

REVIEW ARTICLE

Genetic causes of preimplantation embryo developmental failure

Mariagrazia Paonessa^{1,2} | Andrea Borini¹ | Giovanni Coticchio¹¹9.Baby, Family and Fertility Center, Bologna, Italy²Casa di Cura Candela Spa, Palermo, Italy

Correspondence

Mariagrazia Paonessa, 9.Baby, Family and Fertility Center, Via Dante 15, 40125 Bologna, Italy.

Email: mariagrazia.paonessa@gmail.com

Funding information

9.baby Family and Fertility Center, Bologna, Italy

Abstract

Embryo development requires orchestrated events, finely regulated at the molecular and cellular level by mechanisms which are progressively emerging from animal studies. With progress in genetic technologies—such as genome editing and single-cell RNA analysis—we can now assess embryo gene expression with increased precision and gain new insights into complex processes until recently difficult to explore. Multiple genes and regulative pathways have been identified for each developmental stage. We have learned that embryos with undisturbed and timely gene expression have higher chances of successful development. For example, selected genes are highly expressed during the first stages, being involved in cell adhesion, cell cycle, and regulation of transcription; other genes are instead crucial for lineage specification and therefore expressed at later stages. Due to ethical constraints, studies on human embryos remain scarce, mainly descriptive, and unable to provide functional evidence. This highlights the importance of animal studies as basic knowledge to test and appraise in a clinical context. In this review, we report on preimplantation development with a focus on genes whose impairment leads to developmental arrest. Preconceptional genetic screening could identify loss-of-function mutations of these genes; thereby, novel biomarkers of embryo quality could be adopted to improve diagnosis and treatment of infertility.

KEYWORDS

blastocyst, development, embryos, genes, implantation

1 | INTRODUCTION

1.1 | Preimplantation embryo: The journey from zygote to blastocyst

The zygote, the cell that results from the union of sperm and egg at fertilization, has an immense potential to be totipotent, meaning it can develop into an adult organism and generate offspring (Edwards & Beard, 1997). The zygote is also able to generate the extra-embryonic tissues, such as trophoblast and placenta, which are required to support development to birth. The journey from the zygote to the blastocyst, both in humans and mice, starts with the first cleavage into two daughter cells called blastomeres. During the first

3–4 days embryonic cells divide roughly every 12–18 h. As embryo development progresses, the individual blastomeres are gradually subject to a restriction of their totipotency. When the embryo is formed from approximately eight cells in mice and 8–16 cells in humans, at the morula stage it undergoes a process known as compaction (Chuva de Sousa Lopes & Mummery, 2009). Studies carried out in mice have shown that, at this time, cells make their first developmental decision: becoming part of the trophectoderm (TE) or contributing to the pluripotent inner cell mass (ICM). This cell lineage specification, known as the first cell fate decision, leads to the formation of the blastocyst. In the mouse model, the position of the blastomeres within the morula affects cell fate. Indeed, during morula formation, blastomeres acquire an internal or external

position, with the former creating the ICM and the latter becoming TE cells (Fleming, 1987; reviewed in Coticchio et al., 2019). The internalization of the blastomeres is possible thanks to the compaction of the morula. Blastomeres lose their sphericity; they become elongated and flattened, acquiring an epithelial-like shape. Compaction is therefore crucial for cell fate determination and largely relies on cellular specializations that ensure cell-to-cell contact and adhesion (Haegel et al., 1995; Riethmacher et al., 1995; Torres et al., 1997; Vestweber et al., 1987). If compaction is fundamental for blastomere internalization, cell polarization plays an important role, as well. Before compaction, blastomeres become polarized with the nucleus positioned basally, cell adhesion structures and acetylated microtubules positioned basolaterally, while more dynamic microtubules, actin filaments, and microvilli are localized apically (Suzuki & Ohno, 2006). Cell polarity is finely regulated. In outer cells, it derives from the interplay between the Par3-Par6-aPKC (Par-aPCK) system—which coordinates the formation of the apical domain—and Par1—which instead promotes the assembly of the basolateral domain (Suzuki & Ohno, 2006). This regulatory network is also essential to modulate the Hippo pathway, which is crucially involved in the determination of ICM and TE characteristics as discussed elsewhere in this manuscript. Polarized blastomeres divide and, depending on the orientation of the cleavage plane, they follow a different destiny. If the cleavage plane is parallel to the cell basoapical axis, the resulting daughter cells will be symmetric, maintaining the same polarization and the external position of the mother cell. Alternatively, if the orientation of the cleavage plane is orthogonal to the cell baso-apical axis, the resulting cells will be asymmetric. The cell inheriting the organization and the molecules of the apical domain remains positioned externally, while the other one, inheriting the basolateral organization of the mother cell, retain an internal localization (Korotkevich et al., 2017). The orientation of the cleavage plane is not a random event, but it seems to be controlled by the cortical domain which regulates spindle position (Korotkevich et al., 2017) and therefore cell cleavage. However asymmetric division is only one of the mechanisms through which cell internalization occurs. Recent studies have shown that in mouse embryos, the 60% of the inner cells do not originate from asymmetric division (Samarage et al., 2015). In these cells, internalization could be the result of tensile forces that create apical constriction and expansion of basolateral domain of the cell with consequent repositioning toward the interior of the embryo. In addition, the timing of blastomeres internalization seems to have an effect on the second cell fate decision which occurs within the ICM and results in a two-layered structure: the primitive endoderm (PE) which will form the yolk sac, and the epiblast (EPI) from which the proper embryo originates. Blastomeres internalized in the first round of division are more likely to become EPI, those internalized in the second wave are preferentially directed towards the PE pathway (Morris et al., 2013). In the context of cell fate determination, differences have emerged between human and mouse. In the mouse, TE and ICM fate is initiated in a positional and cell polarization-dependent manner within the morula, followed by a progressive maturation of EPI and PE

(Cockburn & Rossant, 2010) as discussed above. In humans, the establishment of the cell lineages (TE, EPI, and PE) seems to occur at the same time and coincides with blastocyst formation. Indeed, Petropoulos et al. (2016), using single-cell RNA-seq, have shown that, in humans, cells undergo an intermediate state of coexpression of lineage-specific genes and only during blastocyst formation they start to become mutually restrictive. Preimplantation embryo development is therefore a complex process which requires a lot of orchestrated and time-dependent events. The comprehension of the genetic and molecular mechanisms underlining these events remains challenging but would represent a great step ahead for our understanding of human embryo development.

1.2 | Gene expression as a reflection of embryo developmental phases

At the very early stages, embryo development is under the control of the maternal transcriptome. As development begins, maternal transcripts are gradually replaced by embryonic transcripts. This phenomenon known as maternal to zygotic transition (MZT) is the result of the transcription of the zygotic genome. The MZT consists of two major molecular activities. One is the deletion of the maternal messenger RNA (mRNA) and proteins, that are necessary for oocyte maturation, homeostasis, and the first stages of embryogenesis, but that become unnecessary and/or deleterious as the embryo develops. The other is the installation of new zygotic instructions through gene expression, a process that is activated by the maternal program and called zygotic/embryonic genome activation (ZGA/EGA) (Lee et al., 2014). Maternal mRNA translation and degradation are controlled by cytoplasmic polyadenylation and deadenylation, which build up the competence of maturing oocyte to accomplish MZT. EGA occurs very early in human development. Previous studies have identified transcriptional differences between blastomeres at the four- and eight-cell stage (Braude et al., 1988). More recently, with the advent of new DNA technologies, it has been possible to detect the first signs of human embryonic transcription already at the two-cell stage, followed by a more sustained transcription at four- to six-cell stage, with a transcriptional burst at 8–10 cell stage (Vassena et al., 2011). Several mechanisms have been suggested to regulate the MZT: pyruvate availability; titration of maternal repressors; maternal mRNA translation and degradation; accumulation of maternal activators (e.g., TATA binding protein) and expression of embryonic activators (e.g., DUX4) (Sha et al., 2019; Schulz & Harrison, 2019). These changes in gene expression reflect a change in embryo metabolism; the embryo shifts from a relatively quiescent metabolism (Smith & Sturmey, 2013) to a more active one. Indeed, as the embryo develops, its energetic demand changes (Leese, 2012). During the first cleavage stages, energy is required to carry out general homeostatic functions; cells divide but the mass does not increase and genes transcribed in this phase have mainly a role in the translation machinery (Krisner & Prather, 2012). The subsequent wave of transcription affects genes encoding proteins, amino acids,

carbohydrates, and proteins involved in lipid metabolism. The following stages are characterized by a higher energy demand; cells divide more rapidly and, at the blastocyst stage, embryo mass also increases (Smith & Sturmey, 2013). Therefore, in addition to morphogenetic processes, gene transcription also reflects changes in metabolic function, characterized by high oxidative levels and glucose utilization via aerobic glycolysis.

