Morphogenesis of the human preimplantation embryo: bringing mechanics to the clinics

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Abstract

During preimplantation development, the human embryo forms the blastocyst, the structure enabling uterine implantation. The blastocyst consists of an epithelial envelope, the trophectoderm, encompassing a fluid-filled lumen, the blastocoel, and a cluster of pluripotent stem cells, the inner cell mass. This specific architecture is crucial for the implantation and further development of the human embryo. Furthermore, the morphology of the human embryo is a prime determinant for clinicians to assess the implantation potential of in vitro fertilized human embryos, which constitutes a key aspect of assisted reproduction technology. Therefore, it is crucial to understand how the human embryo builds the blastocyst. As any material, the human embryo changes shape under the action of forces. Here, we review recent advances in our understanding of the mechanical forces shaping the blastocyst. We discuss the cellular processes responsible for generating morphogenetic forces that were studied mostly in the mouse and review the literature on human embryos to see which of them may be conserved. Based on the specific morphological defects commonly observed in clinics during human preimplantation development, we discuss how mechanical forces and their underlying cellular processes may be affected. Together, we propose that bringing tissue mechanics to the clinics will advance our understanding of human preimplantation development, as well as our ability to help infertile couples to have babies.

Introduction:

In the first days after fertilization, the human embryo needs to acquire implantation competencies [1–4]. The structure enabling the human embryo to implant is called the blastocyst, which has a very characteristic architecture [5,6]. It consists of an epithelium, the trophectoderm (TE), enveloping a fluid-filled lumen, the blastocoel, and a cluster of pluripotent stem cells, the inner-cell mass (ICM). The TE invades the maternal uterus and implants the embryonic tissues deriving from the ICM [7]. Until implantation, the human embryo can develop without intervention from the mother. As a consequence, the self-organisation of the human embryo into the blastocyst can be studied ex vivo and offers unique opportunities to study human embryonic development.

To form the blastocyst, the human embryo initially undergoes cleavage divisions, *i.e.* there is no cell growth during the interphase (Fig 1). Essentially, cells halve their volume with each cleavage and cells rearrange themselves to shape the blastocyst without changing the total cellular volume of the embryo, as measured accurately in the mouse [8]. The series of cellular rearrangements sculpting the blastocyst can be broken down into three steps: compaction, internalisation and lumen formation [5]. Compaction is the process by which the loosely attached blastomeres enlarge their cell-cell contacts and reduce their surface exposed to the outside medium [9–12]. Internalisation occurs when a subset of cells become entirely surrounded by neighbouring blastomeres and isolated from the outside medium [13–17]. This differential positioning of cells is a prerequisite to the first lineage specification into ICM for inner cells and TE for those remaining at the embryo surface [18–21]. Finally, the blastocoel appears when TE cells pump fluid, which pushes the ICM into one quadrant of the blastocyst [22–24]. The lumen breaks the radial symmetry of the embryo, which is key to the formation of the axes of symmetry after implantation [23,25,26]. The lumen also serves as a new interface for the differentiation of the ICM into primitive endoderm (PrE) and epiblast (Epi) and of the TE into mural and polar TE (mTE and pTE respectively) [27–30]. Sandwiched between the pTE and PrE, the Epi will provide all cells of the human body while the PrE contributes to most of the yolk sac [31,32]. The pTE is thought to mediate uterine implantation in human whereas this role seems devoted to the mTE in mice [28,29,31,33,34].

Since the advent of in vitro culture, the morphology of the human embryo has been observed and described in numerous studies. In fact, together with cell number, the morphology of the human embryo is one of the prime determinant in clinicians assessment of the implantation potential of human embryos [35,36]. For example, poor compaction or slow lumen growth are associated with lower implantation rates [10,37–39]. Therefore, the current and future efforts in developing algorithms able to better predict the implantation potential of human embryos grown in vitro will undoubtedly rely on morphological criteria [40–42].

