critical to establishing distinct cell lines. The first polarized cells along the outer rim of the embryo will generate the trophectoderm (TE), and the central nonpolar cells will form the pluripotent inner cell mass (ICM). **OCT4** and **NANOG** are genes with localized expression to the ICM. **OCT4** was discussed earlier in its relation to the ZGA where it is vital in maintaining pluripotency and is thought to function in a similar manner for cells of the ICM [23].

As compaction occurs, organelles, cytoskeletal elements, and cell adhesion molecules begin to preferentially localize in different regions of the cell, generating the apicobasal polarity. A lack of compaction will result in no blastocyst formation and early arrest of the embryo [24]. Several adhesion and cytoskeletal proteins have been shown to play key roles in the process of compaction and polarization in the morula stage. In mice, a lack of E-cadherin, α-catenin, and β-catenin causes embryo arrest [24]. Specifically, **E-cadherin** and **β-catenin** have been shown to play a vital role in humans as well [25]. **E-cadherin** is a cellular adhesion molecule, and α-catenin links the **E-cadherin/β-catenin** complex to the intracellular cytoskeleton composed of actin. The localization of these adherens junction (AJs) is critical for initiating a signaling cascade generating cell heterogeneity and differentiation [25]. Additionally, phospholipase C (**PLC**) has recently been shown to contribute to apicobasal polarity [22]. The mechanism involves recruiting actin–myosin complexes to the apical membrane [22]. RNA interference repressing **PLC** demonstrated that a lack of **PLC** leads to a lack of cell polarity [22]. The establishment of cell polarity is critical for Hippo pathway signaling which stimulates cell fate differentiation.

In mice, the Hippo pathway is central in the first differentiation of blastomeres to either the trophectoderm or inner cell mass. Hippo signaling inhibits two major transcriptional activators of a trophectoderm fate program, YAP1 (Yes-associated protein 1) and TEAD4 (TEA-domain family member 4) [25]. YAP1 is a transcriptional cofactor that localizes to the nucleus and interacts with transcription factor TEAD4 to promote differentiation to the trophectoderm. The Hippo pathway results in phosphorylation of *YAP1* which prevents its localization to the nucleus. Without the YAP1 nuclear localization, TEAD4-mediated transcription of GATA3 and CDX2 does not occur [5, 25]. GATA3 and CDX2 are both transcription factors that promote the trophectoderm lineage [5]. Thus, activation of the Hippo pathway leads to an ICM fate, and inhibition of the Hippo pathway leads to a TE fate. Hippo pathway signaling is modulated via angiomotin (AMOT). The major regulator of AMOT is cell polarity and adherens junction localization [26]. In inner cells, AMOT binds to AJs via an E-cadherin and NF2 complex [24]. When bound to AJs, AMOT is phosphorylated by large tumor suppressor kinase (LATS) which acts as the molecular switch to turn on the Hippo pathway preventing TE differentiation[24]. In outer cells, atypical protein kinase C (aPKC) sequesters AMOT to the apical membrane. Apical cellular domains are rich in f-actin which binds AMOT sequestering it from adherens junctions [26]. Without AMOT activating the Hippo cascade, YAP1 translocates to the nucleus activating transcription of the TE fate program. Altogether, Hippo pathway signaling in mice is modulated by cell polarity. The presence of an apical domain suppresses Hippo signaling resulting in a trophectoderm fate, whereas lack of an apical domain maintains pluripotency required for the formation of the ICM. The Hippo pathway's role in embryo differentiation was first discovered in mice, but evidence are emerging to suggest a conserved role in cows and humans [25].

2.2.3 2nd differentiation

As the blastocyst develops, the second round of cell differentiation occurs. The ICM differentiates into either epiblast or hypoblast. The hypoblast is what will eventually form the lining of the yolk sac. Epiblast will give rise to the primary germ cell layers which are the endoderm, mesoderm, and ectoderm. Epiblast must maintain a pluripotent state to generate such diversity of tissue. Several genes in mice and humans have been linked to formation of the epiblast. In mice and humans, the

genes **GATA4**, GATA6, and **NANOG** have been associated with the second cell fate decisions. **NANOG** is associated with epiblast formation while **GATA4** and GATA6 are associated with hypoblast lineage. In mice, FGF/MAP Kinase signaling modulates the transcription factors NANOG and GATA6 Interestingly, human epiblast is also associated with increased **NANOG**, but it is not dependent on FGF signaling as in mice [27]. **TGF-β** is another marker highly associated with the epiblast. Inhibition of **TGF-β** leads to decreased amounts of **NANOG** suggesting it is also required for pluripotency of the epiblast [28]. **KLF17** is a protein that has been observed to colocalize with **NANOG** within the epiblast [28]. Recently **KLF17** has been shown to stimulate pluripotency which is not surprising given its relative localization within epiblast [29].