1.3 | Expression of general developmental pathways in human embryos

Several research groups aimed to analyse the gene expression profile of human embryos at different stages of development. Using the microarray technology, Zhang et al. (2009) studied the transcriptome of human oocytes and embryos at six developmental stages; their results suggest that genes predominantly expressed during preimplantation development are well conserved during evolution. An example is given by the transcription factor NR2F2, which was found to be highly expressed in day-5 embryos. NR2F2 mediates progesterone regulation of uterine implantation. It has been shown that *Nr2f2*-null mutant mice die during the early embryonic development due to defects in angiogenesis and heart development (Pereira et al., 1999). In another study, Assou et al. analysed the transcriptome of human oocytes, day-3 embryos, and human embryonic stem cells (hESCs). They found known components of the WNT pathway to be highly representative of all stages. Specifically, *DVL2*, *CTNNB1*, *CTNNA2*, *CTNND1*, *LEF1*, *CSNK1G3*, and *DAAM1* genes were found to be overexpressed in MII oocytes; *FZD4*, *GSK3B*, *CTNND1*, *APC*, *SENP5*, *SENP8*, *CSNK2A2*, and *CSNK1E* in day-3 embryos; *FZD3*, *FZD7*, *SNP6*, *CXNC1*, *AXIN*, and *SFRP1* in hESCs. Overall these findings imply an important role of the WNT pathway in early embryonic development (Assou et al., 2011). Galan et al. (2013) used a genome-wide approach to compare the genome expression profile of human blastomeres from day-3 embryos versus ICMs from blastocysts. They found that blastomeres and ICM display unique transcriptomes. Blastomeres showed an upregulation of genes like *CCNB1*, *GBX2*, *WT1*, *FOXD3*, and *UTF1* that are mainly involved in cell adhesion, cell cycle, and regulation of transcription; and a downregulation of genes involved in cell metabolism, signaling pathways, cell transcription, cell proliferation, pluripotency, and telomerase activity like *GAPDH*, *IMPDH2*, *GABRB3*, *LIN28*, *ZFP42*, *IFITM2*, *PRKDC*, *TOP2A*, and *RPL*. On the other hand, the ICMs gene expression profile showed an upregulation of genes involved in genome replication such as *MCM10* and in genome methylation like *DNMT3L*; an upregulation of transcriptional factors like *GDF3* and *HMGB2*; cell adhesion molecules such as *MUC15* and the protein membrane transporter *ABCG2*; signaling component like *IL6*, *SMAD4*, and the receptor *PPARG*; cytoskeleton components like *ACTB* and *KRT8*. On the contrary, ICM down-regulated genes included the heat shock protein *HSPB1*, which inhibits translation, cell cycle markers such as *GTSE1* and the transcription regulator *EP300*.

2 | CAUSES OF EMBRYO ARREST

Several studies have investigated the gene expression of the human preimplantation embryo to get new insights into the regulation of early embryonic development and determine the genetic causes that lead to growth arrest and consequent embryo loss (a schematic description of representative genes playing an important role in preimplantation development is reported in Figure 1). Embryonic arrest is a very common phenomenon; about 50-70% of embryos fail to make it to the blastocyst stage (French et al., 2010; Gardner et al., 2000). Since survival of the embryo immediately or shortly after fertilization totally depends on stored maternal factors in the egg, it is obvious that embryonic arrest before EGA can be due to an inadequate maternal mRNA content or mutations in relevant genes. Numerous maternal effect genes (MEGs), that is, genes expressed during oogenesis and determining an embryonic phenotype, are known in animal model. In the human, similar evidence is starting to emerge. The *TUBB8* gene is representative of human MEGs. *TUBB8* encodes for a special β -tubulin isotype present only in primates and expressed exclusively in oocytes as the major structural component of the meiotic spindle. *TUBB8* seems to play a crucial role in ensuring cytoskeletal functions that underpin the completion of meiosis during fertilization and the first mitotic divisions. In fact, loss-of-function mutations of this gene cause extensive spindle-assembly defects during oocyte maturation and fertilization, as well as widespread cleavage aberrations and arrest in early embryos (Chen et al., 2017; Feng, Sang, et al., 2016; Feng, Yan, et al., 2016). Polo-like kinase 4 (*PLK4*) is another putative human MEG. The *PLK4* product is a highly regulated kinase controlling the assembly of the daughter centriole in the vicinity of a pre-existing centriole. Predictably, dysregulation of *PLK4* leads to alterations of centriole formation and often centriole duplication. Genetic investigations in assisted reproduction patients revealed the existence of a common female, but not male, haplotype spanning *PLK4*. Strikingly, this haplotype is highly associated with aberrant embryo cleavage at early postfertilization stages. Therefore, it is postulated that maternal mutations lead to altered expression of *PLK4*, which results in the formation of extra copies of centrosomes. Fatally, a tri- or multipolar mitotic spindle is assembled, followed by the division of the fertilized egg into three or more blastomeres and the creation of a condition of chaotic mosaicism due to random chromosome segregation (McCoey et al., 2018). Other maternally regulated genes are those encoding for components of the subcortical maternal complex (SCMC), including *MATER* (Tong et al., 2000), *TLE6* (Alazami et al., 2015), *PADI6* (Xu et al., 2016), and *KHDC3L* (X. Wang et al., 2018). The SCMC is a multiprotein complex uniquely expressed in mammalian oocytes and early embryos that is essential for zygote progression beyond the first embryonic cell divisions (Bebbere et al., 2016). Another example is offered by *ZAR1* (zygotic arrest 1) which is one of the first identified oocyte-specific MEGs that function during the oocyte-to-embryo transition. Studies on mice have shown that embryos from *Zar1*^{-/-} females do not develop beyond the four-cell stage (X. Wu et al., 2003). Similarly, in mouse embryos, the inhibition of maternal stores of the eukaryotic translation initiation factor 4E (eIF4E), results in a

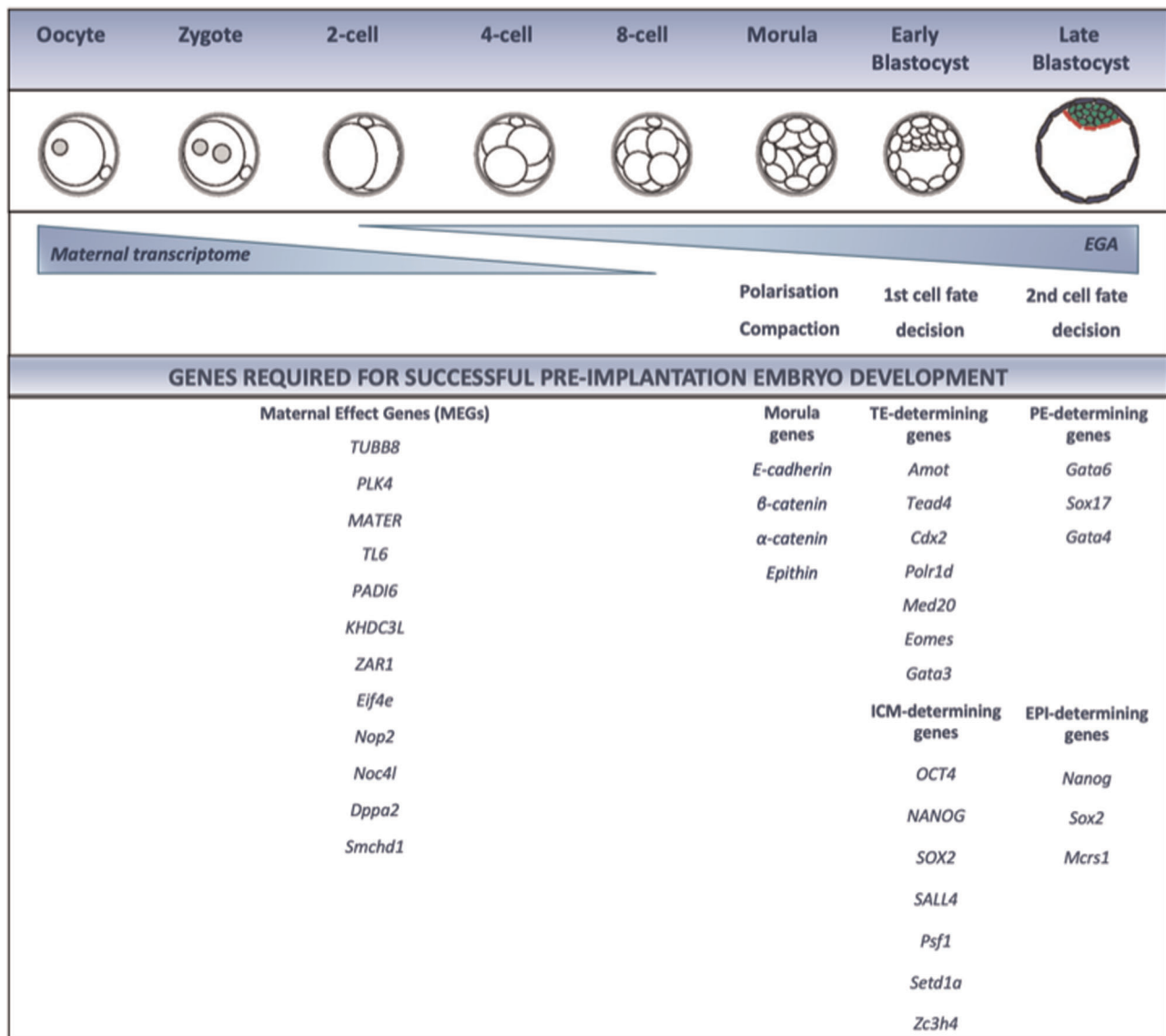


FIGURE 1 Genes required for successful preimplantation embryo development. Sets of genes, transcribed during oogenesis or early development, operate at specific developmental stages, especially during the oocyte-zygote transition, compaction of the morula, and specification of the blastocyst cell lineages. Human and mouse genes are reported in upper and lower case, respectively