Despite the importance of human embryo morphology for its development, we know little of the mechanisms responsible for shaping the human embryo [1–3]. When it comes to shaping any materials, forces are necessarily in action [43,44]. In the past decades, major discoveries regarding the nature of forces that shape animals were made using model organisms such as the worm, fly, fish, frog and, more recently, mouse [11,45–48]. The same forces are most likely responsible for the shaping of the human embryo. Of particular relevance to the shaping of the human blastocyst are surface tension [49–51], adhesive coupling between cells [52–54] and, osmotic pressure [55,56]. In animal cells, surface tension is governed by the contractility of the acto-myosin cortex, a thin layer of cross-linked actin filaments underneath the plasma membrane that is put under tension by the action of non-

muscle myosin II motors [57,58]. The mechanical stresses exerted by the acto-myosin cortex is a prime determinant of cell shape as it drives cell rounding and cytokinetic cleavage in half during cytokinesis, or cell body retraction during migration or blebbing [59–63]. Adhesive coupling between cells resists their separation [23,64,65]. It is mediated by cadherin adhesion molecules and associated proteins, the catenins, which anchor the adhesion complex to the actin cytoskeleton. By doing so, the adhesion complex connects the acto-myosin cortex of contacting cells and transmits its tension throughout the tissue [66,67]. Osmotic pressure dictates the movement of water between sealed compartments such as the outside medium, the cytoplasm and fluid-filled lumens. Osmotic pressure relies on tight junctions, which seal intercellular spaces, and ion pumps, which actively change the osmolarity of sealed compartments [68,69]. Osmotic gradients will then trigger the passive movement of water between compartments through aquaporin water channels [70].

In this review, we discuss how learning about the mechanics of tissues can help understanding the shaping of the human blastocyst. We consider how defective morphologies could be explained by aberrant force patterns and point to specific cellular process underlying them. In particular, we examine defects in cleavage divisions, compaction, internalisation and lumen formation. Since defects in the morphology of human embryos is a prime determinant of their health, bringing mechanics to the clinics will be key to improve assisted reproduction technologies. For reviews covering the patterning of blastocyst lineages or general clinical aspects, we recommend alternative reviews [1–4].

Cleavages

Before human morphogenesis begins, cleavage divisions provide the only changes in the morphology of the embryo (Fig 1). The first division takes place after a day and the second half a day later [71,72]. Then, sister blastomeres divide every day in waves of progressively decreasing synchrony (Fig 1). The early cleavages are notoriously error prone [4,73]. They frequently lead to chromosome segregation errors [74] and are at the origin of aneuploidy that can lead to developmental arrest [73]. Alternatively, these errors can be corrected by excluding aneuploid cells from the embryonic tissue, which are later found in the placenta [75]. Aneuploid cells can also contribute to the epiblast with or without further consequences on embryonic development [76]. In addition to chromosome mis-segregation, cytokinesis can also be faulty. Cytokinesis should split cells in two equal volumes, forming embryos with even cell number and size. However, this is frequently not the case and has dire consequences for the embryo. Indeed, human embryos with unequally sized blastomeres at the 4- and 8-cell stages were found to have more frequent multinucleation and ploidy issues, which eventually reduces their implantation rate [77–79]. Therefore, unequally sized blastomeres can be used as an indicator of the genome integrity of the embryo and of its health in general.

Unequal blastomere sizes can result from distinct events (Fig 2). Cytokinesis itself can produce sister cells with uneven sizes. Skewing the cleavage furrow during cytokinesis, for example by contractility unbalance, will result in uneven cleavage [62]. Alternatively, blastomeres can undergo “reverse cleavages”, fusing sister cells back together after the cleavage furrow completed the separation of the cellular volumes of the sister cells [72,80]. In addition to reducing the total expected number of cell, this results in the formation of polyploid blastomeres with twice the size of their neighbouring cells, which can easily be detected. Incomplete cytokinesis could result from defects in abscission, the process sealing apart the cytoplasm of sister cells and leading to the formation of the midbody [81]. Abscission requires