3. Early embryo pathology

3.1 Cellular & molecular changes

3.1.1 Overview

Human pregnancy loss is much more common than in other species [30]. Estimates of natural human pregnancy loss frequency have varied greatly over the years. This is due to the difficulty in the availability of data on fertilization efficiency. Some sources have estimated the pregnancy loss rate to be as high as 70% from fertilization until birth, but recently a range of 40–60% seems to be more widely agreed upon. Of these, 10–40% are due to preimplantation embryo loss [31]. Furthermore, assisted reproduction technology (ART) has a live birth rate of ~23% [32]. Understanding the pathologic mechanisms for these failures has critical significance for the field of reproductive science. Generally, these failures are due to cellular or molecular etiologies.

3.1.2 Cellular Pathology

Fragmentation, also known as blebbing, occurs when a portion of an embryo's cell breaks off from the rest of the cell resulting in a membrane-bound cell fragment. Fragmentation may occur as early as the first embryo cell division [33]. Fragmentation is a common occurrence in embryos; however, high levels of fragmentation are associated with embryo loss. Specifically, embryos with less than 10% fragmentation have the highest likelihood of implantation. Fragmentation has been shown to be a dynamic process in that cytoplasmic fragments can be resorbed back into blastomeres. There is considerable variability in the size and cellular and molecular composition of these fragments [33]. Several etiologies of these fragments have been proposed.

Blastomere exclusion is one phenomenon that could generate these fragments [33, 34]. Recent time-lapse monitoring of embryo development has shown that blastomere exclusion is a common occurrence (64%) [34] Chromosomal analyses of these excluded blastocysts show a higher frequency of aneuploidy which is consistent with the hypothesis that this could be a mechanism of self-correction [33].

Chromosome-containing micronuclei are another potential origin of embryo fragments. Micronuclei are nuclear membrane-bound structures located in the cytoplasm which contain damaged chromosomes or chromosome fragments [35].

Apoptotic bodies are also the possible origin for embryo fragments. Apoptosis has been suggested as playing a key role in preimplantation development. During apoptosis, the cell shrinks as plasma membrane blebs to form apoptotic bodies which break off from the cell [36, 37].

Persisting polar bodies also may make up some embryonic fragments. Normally polar bodies undergo apoptosis within 1 day of formation. However, evidence exist for their persistence. DNA analysis of some fragments has shown that all of the fragment DNA is maternally derived, specifically from the second polar body [38].

Another potential source of embryonic fragments is extracellular vesicles (EVs). Recently, EVs have been identified as being released from blastomeres of the preimplantation embryo and within blastocoel fluid. These vesicles have been implicated in cell-to-cell communication within the embryo [33, 39, 40].

Abnormal cytokinesis has been shown to be one cause of fragmentation [41]. Therefore, it is not surprising that genes regulating microtubule organization have also been linked to embryo fragmentation and arrest. One such gene is *Tubulin B 8 class VIII (TUBB8)* **TUBB8** codes for a β-tubulin specifically in oocytes as a component of the meiotic spindle. In humans, loss of **TUBB8** impairs meiotic divisions that are critical for fertilization and post-fertilization cell divisions [42]. In mice, mutations in TUBB8 result in embryos with high degrees of fragmentation [43].

Fragmentation is theorized to be associated with reduced implantation rates for multiple reasons. One possibility is due to loss of important cytoplasmic contents, such as regulatory proteins, mitochondria, and mRNA [33]. In fact, mitochondria are the most commonly isolated structures in embryo fragments [44]. Another mechanism proposed for impairing embryo development is disruption of the spatial arrangement of blastomeres [33]. As previously discussed, proper topographical positioning is crucial for proper embryo development.