developmental block at two- to four-cell stage, suggesting an important role for this gene during the maternal-to-embryonic transition (Li et al., 2020). During the oocyte-to-embryo transition, another important role is played by the maternal nucleolus. Although, the molecular mechanisms underlying nucleolar maturation and ribosome biogenesis during preimplantation development remains unclear, recently, Wang et al. found that a nucleolar protein, called NOP2, is essential for early embryo development. They showed that NOP2 depletion causes: developmental arrest during the morula-to-blastocyst transition in mouse and bovine models; disruption of the first lineage specification program with reduced level of key lineage-specific genes, including TEAD4, NANOG, and KLF4; disruption of nucleolar size and composition, as well as, reduced levels of rRNAs and several genes encoding ribosome proteins. In conclusion, NOP2 was found to be essential during preimplantation development in

mammals, playing a critical role in nucleolar maturation and ribosome biogenesis (H. Wang et al., 2020). In addition to NOP2, another nucleolar protein, NOC4L, was previously associated with embryonic lethality during the preimplantation stages of development. Indeed, studies on *Noc4l*-deficient mice revealed that lack of functional NOC4L results in increased apoptosis and inhibited proliferation at the morula stage with consequent deficient embryonic growth (Qin et al., 2017). Another possible cause of embryonic arrest could be the inability to activate the global embryonic gene transcription. EGA is, indeed, one of the most important events in embryogenesis, crucial for the establishment of the totipotency and the maintenance of the normal embryonic development. Cell cycle-dependent events and both translational and posttranslational regulatory mechanisms within the cytoplasm are needed to mediate and regulate EGA (Latham, 1999). Different maternal genes, take part in this process.

For example, studies in mice, have highlighted an important role for the maternal developmental pluripotency associated gene 2 (*Dppa2*), as activator of many downstream EGA transcripts. *DDPA2*, acts by directly regulating the expression of *Dux*, which in turn activates the Zinc finger and scan domain containing protein 4 (*Zscan4*) and its downstream genes (Eckersley-Maslin et al., 2019). Another maternally expressed gene, *Smchd1* (Structural maintenance of chromosomes flexible hinge-domain containing protein 1), acting downstream of *DPPA2*, was found to play an important role in controlling EGA, mainly by repressing *DUX* and *ZSCAN4* (Schall et al., 2019). Previous studies on *Smchd1*, had already pointed out an important role for this gene in early embryonic development. Indeed, knockdown *Smchd1* mice showed a compromised long-term embryo viability, a reduced nuclear volume at the morula stage, reduced blastocyst cell number, impaired blastocyst formation and hatching, as well as, a reduced viability to term (Midic et al., 2018). Precise control over the time of EGA is essential for normal embryogenesis. Dobson et al., using DNA microarray analysis, reported the global patterns of gene expression in human embryos through day 3, reporting that, in the vast majority of embryos, genome activation occurs on schedule, by day 3, regardless of morphological quality or cell number. Thus, failure to activate embryo transcription does not explain most cases of embryo arrest. Also, cleavage arrest not linked to chromosomal abnormalities could rather be caused by misregulation of individual genes (Dobson et al., 2004). In particular, these authors observed an upregulation of two genes: *H2AFZ* and *MYC*, known to be associated with embryonic growth control in other organisms. Since mutant mice lacking functional copies of *H2afz* or *Myc* die during embryogenesis, we could speculate a similar role for genes operating during early human embryonic development. Interestingly, in the above study, downregulation of the apoptotic factor *PDCD5* was observed, probably to ensure genome activation. Wong et al. reported evidence suggesting that embryos are predestined to survival or death irrespective of EGA regulation. These authors proposed that successful preimplantation development can be predicted before the major wave of EGA, based on only three parameters: the duration of the first cytokinesis, the time between the first and the second mitotic division, and the synchronicity of the second and third mitoses (Wong et al., 2010). Therefore, human embryo development is largely influenced by inherited maternal/paternal factors. Since maternal-to-embryonic transition is highly dependent on stored maternal factors, oocyte quality is critical in determining the outcome of early embryonic development. Oocytes quality is, indeed, one of the main causes of human reproductive failure and, importantly, is highly dependent upon maternal age.

3 | GENES REQUIRED FOR SUCCESSFUL PREIMPLANTATION EMBRYO DEVELOPMENT

Dynamic fluctuations in gene activity occur throughout preimplantation embryo development, while each developmental stage is characterized by a specific gene expression profile. Wells et al.

analysed the expression profile of nine genes: *BRCA1*, *BRCA2*, *ATM*, *TP53*, *RB1*, *MAD2*, *BUB1*, *APC*, and *Beta-Actin*, during human preimplantation embryo development and defined specific patterns of expression for each developmental stage. These nine genes are, indeed, known to play important roles during preimplantation embryo development in cell cycle regulation, DNA repair, signaling pathways, construction of the cytoskeleton, and apoptosis. They found that the expression of these genes at the appropriate developmental stage may be indicative of embryo viability per se (Wells et al., 2005). Therefore, an improved understanding of gene activity during embryo development, and the determination of characteristic gene expression profiles for each developmental stage, would help to predict embryo developmental outcome.

3.1 | The morula stage

Compaction of the morula is preceded by expression of genes for cytoskeletal, cell adhesion, and cell junction proteins. Among cell adhesion molecules, E-cadherin plays a crucial role. During compaction, it is preferentially localized at intercellular adherens junction, both in human and mouse, ensuring lateral adhesion and contributing to cell polarity. In mice, a null mutation of the *E-cadherin* encoding gene leads to loss of cell polarity; embryos undergo compaction due to residual maternal E-cadherin but fail to form a normal blastocyst able to hatch out of the zona pellucida. Since hatching is fundamental for implantation of the embryos, further development cannot proceed in vivo (Riethmacher et al., 1995). E-cadherin forms a complex with β - and γ -catenin and is anchored to the actin cytoskeleton by α -catenin. In mice, loss of β -catenin results in embryonic lethality, although embryos develop normally until the gastrulation stage when developmental arrest occurs. β -Catenin is, indeed, fundamental for ectoderm formation, and also for mesoderm, highlighting its important role in signaling pathways (Haegel et al., 1995). α -Catenin, which mediates the connection of E-cadherin to the actin filament network, is required for the formation of the TE and, as for E-cadherin null embryos, lack of α -catenin causes developmental arrest at the blastocyst stage (Torres et al., 1997). In human, morphological differences between blastomeres (polarization and/or position) have not been observed before compaction. However, as for other mammalian species, E-cadherin timely expression and distribution is fundamental for normal compaction and blastulation. Indeed, it has been observed that embryos with cleavage asynchronies are characterized by abnormal distribution of E-cadherin, abnormal blastulation and consequent embryo death (Alikani, 2005). Another example of a cell adhesion molecule, involved in compaction and important for normal embryo development, is the transmembrane serine protease Epithin. This component localizes with E-cadherin at areas of cell membrane contact between blastomeres; its ablation hampers cell adhesion, producing embryo death (Khang et al., 2005). The role of *Matriptase/MT-SP1* gene, the human orthologue of *Epithin*, is not very clear and a study conducted by List

et al. (2002) showed that, although *Matriptase/MT-SP1*-deficient mice are capable to develop to term, they die within 48 h of birth.