the coordination of complex cellular machineries and often fails when chromosomes are mis-segregated. Often referred to as “direct cleavage”, blastomeres can cleave into 3 similarly sized daughter cells instead of 2. After the first and second cleavages, embryos can show 3 cells and 5 cells respectively [72,82]. This increases the number of expected cells and results in the formation of blastomeres that are 1/3rd smaller than expected, which can be difficult to notice with insufficient resolution. Direct cleavages are associated with fewer embryos reaching blastocyst stage and reduced implantation rate [82]. Nevertheless, blastocysts produced after direct cleavages can show normal ploidy, suggesting some repairing mechanism, possibly involving the exclusion of defective cells [72,83]. Such abnormal divisions can result from multipolar spindle and supernumerary centrioles caused by defective sperms [84,85]. Finally, human blastomeres often produce cellular fragments during cytokinesis, which wastes cellular material [73,86]. Importantly, fragments sometimes entrap chromosomes, making their cell of origin aneuploid [73,87]. Unsurprisingly, fragmentation is associated with poor prognosis for implantation [88,89]. Fragmentation is observed frequently in human embryos but rarely in mice, which makes the process difficult to study. Why human blastomeres produce so many fragments during cytokinesis is unclear. Blocking the actin and microtubule cytoskeletons reduces fragmentation, suggesting that these generate the forces driving fragmentation [90]. Cellular fragments have been compared to blebs due to their spherical shape. However, blebs are short-lived (around a minute), while fragment are stable over tens of hours [60]. Blebs could persists for longer time if contractility would be hyper-activated [91]. However, there is not enough information available on fragment dynamics to understand how they form and persist. Alternatively, membrane threads connecting the zona pellucida to blastomeres were proposed to pull on blastomeres during cytokinesis [92].

Compaction

Starting as early as during the 8-cell stage, compaction is the first morphogenetic process associated with the formation of the blastocyst (Fig 1). During this process, blastomeres get closer together, forming a tighter structure (Fig 3). This developmentally regulated adhesion process requires the calcium-dependent cell-cell adhesion molecule CDH1 (formerly known as E-cadherin or uvomorulin) [90,93–95]. Removing extracellular calcium prevents CDH1 binding and causes embryos to de-compact [96–98]. The compaction process was long thought to be driven by increased adhesion of cells via modifications of the CDH1-dependent adhesion machinery [1,99]. However, mechanical measurements in the mouse embryo revealed that the forces driving compaction are located at the embryo surface rather than at cell-cell contacts [11]. Micropipette aspiration of mouse blastomeres throughout compaction uncovers raising tensions at the surface of the embryo as a result from the action of the actomyosin cortex (Toolbox 1). Cells literally pull themselves together using their intracellular muscles. The adhesion molecule CDH1 enables compaction by anchoring the actin cytoskeleton of contacting cells so that they can effectively pull onto each other [67]. CDH1 also provides local signals lowering contractility at cell-cell contacts and effectively relaxing them [100]. Whether such mechanism is also responsible for human compaction remains to be tested. Identifying the nature of the forces driving human embryo compaction would help understanding what causes this process to fail in some instances.

Clinical studies found that compaction can be defective in several different ways (Fig 3). Compaction can be globally reduced with all blastomeres simply failing to grow their cell-cell contacts, which is associated with fewer embryos reaching blastocyst stage and lower

implantation rates [38,101]. Several cellular processes could be at fault, such as cell adhesion or contractility. Micropipette aspiration on embryos failing to compact would narrow down the options. Low tensions at the embryo surface would point towards contractility problems, such as in mouse mutants for myosin paralogs [16,102]. High tensions at the embryo surface would instead suggest defective adhesion, such as in mouse mutants for cadherin adhesion molecules [11].

Alternatively, compaction can be delayed. While a minority of embryo start compaction as early as during the 8-cell stage, the majority of human embryos begin compaction during the 4th wave of cleavages, with at least 9 blastomeres and most finish compaction during the 16-cell stage [10]. Embryos compacting less than 80 h after fertilization show higher rate of implantation and live birth than embryos compacting after the 80 h mark [12,101,103]. What controls the timing of compaction remains unclear though. In the mouse, activation of PKC can induce a premature (and transient) compaction [104,105], which has been proposed to promote contractility at the embryo surface [106].

Finally, compaction can be unequal (also referred to as partial compaction), with some blastomeres forming a compact mass while other cells appear excluded from this group (Fig 3). Excluded cells may later on contribute to the TE or be excluded entirely from the blastocyst. The time of exclusion can occur before or after compaction is complete, with higher survival rates for embryos excluding cells during the compaction process [9]. Interestingly, excluded cells tend to be aneuploid while the rest of the embryo seems euploid [72]. This indicates that exclusion of cells during compaction could serve as a repair mechanism ensuring that defective cells do not participate to the blastocyst or to the embryonic tissues. Indeed, cells of the placenta show a high rate of aneuploidy and have long been thought to act as a sink eliminating defective cells from embryonic tissues [75]. However, the mechanism underlying unequal compaction and exclusion of defective cells remain unknown. Again, excluded cells could have lower tension at their surfaces or higher tensions at their cell-cell contacts, which, if measured, would point at the underlying cellular process at fault, contractility and/or adhesion (Toolbox 1).