Fragment removal has recently garnered interest as a technique to improve implantation outcomes. Data supporting this therapy is mixed. Some studies have shown a beneficial effect and others have shown no difference [44, 45]. This variation could be due to different etiologies of fragmentation. If the fragments contain essential organelles such as mitochondria, then removal could deprive blastomeres of vital ATP. In contrast, if the fragments did not contain essential structures, then removal could be beneficial. The most recent study analyzing fragment removal showed a benefit of fragment removal at day 2 which is the safest for the developing embryo. Day 2 embryos have larger blastomeres with more accessible perivitelline space making removal easier and decreasing the time spent outside of the incubator. In addition, day 2 embryo cell membranes are the most resistant to the mechanical stress from manipulation. As time progresses *in vitro* culture media is not able to supply the necessary membrane components and they become stiff and inelastic [45]. More research must be done to evaluate this potential tool for improving pregnancy outcomes.

3.1.3 Molecular Pathology

The major molecular etiology of embryo arrest is aneuploidy. Aneuploidy refers to a cell having an abnormal number of chromosomes. Aneuploidy is the most common cause of pregnancy loss [46, 47]. Aneuploidy is estimated to affect 4–5% of clinical pregnancies and has been shown to have an even higher incidence in preimplantation embryos [46]. The most common cause of embryonic aneuploidy stems from errors meiotic division of oocytes [47]. At 40 years old, approximately 74% of oocytes that

have completed both meiotic divisions are aneuploid. In females less than 36 years old, errors in meiosis I are most common. In females greater than 36, errors in meiosis II are most common [46]. At the cleavage stage, around 83% of embryos are aneuploid. This increase is thought to be due to mitotic errors since aneuploidy is only seen in approximately 5% of sperm [46]. Interestingly, by the blastocyst stage, only 58% of embryos were chromosomally abnormal [46]. The prevailing thought is this is due to apoptosis of nonviable cells. Additionally, some embryos may have completely arrested and not progressed to the blastocyst stage.

Aneuploidy has a very high association with increased maternal age. Several mechanisms such as recombination errors, cohesion dysfunction, spindle assembly failure, and mitochondrial dysfunction are all implicated in the decline of oocyte quality with age.

Evidence shows that the ability to repair recombination errors decreases with maternal age [48]. Experiments on several genes required for recombination such as meiotic recombination protein **REC114** and synaptonemal complex protein SCP3 highlight the importance of the process in preventing aneuploidy. REC114 is a maternally derived gene that encodes a protein that forms double-stranded breaks initiating homologous recombination, which is critical for proper segregation of homologs in meiosis. Mutations in the gene **REC114** result in multiple pronuclei formation and have been linked to early embryonic arrest and female infertility [49]. SCP3 is a component of the synaptonemal complex formed between homologous chromosomes during the prophase of meiosis. In mice, loss of SCP3 results in aneuploidy due to abnormal chromosomal segregation during oocyte meiosis [50].

Cohesins are protein complexes required for meiosis and mitosis via holding sister chromatids together. In mice, knockouts of cohesin subunit proteins Smc1β or Rec8 result in aneuploidy and lethality in early embryos. In humans, levels of **Smc1β** and **Rec8** have been shown to decrease with age [48]. Additionally, the intrakinetochore distance (iKT) of sister chromatids increases with age. As the distance increases, the sister chromatids no longer function as a single unit which results in abnormal separation and increases the likelihood of aneuploidy [48].

The spindle assembly checkpoint (SAC) is another maternal factor thought to decrease in quality with age. The SAC functions to ensure proper chromosome segregation by preventing separation of chromosomes until each is properly attached to the spindle apparatus. The SAC is made up of multiple proteins, and one of the most studied is mitotic arrest deficient protein 2 (MAD2). MAD2 specifically inhibits the anaphase-promoting complex (APC/C). The APC/C is a ubiquitin ligase that normally degrades cyclin B1 stimulating the metaphase to anaphase transition. MAD2 associates with APC/C preventing it from degrading cyclin B1 and thus halting the cell in metaphase. In mice, RNAi inhibition of MAD2 led to increased levels of aneuploidy along with increased levels of chromosomal misaggregation and reduced fertility [48]. In a related manner, genes regulating microtubule organization have also been linked to aneuploidy and early embryo arrest. PLK-4 is a serine/threonine kinase that is a major regulator of centriole assembly. In humans, mutations in **PLK-4** are associated with abnormal mitotic spindle formation and embryo cleavage. Specifically, **PLK-4** mutations result in tripolar mitotic spindle assembly which causes abnormal chromosome separation and aneuploidy [51].