3.2 | Early blastocyst stage

In mice, lineage segregation begins after compaction, and lineage-specific genes are transcribed. The Hippo signaling pathway plays a crucial role in setting up the different cell lineages and its activation depends on angiomin (AMOT) protein. In inner cells, AMOT is associated with the adherens junctions, binding to E-cadherin and its junction-associated protein NF2. Phosphorylation of such complex by LATS reduces AMOT's F-actin binding activity in inner cells and stimulates the ability of LATS to activate Hippo. In outer cells, AMOT is found in the apical domain where it binds to F-actin; this prevents the interaction of AMOT with E-cadherin, the subsequent phosphorylation by LATS and, as a consequence, Hippo is not activated (Hirate et al., 2013). AMOT is also an important regulator of the first cell fate specification in the mouse embryo; it prevents expression of TE-marker genes in the ICM and its loss is sufficient to commit blastomeres into the TE fate regardless of their position or polarity (Hirate et al., 2013). *Amot*^{-/-} mouse embryos can survive through implantation and arrest only at early postimplantation stages (Leung & Zernicka-Goetz, 2013). When Hippo pathway is active, the kinases LATS1/2 phosphorylate the transcriptional coactivators YAP/TAZ. YAP/TAZ phosphorylation results in their cytoplasmic sequestering and, consequently their target genes are not expressed. *Tead4* is one of the Hippo target genes. *Tead4* is an activator gene for TE fate, therefore when it is not activated cells take on an ICM fate. Instead, in outside cells YAP/TAZ activates *Tead4* and the TE program is switched on. *Tead4* is required for successful preimplantation development. Indeed, it has been shown that KO mouse embryos for *Tead4* do not express the TE genes *Cdx2* and *Eomes*, causing a developmental arrest at the morula stage (Nishioka et al., 2008). TEAD4 is expressed both in TE and ICM. Home et al. (2012) proposed that, rather than the expression of TEAD4 itself, the mechanism that dictates the first cell fate specification in mammals could be the altered subcellular localization of TEAD4. More specifically, lack of nuclear localization of TEAD4 impairs TE differentiation in inner blastomeres driving them toward an ICM destiny. This is in net contrast with what reported by Nishioka, where TEAD4 protein was detectable in all nuclei of TE and ICM of the mouse blastocyst. In addition, according to Kaneko and De Pamphilis (2013), developmental arrest at the morula stage in *Tead4*^{-/-} embryos occurs not as a consequence of TE specification failure. They observed that *Tead4*^{-/-} mouse embryos were able to develop to the blastocyst stage and express TE-specific genes when cultured under condition that reduce oxidative stress. Therefore they proposed a novel role for TEAD4, pointing out that the reason TEAD4 is essential for blastocyst development is because TEAD4 is required to establish the energy homeostasis essential for blastocoel formation and expansion. TEAD4 also plays an important role in postimplantation mouse and human embryo development, by promoting self-renewal

and stemness of trophoblasts progenitors to ensure placentation (Saha et al., 2020).

The transcription factor CDX2 acts early in the lineage-determining hierarchy. *Cdx2* is a TE master regulator gene and its expression in the TE represses the ICM genes *Oct4* (*Pou5f1*) and *Nanog*. The TE of *Cdx2* mutant embryos do not form properly and in addition, it acquires ICM characteristics. Contrary, overexpression of *Cdx2* in ESCs results in cells acquiring properties of trophoblast stem cells (Ralston & Rossant, 2008). Therefore, *Cdx2* expression appears to be required for TE cell fate specification in the early mouse embryo, and loss of zygotic *Cdx2* leads to preimplantation lethality. However, it has been shown that *Cdx2* zygotic mutants can still initiate TE differentiation and blastulation (Strumpf et al., 2005), leading to the conclusion that *Cdx2* is important for the maintenance and regulation of TE function rather than specification.

Contrary to the zygotic, ablation of the maternal *Cdx2*, using a Cre/lox strategy, revealed that maternal *Cdx2* is dispensable for mouse development (Blij et al., 2012). Wu et al. (2010) used an RNA interference approach to eliminate both maternal and zygotic *Cdx2*. The resulting embryos failed to hatch and implant, but they developed to the blastocyst stage indicating that TE specification had been initiated. Jedrusik et al. (2015) contradicted this study by showing that *Cdx2* maternal-zygotic null embryos, exhibit reduced developmental potential to the blastocyst stage, increased cell death and failure of TE specification; concluding that *Cdx2* is required for the correct specification of TE from the morula stage onwards. In addition, it seems that CDX2 plays a role in cell allocation to inside and outside positions and thereby it has a fundamental role in cell fate determination (Jedrusik et al., 2008). CDX2 expression also relies on POLR1D, which has been found to be essential for early murine preimplantation development. Loss of POLR1D results in severe DNA damage, delayed cellular proliferation, impaired CDX2 expression, and TE specification and therefore, failure to form a blastocyst (Miao, Sun, Golan, et al., 2020). Contrary, *Med20* mutant mouse blastocysts exhibit a normal morphology but they cannot hatch from the zona pellucida, indicating impaired blastocyst function. MED20, a subunit of the Mediator complex, is indeed crucial for early mammalian embryo development and cell lineage specification. In addition, the ectopic expression of the EPI marker *Nanog* in the TE of *Med20* mutants, is indicative of defects in trophoblast specification (Cui et al., 2019). Other important genes in lineage specification of TE and ICM during mouse preimplantation development are: *Hdac1* and *Hdac2*. Double knockdown of these genes results in embryonic arrest during the morula to blastocyst transition with defects including suppressed *Cdx2* expression and aberrant Hippo pathway (Zhao et al., 2020). EOMES is a T-box-specific factor and murine embryos null for *Eomes* fail to form a functional TE (Strumpf et al., 2005). GATA3 is another important transcription factor in TE specification; it acts in parallel to CDX2 and downstream of TEAD4 (Ralston et al., 2010). In mouse trophoblast stem cells, knockdown of *Gata3* by RNA interference results in CDX2 downregulation and impairment of morula-to-blastocyst transition (Home et al., 2009).

Apart from Hippo pathway, Notch signaling also appears involved in the first cell fate decision cooperating with TEAD4 to ensure sufficient activation of *Cdx2* in the TE (Leung & Zernicka-Goetz, 2015). Finally, also the PAR complex can be defined as another TE determinant as its downregulation results in apolar distribution of AMOT (Hirate et al., 2013). The emergence of pluripotent ICM lineages from the morula is controlled by metabolic and signaling pathways including WNT, MAPK, TGF β , NOTCH, integrin-mediated cell adhesion, and apoptosis-signaling pathways (Adjaye, 2005). Furthermore, when comparing gene expression profiles of ICM and TE from human blastocysts, Adjaye et al. identified genes specific to the ICM such as *OCT4*, *NANOG*, *HMGB1*, and *DPPA5*. Essential genes for the development of the ICM are, indeed, the ones encoding for the master regulators such as the octamer-binding transcription factor 3/4 (*OCT4* or *POU5-FI*), *NANOG*, *SRY-box 2* (*SOX2*), and the Spalt-like transcription factor 4 (*SALL4*). They maintain pluripotency during the pre-implantation stages of mammalian embryonic development and positively regulate one another. These genes have been described in hESCs. *NANOG* expression is restricted to ICM cells (Niakan & Eggan, 2013). *OCT4* expression has also been detected in the TE cells, where it colocalizes with *CDX2*; this colocalization occurs only for a short period of time, at the onset of expansion, and it seems to be protracted in human and bovine embryos compared to mouse and rhesus monkey, highlighting once again species-specific differences (Berg et al., 2011; A. E. Chen et al., 2009). By day 8, *OCT4* expression becomes restricted to a small population of EPI cells. *OCT4* is a critical regulator of cellular pluripotency, as shown by a zygotic *Oct4*-knockout study in which deletion of *Oct4* drives inner cells into a trophoblast rather than ICM destiny (Nichols et al., 1998). However, additional studies have shown that knockout of both maternal and zygotic *Oct4* do not prevent ICM-TE lineage segregation and ICM markers such as *NANOG* and *GATA6*, still localize to the inside cells (Frum et al., 2013; G. Wu et al., 2013). In addition, depletion of *OCT4* reduces the binding of *TFCP2L1*, *DAX1*, *ESRRB* (Ding et al., 2012; J. Wang et al., 2006) as well as the binding of *SMAD1* and *STAT3* to their target genes (X. Chen et al., 2008). *OCT4* also regulates the expression of *Nanog* in conjunction with *SOX2*, though *SOX2* is dispensable (Rodda et al., 2005). Overexpression of *Oct4* can, indeed, compensate for *SOX2* absence as shown by the rescue of *Sox2*-null ESCs (Masui et al., 2007). *Sox2* expression starts from the eight-cell stage and it does not become restricted to the ICM of the blastocyst, being only downregulated in the TE cells. Contrary, *Sall4* which is expressed from compaction till blastocyst stage, become restricted to the ICM (de Paepe et al., 2014). Defective ICM has also been reported with loss-of-function in other genes such as *Psf1*, whose absence causes embryonic lethality around the implantation stage, with *PSF1*^{-/-} mouse embryos showing impaired proliferation of both ICM and trophoblasts (Ueno et al., 2005); *Setd1a*, which is required for the generation of *Oct4*-positive ICM (Fang et al., 2016) and *Zc3h4*, where loss of *ZC3H4* results in defective ICM with consequent reduction of both EPI and PE lineages, many

DNA breaks and reduced cell proliferation with consequent implantation failure and early lethality (Su et al., 2020).