Internalisation of the ICM precursors

The positioning of cells within the human embryo away from the cell-medium interface is a critical morphogenetic step for the formation of the TE and ICM lineages (Fig 1). Experimental manipulation of cell position reveals that outside cells from early human blastocyst can adopt ICM fate if transplanted onto the ICM, away from the cell-medium interface [18]. This apparent plasticity is explained by the fact that human lineages are definitely set in the late blastocyst stage [20,31,107]. Until then, the position of cells within the human embryo will guide their differentiation into either TE or ICM. How do blastomeres adopt different positions within the human embryo in the first place?

Regardless of the molecular and cellular mechanisms that may control cell internalisation, with sufficient cleavages, blastomeres could end up on the inside of the embryo for geometrical reasons only [108]. Indeed, the packing of spherical objects into a sphere would necessarily position cells on the inside when they become sufficiently small as compared to the size of the embryo. If human embryos would be relying on geometrical packing only, then reducing the embryo size would prevent the formation of an ICM. Dissociating human embryos at the 4-cell stage reveals that 4-cell stage blastomeres are able to form smaller blastocyst [21]. Quarter embryos compact, grow their lumen and contain inner cells expressing the ICM

marker NANOG. This implies that mechanisms other than geometrical packing drive cell internalisation in human embryos.

In the mouse, at least two distinct mechanisms can internalise cells: oriented cell divisions and contractility-meditated cell sorting. Oriented cell division consists in aligning the mitotic spindle perpendicularly to the embryo surface so that one daughter cell is pushed inward after cytokinesis [15,109,110]. Cell sorting occurs during the interphase and relies on differences in surface tensions between cells, which, if sufficiently high, allow the strongest cells to pull their way inside the embryo [16,17]. Both positioning mechanisms rely on the establishment of apicobasal polarity with the apical domain facing the cell-medium interface [13–16]. Division orientation is guided by the tethering of one of the spindle pole to the apical domain [15]. This also facilitates the asymmetric inheritance of the apical domain, including sub-apical components such as intermediate filaments when present [111]. The asymmetric division of apical components, such as the apical kinases PRKCz and PRKCi (also known as aPKCι and aPKCζ) that downregulate actomyosin contractility, among sister cells is key to generate cell populations with different mechanical properties and drive cell sorting (Toolbox 1) [16,112]. Last but not least, apicobasal polarity governs the positional signals guiding the differentiation of TE and ICM lineages (Toolbox 2). Apical signals promote TE lineages by enforcing the nuclear localisation of the co-transcriptional activator YAP while cell-cell contacts favour its cytoplasmic degradation [112,113]. Therefore, the apical domain is a master regulator of lineage positioning and specification in the mouse embryo.

How much of this mechanism is conserved in human embryos is still unclear. Recently, the presence and function of the core components of the apical machinery controlling TE fate were confirmed in human and cow embryos [20]. Apical markers such as PRKCz, PARD6B or AMOT are observed at the apical domain of human embryos (Toolbox 2). The accumulation of PARD6B also seems to occur about 90 h post fertilization [114]. The human embryo then consists of at least 16 cells and compaction has completed [10,12,103]. This is later than in the mouse, which compacts and polarises during the 8-cell stage [11,20]. Similarly to the mouse [112], inhibition of PRKCz using a chemical inhibitor or TRIM-AWAY, which targets proteins to the proteasome using antibodies [115], impacts lineage specification in human embryos [20]. When apical signals are reduced, YAP nuclear localisation is compromised and the level of the TE-specific transcription factor GATA3 in outer cells is reduced [20]. Therefore, the TE specification module by apical signals seems conserved in human embryos. However, whether human embryos also rely on apicobasal polarity to positions inner and outer cells remains to be determined.

At that point, the human embryo forms what is called the morula (Fig 1). The morula is compact, surface cells have an apical domain facing the outside medium, which initiate their TE differentiation, while inner cells do not have apical material and start adopting an ICM fate. Importantly, compaction is functionally independent from polarisation (and cell internalisation). This is best illustrated with mouse mutant embryos in which the processes can be impaired selectively. Lacking essential apical kinases, *Prkcz;Prkci* mutants fail to form normal apical domains, which prevents the correct specification of TE and ICM lineages but show a normal, or even enhanced, compaction [112]. On the other hand, *Myh9* or *Myh9;Myh10* mutants fail to compact due to the absence of sufficient actomyosin contractility but polarise correctly and form ICM and TE lineages in the correct proportions [102]. Therefore, despite their apparent synchrony, compaction and polarisation (and cell internalisation) can occur independently from

one another in mouse mutants. This also seems to be the case for human embryos, which appear to compact normally when apical polarisation is impaired using inhibitors or TRIM-AWAY [20]. Whether compaction is required for human embryo polarisation remains to be tested.