Mitochondrial dysfunction also plays a role in the age-related decline of oocyte quality. Meiotic errors have been the most implicated so far in this discussion of aneuploidy. It should be no surprise that mitochondria have responsibility as well

since they are the key energy providers for the meiotic processes such as microtubule assembly, chromosome segregation, cytokinesis, etc. Mitochondrial damage from reactive oxygen species (ROS) is thought to play a major role in this dysfunction. Mitochondria are the major generators of ROS but also have the least defenses against these ROS due to limited DNA repair mechanisms. Thus, ROS damages mitochondrial DNA (mtDNA) which causes mitochondrial dysfunction which then causes an increase in ROS and ultimately creates a repetitive cycle leading to more mitochondrial dysfunction. Mitochondria DNA mutations are estimated to accumulate at a rate of 25 times more than nuclear DNA [52]. Antioxidant levels have been shown to decrease with maternal age. In mice, the antioxidants peroxiredoxin 3 (Prdx3), thioredoxin 2 (Txn2), glutaredoxin 1 (Glrx1), glutathione S-transferase mu 2 (Gstm2), and superoxide dismutase 1 (Sod1) have all been shown to be decreased in advanced age [53]. Mitochondrial dysfunction leads to ATP depletion which ultimately causes failure of meiotic spindle assembly resulting in aneuploidy [53].

A recently discovered phenomenon that is closely related to aneuploidy is the relatively high incidence of mosaicism in the embryo. Mosaicism is defined as the presence of more than one genetic cell line. In contrast to the meiotic errors causing aneuploidy in all cells, mosaicism typically arises from mitotic errors after fertilization. The two most common etiologies of mosaicism are nondisjunction and anaphase lag [54]. Importantly, mosaicism and aneuploidy are not mutually exclusive, and mosaicism is associated with a spectrum of aneuploidy.

Embryonic mosaicism has important ramifications for preimplantation genetic testing used in assisted reproduction technology (ART). Preimplantation genetic testing for aneuploidy (PGT-A) is currently a widely used screening test for embryo selection in *in vitro* fertilization (IVF); however, its validity has recently been called into question for several reasons. PGT-A relies on a single trophectoderm biopsy which some argue cannot be representative of the entire embryo genetic profile since mosaicism is common [55]. In addition, there is evidence of self-correction of aneuploidy in preimplantation embryos. A recent study showed that transfer of aneuploid embryos can result in euploid pregnancies at delivery. Interestingly this study also showed that aneuploid cells were preferentially localized within the trophectoderm [56]. This phenomenon supports the theory that blastomere exclusion takes place and could serve as a self-corrective mechanism. Additionally, the presence of a corrective mechanism implies that PGT-A testing may result in a disproportionate number of false-positives since the aneuploidy may have been corrected later in development.

4. Conclusion

The development of the embryo from fertilization to implantation is a highly regulated and incredibly complex phenomenon. A zygote resulting from the joining of two terminally differentiated cells must be able to give rise to the diverse groups of cell types and tissues making up the human. For this to occur, the zygotic genome must be reprogrammed back to a state of totipotency. As this is happening, there is a shift from maternal to zygotic genome control. As the zygotic genome becomes the dominant effector, molecular heterogeneities begin to appear which will eventually result in the first cell fate decisions. Cells will differentiate to either the trophectoderm or the ICM, resulting in the formation of a blastocyst. The trophectoderm is

required for implantation and some parts will eventually become the placenta. Cells of the ICM will maintain their pluripotency and continue to develop as the embryo.

Understanding the cellular and molecular mechanisms controlling this process has important implications in the field of assisted reproductive technology, especially IVF. IVF generally produces 6–10 embryos in a cycle, and a major challenge of the process is deciding how to select the best embryo for transfer. A more complete knowledge of what makes a preimplantation embryo viable would allow for better selection and improve IVF rates. This would dramatically lower costs for patients by preventing the need for additional cycles of therapy. Additionally, it would decrease the psychological toll of repeated failed pregnancies despite IVF treatment.

Conflict of interest

The authors declare no conflict of interest.

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