3.3 | Late blastocyst stage

After the first cell fate decision, the ICM differentiates into PE and EPI. This is the second lineage specification. It results from mutual interaction between *NANOG* and *GATA6* and it is regulated by the FGF/MAPK signaling pathway (Chazaud et al., 2006). FGF signaling acts by repressing transcription factors specific for the EPI and PE lineages (Frum & Ralston, 2015) and *NANOG* and *GATA6* become restricted to EPI and PE, respectively. Other transcription factors involved in the second lineage segregation include *GATA4* and *SOX17* (Chazaud et al., 2006). At the blastocyst stage, *Sox17* is expressed in all the ICM cells; its expression becomes then restricted to the putative PE cells and coincides with *Gata4* expression (Roode et al., 2012). Suppression/overexpression of FGF signaling in mouse embryos results in a shift of ICM fate towards EPI and PE respectively (Piliszek et al., 2016).

3.4 | Lineage segregation in human embryos

Even though human and mouse embryos seem to be morphologically similar during preimplantation development, important differences exist. Therefore, the molecular mechanisms that mediate lineage segregation in human embryos remain largely unknown. For example, in humans, the information is lacking about the Hippo signaling pathway which is essential for TE/ICM lineage segregation in mice. Similarly, while in the mouse the FGF signaling pathway plays a crucial role in EPI/PE lineage segregation, human embryos are not dependent upon FGF/MAPK signaling (Kuijk et al., 2012; Roode et al., 2012). In addition, it seems that lineage-associated genes are expressed in human embryos later than in mouse and, more important, differences in gene expression itself have been found. For example, Petropoulos et al. showed that even if *CDX2* is expressed in human TE, it is not one of the top ranked genes. This supports the notion that in human, *CDX2* is probably not involved in TE specification. Alternatively, it is possible that genes like *Eomes*, playing an important role in the mouse embryo, are not expressed at all in human (Petropoulos et al., 2016). In addition, a study conducted by Krivega et al. (2015) attributes a role for *WNT3* and *B-Catenin* in propagation of human TE, whereas in mouse *WNT*-dependent events occur only at the gastrulation stage. Other gene expression studies highlighted other genes that could play an important role in human preimplantation development. Among the TE-specific genes, *PTGES*, *EMP2*, *TGFBR3*, and *PDGFA*; *DPPA5*, *ESRG*, *KLF17*, and *ARGFX* were found to be EPI-specific genes. In addition, *LINC00261*, *FRZB*, *AMOTL1*, and *DPPA* were identified among the top ranked PE-specific genes (Petropoulos et al., 2016). By using an advanced single-cell gene-expression and 3D mapping analysis, Durruthy-Durruthy et al. (2016) identified the microspherule protein 1 (*MCRS1*) as another putative EPI-specific marker in human

blastocysts. MCRS1, also known as MSP58, is an evolutionarily conserved protein that has been implicated in various biological processes and reported to be essential during early mammalian embryogenesis. The role of MCRS1 has not yet been assessed during human development, due to limitations of working with human embryos, but analysis of lineage specification in mutant mouse blastocysts revealed that while the trophoblast and PE are properly specified, the EPI lineage is compromised and exhibits a severe reduction in cell number (Cui et al., 2020). The retinoblastoma-binding protein 4 (RBBP4), similarly to MCRS1, is an evolutionarily conserved protein which has been proved to play an important role during early mouse embryo development in vivo. It is essential for early embryo survival and successful implantation and its loss leads to apoptosis, defective ICM specification and hyperacetylated histones (Miao, Sun, Barletta, et al., 2020). Another difference between mouse and human blastocyst is the expression of laminin in PE and TE cells respectively. Moreover, although GATA4, GATA6, and SOX17 are expressed in the PE of both mouse and human embryos, their temporal and spatial localization throughout human preimplantation development is still not completely understood (Niakan & Eggan, 2013).

4 | CONCLUSIONS

From fertilization to blastocyst, embryo development may arrest at all intermediate stages. Being crucial regulatory transition underpinning preimplantation development, EGA is major potential source of developmental failure. Yet, available data do not support the hypothesis that large-scale failure of embryonic gene expression is a frequent cause of developmental arrest. Rather, null mutations or incorrect expression of individual genes active during oogenesis or early development can impair crucial embryonic functions at specific stages and ultimately cause cleavage arrest or widespread cell death. Identification of such genes and their function will undoubtedly be instrumental to gain a broader insight into the genetic wiring early human development. It will also open unprecedented opportunities to develop novel screening tools for the assessment of genetic risk in reproduction. Until recently, due to technical constraints, the genetic causes of infertility have been investigated solely in relation to monogenic traits. But novel low-cost genome-wide sequencing and analysis techniques promise to cause a paradigm shift. Infertility causes, previously classified as “idiopathic,” will be investigated by screening hundreds or thousands of genes playing a role in gamete and embryo competence. By learning the causes of their reproductive failure, infertile couples will make a more informed choice on possible alternative treatments. Men and women will become more aware of their reproductive risk, even before conception. Individuals suffering from genetic conditions causing premature decline in infertility will proactively undertake fertility preservations measures. The genetic of early human development will then contribute to the establishment of precision medicine in infertility.

PEER REVIEW

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REFERENCES

- Adjaye, J. (2005). Whole-genome approaches for large-scale gene identification and expression analysis in mammalian preimplantation embryos. *Reproduction, Fertility and Development*, 17(2), 37–45. <https://doi.org/10.1071/rd04075>
- Alazami, A. M., Awad, S. M., Coskun, S., Al-Hassan, S., Hijazi, H., Abdulwahab, F. M., Poizat, C., & Alkuraya, F. S. (2015). TLE6 mutation causes the earliest known human embryonic lethality. *Genome Biology*, 16(1), 240. <https://doi.org/10.1186/s13059-015-0792-0>
- Alikani, M. (2005). Epithelial cadherin distribution in abnormal human pre-implantation embryos. *Human Reproduction*, 20(12), 3369–3375. <https://doi.org/10.1093/humrep/dei242>
- Assou, S., Ne Boumela, I., Haouzi, D., Anahory, T., Dechaud, H., De Vos, J., & Hamamah, S. (2011). Dynamic changes in gene expression during human early embryo development: From fundamental aspects to clinical applications. *Human Reproduction Update*, 17(2), 272–290. <https://doi.org/10.1093/humupd/dmq036>
- Bebbere, D., Masala, L., Albertini, D. F., & Ledda, S. (2016). The subcortical maternal complex: Multiple functions for one biological structure? *Journal of Assisted Reproduction and Genetics*, 33(11), 1431–1438. <https://doi.org/10.1007/s10815-016-0788-z>
- Berg, D. K., Smith, C. S., Pearton, D. J., Wells, D. N., Broadhurst, R., Donnison, M., & Pfeffer, P. L. (2011). Trophoblast lineage determination in cattle. *Developmental Cell*, 20(2), 244–255. <https://doi.org/10.1016/j.devcel.2011.01.003>
- Blij, S., Frum, T., Akyol, A., Fearon, E., & Ralston, A. (2012). Maternal Cdx2 is dispensable for mouse development. *Development*, 39(21), 3969–3972. <https://doi.org/10.1242/dev.086025>
- Braude, P., Bolton, V., & Moore, S. (1988). Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature*, 332(6163), 459–461. <https://doi.org/10.1038/332459a0>
- Chazaud, C., Yamanaka, Y., Pawson, T., & Rossant, J. (2006). Early lineage segregation between epiblast and primitive endoderm in mouse blastocysts through the Grb2-MAPK pathway. *Developmental Cell*, 10(5), 615–624. <https://doi.org/10.1016/j.devcel.2006.02.020>
- Chen, A. E., Egli, D., Niakan, K., Deng, J., Akutsu, H., Yamaki, M., Cowan, C., Fitz-Gerald, C., Zhang, K., Melton, D. A., & Eggan, K. (2009). Optimal timing of inner cell mass isolation increases the efficiency of human embryonic stem cell derivation and allows generation of sibling cell lines. *Cell Stem Cell*, 4(2), 103–106. <https://doi.org/10.1016/j.stem.2008.12.001>
- Chen, B., Li, B., Li, D., Yan, Z., Mao, X., Xu, Y., Mu, J., Li, Q., Jin, L., He, L., Kuang, Y., Sang, Q., & Wang, L. (2017). Novel mutations and structural deletions in TUBB8: Expanding mutational and phenotypic spectrum of patients with arrest in oocyte maturation, fertilization or early embryonic development. *Human Reproduction*, 32(2), 457–464. <https://doi.org/10.1093/humrep/dew322>
- Chen, X., Xu, H., Yuan, P., Fang, F., Huss, M., Vega, V. B., Wong, E., Orlov, Y. L., Zhang, W., Jiang, J., Loh, Y. H., Yeo, H. C., Yeo, Z. X., Narang, V., Govindarajan, K. R., Leong, B., Shahab, A., Ruan, Y., ... Ng, H. H. (2008). Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. *Cell*, 133(6), 1106–1117. <https://doi.org/10.1016/j.cell.2008.04.043>
- Chuva de Sousa Lopes, S. M., & Mummery, C. L. (2009). Differentiation in early development. In R. Lanza, J. Gearhart, B. Hogan, D. Melton, R. Pedersen, E. D. Thomas, J. Thomson, & I. Wilmut (Eds.), *Essentials of stem cell biology* (pp. 117–129). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-374729-7.00013-5>

- Cockburn, K., & Rossant, J. (2010). Making the blastocyst: Lessons from the mouse. *Journal of Clinical Investigation*, 120(4), 995–1003. <https://doi.org/10.1172/JCI41229>
- Coticchio, G., Lagalla, C., Sturmey, R., Pennetta, F., & Borini, A. (2019). The enigmatic morula: Mechanisms of development, cell fate determination, self-correction and implications for ART. *Human Reproduction Update*, 25(4), 422–438. <https://doi.org/10.1093/humupd/dmz008>
- Cui, W., Cheong, A., Wang, Y., Tsuchida, Y., Liu, Y., Tremblay, K. D., & Mager, J. (2020). MCRS1 is essential for epiblast development during early mouse embryogenesis. *Reproduction*, 159, 1–13. <https://doi.org/10.1530/REP-19-0334>
- Cui, W., Marcho, C., Wang, Y., Degani, R., Golan, M., Tremblay, K. D., Rivera-Pérez, J. A., & Mager, J. (2019). MED20 is essential for early embryogenesis and regulates NANOG expression. *Reproduction*, 157, 215–222. <https://doi.org/10.1530/REP-18-0508>
- de Paepe, C., Krivega, M., Cauffman, G., Geens, M., & van de Velde, H. (2014). Totipotency and lineage segregation in the human embryo. *Molecular Human Reproduction*, 20, 599–618. <https://doi.org/10.1093/molehr/gau027>
- Ding, J., Xu, H., Faiola, F., Ma'Ayan, A., & Wang, J. (2012). Oct4 links multiple epigenetic pathways to the pluripotency network. *Cell Research*, 22(1), 155–167. <https://doi.org/10.1038/cr.2011.179>
- Dobson, A. T., Raja, R., Abeyta, M. J., Taylor, T., Shen, S., Haqq, C., & Reijo Pera, R. A. (2004). The unique transcriptome through day 3 of human preimplantation development. *Human Molecular Genetics*, 13(14), 1461–1470. <https://doi.org/10.1093/hmg/ddh157>
- Durruthy-Durruthy, J., Wossidlo, M., Pai, S., Takahashi, Y., Kang, G., Omberg, L., Chen, B., Nakauchi, H., Reijo Pera, R., & Sebastiano, V. (2016). Spatiotemporal reconstruction of the human blastocyst by single-cell gene-expression analysis informs induction of naive pluripotency. *Developmental Cell*, 38, 100–115. <https://doi.org/10.1016/j.devcel.2016.06.014>
- Eckersley-Maslin, M., Alda-Catalinas, C., Blotenburg, M., Kreibich, E., Krueger, C., & Reik, W. (2019). Dppa2 and Dppa4 directly regulate the Dux-driven zygotic transcriptional program. *Genes and Development*, 33, 194–208. <https://doi.org/10.1101/gad.321174.118>
- Edwards, R. G., & Beard, H. K. (1997). Oocyte polarity and cell determination in early mammalian embryos. *Molecular Human Reproduction*, 3(10), 863–905. <https://doi.org/10.1093/molehr/3.10.863>
- Fang, L., Zhang, J., Zhang, H., Yang, X., Jin, X., Zhang, L., Skalnik, D. G., Jin, Y., Zhang, Y., Huang, X., Li, J., & Wong, J. (2016). H3K4 methyltransferase set1a is a key Oct4 coactivator essential for generation of Oct4 positive inner cell mass. *Stem Cells*, 34, 565–580. <https://doi.org/10.1002/stem.2250>
- Feng, R., Sang, Q., Kuang, Y., Sun, X., Yan, Z., Zhang, S., Shi, J., Tian, G., Luchniak, A., Fukuda, Y., Li, B., Yu, M., Chen, J., Xu, Y., Guo, L., Qu, R., Wang, X., Sun, Z., ... Wang, L. (2016). Mutations in TUBB8 and human oocyte meiotic arrest. *New England Journal of Medicine*, 374(3), 223–232. <https://doi.org/10.1056/nejmoa1510791>
- Feng, R., Yan, Z., Li, B., Yu, M., Sang, Q., Tian, G., Xu, Y., Chen, B., Qu, R., Sun, Z., Sun, X., Jin, L., He, L., Kuang, Y., Cowan, N. J., & Wang, L. (2016). Mutations in TUBB8 cause a multiplicity of phenotypes in human oocytes and early embryos. *Journal of Medical Genetics*, 53(10), 662–671. <https://doi.org/10.1136/jmedgenet-2016-103891>
- Fleming, T. P. (1987). A quantitative analysis of cell allocation to trophoblast and inner cell mass in the mouse blastocyst. *Developmental Biology*, 119(2), 520–531. [https://doi.org/10.1016/0012-1606\(87\)90055-8](https://doi.org/10.1016/0012-1606(87)90055-8)
- French, D. B., Sabanegh, E. S., Goldfarb, J., & Desai, N. (2010). Does severe teratozoospermia affect blastocyst formation, live birth rate, and other clinical outcome parameters in ICSI cycles? *Fertility and Sterility*, 93(4), 1097–1103. <https://doi.org/10.1016/j.fertnstert.2008.10.051>
- Frum, T., Halbisen, M. A., Wang, C., Amiri, H., Robson, P., & Ralston, A. (2013). Oct4 Cell-autonomously promotes primitive endoderm development in the mouse blastocyst. *Developmental Cell*, 25(6), 610–622. <https://doi.org/10.1016/j.devcel.2013.05.004>
- Frum, T., & Ralston, A. (2015). Cell signaling and transcription factors regulating cell fate during formation of the mouse blastocyst. *Trends in Genetics*, 31(7), 402–410. <https://doi.org/10.1016/j.tig.2015.04.002>
- Galan, A., Diaz-Gimeno, P., Poo, M. E., Valbuena, D., & Sanchez, E. (2013). Defining the genomic signature of totipotency and pluripotency during early human development. *PLOS One*, 8(4), 62135. <https://doi.org/10.1371/journal.pone.0062135>
- Gardner, D. K., Lane, M., & Schoolcraft, W. B. (2000). Culture and transfer of viable blastocysts: A feasible proposition for human IVF. *Human Reproduction*, 15(SUPPL. 6), 9–23.
- Haegel, H., Larue, L., Ohsugi, M., Fedorov, L., Herrenknecht, K., & Kemler, R. (1995). Lack of β -catenin affects mouse development at gastrulation. *Development*, 121(11), 3529–3537.
- Hirate, Y., Hirahara, S., Inoue, K. I., Suzuki, A., Alarcon, V. B., Akimoto, K., Hirai, T., Hara, T., Adachi, M., Chida, K., Ohno, S., Marikawa, Y., Nakao, K., Shimono, A., & Sasaki, H. (2013). Polarity-dependent distribution of angiominin localizes hippo signaling in preimplantation embryos. *Current Biology*, 23(13), 1181–1194. <https://doi.org/10.1016/j.cub.2013.05.014>
- Home, P., Ray, S., Dutta, D., Bronshteyn, I., Larson, M., & Paul, S. (2009). GATA3 is selectively expressed in the trophectoderm of peri-implantation embryo and directly regulates Cdx2 gene expression. *Journal of Biological Chemistry*, 284, 28729–28737. <https://doi.org/10.1074/jbc.M109.016840>
- Home, P., Saha, B., Ray, S., Dutta, D., Gunewardena, S., Yoo, B., Pal, A., Vivian, J. L., Larson, M., Petroff, M., Gallagher, P. G., Schulz, V. P., White, K. L., Golos, T. G., Behr, B., & Paul, S. (2012). Altered subcellular localization of transcription factor TEAD4 regulates first mammalian cell lineage commitment. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 7362–7367. <https://doi.org/10.1073/pnas.1201595109>
- Jedrusik, A., Cox, A., Wicher, K., Glover, D. M., & Zernicka-Goetz, M. (2015). Maternal-zygotic knockout reveals a critical role of Cdx2 in the morula to blastocyst transition. *Developmental Biology*, 398, 147–152. <https://doi.org/10.1016/j.ydbio.2014.12.004>
- Jedrusik, A., Parfitt, D. E., Guo, G., Skamagki, M., Grabarek, J. B., Johnson, M. H., Robson, P., & Zernicka-Goetz, M. (2008). Role of Cdx2 and cell polarity in cell allocation and specification of trophectoderm and inner cell mass in the mouse embryo. *Genes and Development*, 22(19), 2692–2706. <https://doi.org/10.1101/gad.486108>
- Kaneko, K. J., & De Pamphilis, M. L. (2013). TEAD4 establishes the energy homeostasis essential for blastocoel formation. *Development*, 140, 3680–3690. <https://doi.org/10.1242/dev.093799>
- Khang, I., Sonn, S., Park, J. H., Rhee, K., Park, D., & Kim, K. (2005). Expression of epithin in mouse preimplantation development: Its functional role in compaction. *Developmental Biology*, 281(1), 134–144. <https://doi.org/10.1016/j.ydbio.2005.02.026>
- Korotkevich, E., Niwayama, R., Courtois, A., Friese, S., Berger, N., Buchholz, F., & Hiiragi, T. (2017). The apical domain is required and sufficient for the first lineage segregation in the mouse embryo. *Developmental Cell*, 40(3), 235–247. <https://doi.org/10.1016/j.devcel.2017.01.006>
- Krisher, R. L., & Prather, R. S. (2012). A role for the Warburg effect in preimplantation embryo development: Metabolic modification to support rapid cell proliferation. *Molecular Reproduction and Development*, 79, 311–320.
- Krivega, M., Essahib, W., & Van de Velde, H. (2015). WNT3 and membrane-associated β -catenin regulate trophectoderm lineage differentiation in human blastocysts. *Molecular Human Reproduction*, 21(9), 711–722. <https://doi.org/10.1093/molehr/gav036>

- Kuijck, E. W., van Tol, L. T. A., van de Velde, H., Wubbolts, R., Welling, M., Geijsen, N., & Roelen, B. A. J. (2012). The roles of FGF and MAP kinase signaling in the segregation of the epiblast and hypoblast cell lineages in bovine and human embryos. *Development*, 139(5), 871–882. <https://doi.org/10.1242/dev.071688>
- Latham, K. E. (1999). Mechanisms and control of embryonic genome activation in mammalian embryos. *International Review of Cytology*, 193, 71–124. [https://doi.org/10.1016/S0074-7696\(08\)61779-9](https://doi.org/10.1016/S0074-7696(08)61779-9)
- Lee, M. T., Bonneau, A. R., & Giraldez, A. J. (2014). Zygotic genome activation during the maternal-to-zygotic transition. *Annual Review of Cell and Developmental Biology*, 30, 581–613. <https://doi.org/10.1146/annurev-cellbio-100913-013027>
- Leese, H. J. (2012). Metabolism of the preimplantation embryo: 40 years on. *Reproduction*, 143, 417–427.
- Leung, C. Y., & Zernicka-Goetz, M. (2013). Angiomotin prevents pluripotent lineage differentiation in mouse embryos via Hippo pathway-dependent and -independent mechanisms. *Nature Communications*, 4(1), 2251. <https://doi.org/10.1038/ncomms3251>
- Leung, C. Y., & Zernicka-Goetz, M. (2015). Mapping the journey from totipotency to lineage specification in the mouse embryo. *Current Opinion in Genetics and Development*, 34, 71–76. <https://doi.org/10.1016/j.gde.2015.08.002>
- Li, Y., Huang, X., Tang, J., Ji, X., Liu, M., Chang, L., Liu, J., Gu, Y., Shi, C., Ni, W., Shi, H., O'Neill, C., & Jin, X. (2020). The regulation of mammalian maternal-to-embryonic transition by eukaryotic translation initiation factor 4E. *bioRxiv*. <https://doi.org/10.1101/2020.01.16.908582>
- List, K., Haudenschild, C. C., Szabo, R., Chen, W., Wahl, S. M., Swaim, W., Engelholm, L. H., Behrendt, N., & Bugge, T. H. (2002). Matriptase/MT-SP1 is required for postnatal survival, epidermal barrier function, hair follicle development, and thymic homeostasis. *Oncogene*, 21, 3765–3779. <https://doi.org/10.1038/sj/onc.1205502>
- Masui, S., Nakatake, Y., Toyooka, Y., Shimosato, D., Yagi, R., Takahashi, K., Okochi, H., Okuda, A., Matoba, R., Sharov, A. A., Ko, M. S. H., & Niwa, H. (2007). Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nature Cell Biology*, 9(6), 625–635. <https://doi.org/10.1038/ncb1589>
- Mccoy, R. C., Newnham, L. J., Ottolini, C. S., Hoffmann, E. R., Chatzimeletiou, K., Cornejo, O. E., Zhan, Q., Zaninovic, N., Rosenwaks, Z., Petrov, D. A., Demko, Z. P., Sigurjonsson, S., & Handyside, A. H. (2018). Tripolar chromosome segregation drives the association between maternal genotype at variants spanning PLK4 and aneuploidy in human preimplantation embryos. *Human Molecular Genetics*, 27(14), 2573–2585. <https://doi.org/10.1093/hmg/ddy147>
- Miao, X., Sun, T., Barletta, H., Mager, J., & Cui, W. (2020). Loss of RBBP4 results in defective inner cell mass, severe apoptosis, hyperacetylated histones and preimplantation lethality in mice. *Biology of Reproduction*, 103, 13–23. <https://doi.org/10.1093/biolre/iaaa046>
- Miao, X., Sun, T., Golan, M., Mager, J., & Cui, W. (2020). Loss of POLR1D results in embryonic lethality prior to blastocyst formation in mice. *Molecular Reproduction and Development*, 87, 1152–1158. <https://doi.org/10.1002/mrd.23427>
- Midic, U., Vincent, K. A., Wang, K., Lokken, A., Severance, A. L., Ralston, A., Knott, J. G., & Latham, K. E. (2018). Novel key roles for structural maintenance of chromosome flexible domain containing 1 (Smchd1) during preimplantation mouse development. *Molecular Reproduction and Development*, 85, 635–648. <https://doi.org/10.1002/mrd.23001>
- Morris, S. A., Graham, S. J. L., Jedrusik, A., & Zernicka-Goetz, M. (2013). The differential response to Fgf signalling in cells internalized at different times influences lineage segregation in preimplantation mouse embryos. *Open Biology*, 3, 130104. <https://doi.org/10.1098/rsob.130104>
- Niakan, K. K., & Eggan, K. (2013). Analysis of human embryos from zygote to blastocyst reveals distinct gene expression patterns relative to the mouse. *Developmental Biology*, 375(1), 54–64. <https://doi.org/10.1016/j.ydbio.2012.12.008>
- Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Schöler, H., & Smith, A. (1998). Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell*, 95(3), 379–391. [https://doi.org/10.1016/S0092-8674\(00\)81769-9](https://doi.org/10.1016/S0092-8674(00)81769-9)
- Nishioka, N., Yamamoto, S., Kiyonari, H., Sato, H., Sawada, A., Ota, M., Nakao, K., & Sasaki, H. (2008). Tead4 is required for specification of trophectoderm in pre-implantation mouse embryos. *Mechanisms of Development*, 125(3–4), 270–283. <https://doi.org/10.1016/j.mod.2007.11.002>
- Pereira, F. A., Yuhong, Q., Zhou, G., Tsai, M. J., & Tsai, S. Y. (1999). The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. *Genes and Development*, 13(8), 1037–1049. <https://doi.org/10.1101/gad.13.8.1037>
- Petropoulos, S., Edsgård, D., Reinus, B., Deng, Q., Panula, S. P., Codeluppi, S., Plaza Reyes, A., Linnarsson, S., Sandberg, R., & Lanner, F. (2016). Single-cell RNA-Seq reveals lineage and X chromosome dynamics in human preimplantation embryos. *Cell*, 165(4), 1012–1026. <https://doi.org/10.1016/j.cell.2016.03.023>
- Piliszek, A., Grabarek, J. B., Frankenberg, S. R., & Plusa, B. (2016). Cell fate in animal and human blastocysts and the determination of viability. *Molecular Human Reproduction*, 22(10), 681–690. <https://doi.org/10.1093/molehr/gaw002>
- Qin, Y., Li, H., Jia, L., Yan, J., Gao, G. F., & Li, X. (2017). Targeted disruption of Noc4l leads to preimplantation embryonic lethality in mice. *Protein and Cell*, 8, 230–235. <https://doi.org/10.1007/s13238-016-0335-9>
- Ralston, A., Cox, B. J., Nishioka, N., Sasaki, H., Chea, E., Rugg-Gunn, P., Guo, G., Robson, P., Draper, J. S., & Rossant, J. (2010). Gata3 regulates trophoblast development downstream of Tead4 and in parallel to Cdx2. *Development*, 137(3), 395–403. <https://doi.org/10.1242/dev.038828>
- Ralston, A., & Rossant, J. (2008). Cdx2 acts downstream of cell polarization to cell-autonomously promote trophectoderm fate in the early mouse embryo. *Developmental Biology*, 313(2), 614–629. <https://doi.org/10.1016/j.ydbio.2007.10.054>
- Riethmacher, D., Brinkmann, V., & Birchmeier, C. (1995). A targeted mutation in the mouse E-cadherin gene results in defective preimplantation development. *Proceedings of the National Academy of Sciences of the United States of America*, 92(3), 855–859. <https://doi.org/10.1073/pnas.92.3.855>
- Rodda, D. J., Chew, J. L., Lim, L. H., Loh, Y. H., Wang, B., Ng, H. H., & Robson, P. (2005). Transcriptional regulation of Nanog by OCT4 and SOX2. *Journal of Biological Chemistry*, 280(26), 24731–24737. <https://doi.org/10.1074/jbc.M502573200>
- Roode, M., Blair, K., Snell, P., Elder, K., Marchant, S., Smith, A., & Nichols, J. (2012). Human hypoblast formation is not dependent on FGF signalling. *Developmental Biology*, 361(2), 358–363. <https://doi.org/10.1016/j.ydbio.2011.10.030>
- Saha, B., Ganguly, A., Home, P., Bhattacharya, B., Ray, S., Ghosh, A., Karim Rumi, M. A., Marsh, C., French, V. A., Gunewardena, S., & Paul, S. (2020). TEAD4 ensures postimplantation development by promoting trophoblast self-renewal: An implication in early human pregnancy loss. *Proceedings of the National Academy of Sciences of the United States of America*, 117, 17864–17875. <https://doi.org/10.1073/pnas.2002449117>
- Samarage, C. R., White, M. D., Álvarez, Y. D., Fierro-González, J. C., Henon, Y., Jesudason, E. C., Bissiere, S., Fouras, A., & Plachta, N. (2015). Cortical tension allocates the first inner cells of the mammalian embryo. *Developmental Cell*, 34(4), 435–447. <https://doi.org/10.1016/j.devcel.2015.07.004>
- Schall, P. Z., Ruebel, M. L., & Latham, K. E. (2019). A new role for SMCHD1 in life's master switch and beyond. *Trends in Genetics*, 35, 948–955. <https://doi.org/10.1016/j.tig.2019.10.001>

- Schulz, K. N., & Harrison, M. M. (2019). Mechanisms regulating zygotic genome activation. *Nature Reviews Genetics*, 20, 221–234.
- Sha, Q. Q., Zhang, J., & Fan, H. Y. (2019). A story of birth and death: mRNA translation and clearance at the onset of maternal-To-zygotic transition in mammals. *Biology of Reproduction*, 101, 579–590. <https://doi.org/10.1093/biolre/iox012>
- Smith, D. G., & Sturmey, R. G. (2013). Parallels between embryo and cancer cell metabolism. *Biochemical Society Transactions*, 41(2), 664–669. <https://doi.org/10.1042/BST20120352>
- Strumpf, D., Mao, C. A., Yamanaka, Y., Ralston, A., Chawengsaksophak, K., Beck, F., & Rossant, J. (2005). Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. *Development*, 132(9), 2093–2102. <https://doi.org/10.1242/dev.01801>
- Su, J., Miao, X., Archambault, D., Mager, J., & Cui, W. (2020). ZC3H4—a novel Cys-Cys-Cys-His-type zinc finger protein—Is essential for early embryogenesis in mice. *Biology of Reproduction*, 104(2), 325–335. <https://doi.org/10.1093/biolre/iaaa215>
- Suzuki, A., & Ohno, S. (2006). The PAR-aPKC system: Lessons in polarity. *Journal of Cell Science*, 119, 979–987.
- Tong, Z.B.in, Gold, L., Pfeifer, K. E., Dorward, H., Lee, E., Bondy, C. A., Dean, J., & Nelson, L. M. (2000). Mater, a maternal effect gene required for early embryonic development in mice. *Nature Genetics*, 26(3), 267–268. <https://doi.org/10.1038/81547>
- Torres, M., Stoykova, A., Huber, O., Chowdhury, K., Bonaldo, P., Mansouri, A., Butz, S., Kemler, R., & Gruss, P. (1997). An α -E-catenin gene trap mutation defines its function in preimplantation development. *Proceedings of the National Academy of Sciences of the United States of America*, 94(3), 901–906. <https://doi.org/10.1073/pnas.94.3.901>
- Ueno, M., Itoh, M., Kong, L., Sugihara, K., Asano, M., & Takakura, N. (2005). PSF1 is essential for early embryogenesis in mice. *Molecular and Cellular Biology*, 25, 10528–10532. <https://doi.org/10.1128/mcb.25.23.10528-10532.2005>
- Vassena, R., Boué, S., González-Roca, E., Aran, B., Auer, H., Veiga, A., & Belmonte, J. C. I. (2011). Waves of early transcriptional activation and pluripotency program initiation during human preimplantation development. *Development*, 138(17), 3699–3709. <https://doi.org/10.1242/dev.064741>
- Vestweber, D., Gossler, A., Boller, K., & Kemler, R. (1987). Expression and distribution of cell adhesion molecule uvomorulin in mouse preimplantation embryos. *Developmental Biology*, 124, 451–456.
- Wang, H., Wang, L., Wang, Z., Dang, Y., Shi, Y., Zhao, P., & Zhang, K. (2020). The nucleolar protein NOP2 is required for nucleolar maturation and ribosome biogenesis during preimplantation development in mammals. *FASEB Journal*, 34(2), 2715–2729. <https://doi.org/10.1096/fj.201902623R>
- Wang, J., Rao, S., Chu, J., Shen, X., Levasseur, D. N., Theunissen, T. W., & Orkin, S. H. (2006). A protein interaction network for pluripotency of embryonic stem cells. *Nature*, 444(7117), 364–368. <https://doi.org/10.1038/nature05284>
- Wang, X., Song, D., Mykytenko, D., Kuang, Y., Lv, Q., Li, B., Chen, B., Mao, X., Xu, Y., Zukin, V., Mazur, P., Mu, J., Yan, Z., Zhou, Z., Li, Q., Liu, S., Jin, L., He, L., ... Wang, L. (2018). Novel mutations in genes encoding subcortical maternal complex proteins may cause human embryonic developmental arrest. *Reproductive BioMedicine Online*, 36(6), 698–704. <https://doi.org/10.1016/j.rbmo.2018.03.009>
- Wells, D., Bermudez, M. G., Steuerwald, N., Thornhill, A. R., Walker, D. L., Malter, H., Delhanty, J. D. A., & Cohen, J. (2005). Expression of genes regulating chromosome segregation, the cell cycle and apoptosis during human preimplantation development. *Human Reproduction*, 20(5), 1339–1348. <https://doi.org/10.1093/humrep/deh778>
- Wong, C. C., Loewke, K. E., Bossert, N. L., Behr, B., De Jonge, C. J., Baer, T. M., & Pera, R. A. R. (2010). Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. *Nature Biotechnology*, 28(10), 1115–1121. <https://doi.org/10.1038/nbt.1686>
- Wu, G., Gentile, L., Fuchikami, T., Sutter, J., Psathaki, K., Esteves, T. C., Araújo-Bravo, M. J., Ortmeier, C., Verberk, G., Abe, K., & Schöler, H. R. (2010). Initiation of trophectoderm lineage specification in mouse embryos is independent of Cdx2. *Development*, 137, 4159–4169. <https://doi.org/10.1242/dev.056630>
- Wu, G., Han, D., Gong, Y., Sebastiano, V., Gentile, L., Singhal, N., Adachi, K., Fishedick, G., Ortmeier, C., Sinn, M., Radstaak, M., Tomilin, A., & Schöler, H. R. (2013). Establishment of totipotency does not depend on Oct4A. *Nature Cell Biology*, 15(9), 1089–1097. <https://doi.org/10.1038/ncb2816>
- Wu, X., Viveiros, M. M., Eppig, J. J., Bai, Y., Fitzpatrick, S. L., & Matzuk, M. M. (2003). Zygote arrest 1 (Zar1) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. *Nature Genetics*, 33(2), 187–191. <https://doi.org/10.1038/ng1079>
- Xu, Y., Shi, Y., Fu, J., Yu, M., Feng, R., Sang, Q., Liang, B., Chen, B., Qu, R., Li, B., Yan, Z., Mao, X., Kuang, Y., Jin, L., He, L., Sun, X., & Wang, L. (2016). Mutations in PADI6 cause female infertility characterized by early embryonic arrest. *American Journal of Human Genetics*, 99(3), 744–752. <https://doi.org/10.1016/j.ajhg.2016.06.024>
- Zhang, P., Zuchelli, M., Bruce, S., Hambiliki, F., Stavreus-Evers, A., Levkov, L., Skottman, H., Kerkelä, E., Kere, J., & Hovatta, O. (2009). Transcriptome profiling of human pre-implantation development. *PLOS One*, 4(11), e7844. <https://doi.org/10.1371/journal.pone.0007844>
- Zhao, P., Wang, H., Wang, H., Dang, Y., Luo, L., Li, S., Shi, Y., Wang, L., Wang, S., Mager, J., & Zhang, K. (2020). Essential roles of HDAC1 and 2 in lineage development and genome-wide DNA methylation during mouse preimplantation development. *Epigenetics*, 15, 369–385. <https://doi.org/10.1080/15592294.2019.1669375>

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