Lumen formation

Apicobasal polarisation is also key for the formation of the blastocoel, the first mammalian lumen, which is the last morphogenetic step shaping the blastocyst (Fig 1). Fluid accumulates within the morula and inflates the embryo to almost 10 times its original volume [39]. Fluid is pumped into the intercellular space through the polarised surface epithelium while inner cells cluster into a bud against the epithelium. This bud forms the embryonic pole of the blastocyst, where the human embryo will mediate the uterine implantation [28,29,33,34]. In the expanded blastocyst, surface cells are now differentiated into TE and inner cells into ICM, which further specifies into PrE and Epi as the blastocyst prepares for implantation.

The formation of the blastocoel relies on the tight sealing of the TE, polarised transport and the ordered detachment of the ICM into one pole. Recent studies in the mouse provided mechanistic details on how these architectural changes proceed. Initially forming a domain in the centre of the cell-medium interface of surface cells, the apical domain expands [15]. When the apical domain hits the apical edge of cell-cell junctions, tight junctions seal the TE [24]. The expansion of the apical domain relies on coordinated cytoskeletal actions of the microtubules signalling to exclude acto-myosin from the apical domain, as observed in PRKC mutants [16,24]. This sealing can be challenged during cell divisions, which requires cells to round up and pull on their neighbouring cells, putting junctions under mechanical stress [22,116]. Therefore, tight junctions must reinforce themselves to prevent the embryo from collapsing under the increasing pressure of the blastocoel and during the waves of divisions of the TE. Polarised transport occurs through the cells rather than via junctions, as suggested by mouse mutants that, after failing all successive divisions, form a single-celled embryo, which nevertheless initiates blastocoel formation [102]. Indeed, as long as osmolytes transporters, such as the Na/K pump, and aquaporins are polarised along the apicobasal axis, blastomeres pump fluid from their apical to their basolateral compartment. This basolateral compartment is enriched in adhesion molecules, such as CDH1, and mechanically opposes detachment and fluid accumulation [23]. However, the fluid pressure, between 5-10 times higher than that of cells, is large enough to fracture cell-cell contacts. This includes contacts between ICM cells, which are transiently broken [23]. Contacts then repair themselves thanks to the action of their actomyosin cytoskeleton [117]. Interestingly, patterning the contractility of cells is sufficient to direct the positioning of the lumen and ICM, effectively dictating the first axis of symmetry of the mammalian embryo, which guides its implantation.

How much of these mechanisms are conserved in human embryos is unclear. The presence of key components from the tight junctions and fluid pumping machineries are likely [19,118–121]. More generally, the differentiation of the TE into a functional epithelium is required for the integrity of the human blastocoel, which fails when key transcription factor POU5F1 is mutated [19]. If conserved in human embryos, these mechanisms could have important clinical implications for better assessing embryos. The rate of blastocyst expansion is thought to provide information on its implantation potential, with implanting embryos showing higher expansion rates than non-implanting embryos [37,39]. Interestingly, the expansion rate is also an indicator of the ploidy state of the embryo, with aneuploid embryos inflating slower

than euploid ones [122]. Aneuploidies seem to be more frequent in the TE directly lying over the ICM, the polar TE, which attaches to the uterus [123]. The reason for the slower expansion rate could be linked to leaky TE, for example as a result of poor sealing of tight junctions or could be due to weaker polarised transport. Non-invasive methods allowing measuring the sealing and transport of human embryos would be very helpful to assess these properties and better understand the causes underlying slower expansion. Fluorescence based assays can be used to detect leakage [22,68]. Measuring volume changes after osmotic shocks would allow assessing the transport properties [124]. Besides, analysing blastocyst growth rates with higher temporal resolution could give more information on sudden collapses [55,116]. Several studies investigated the relationship between collapses and implantation rates, with sometimes contradictory conclusions [125–127]. As collapses may result from cell divisions transiently disrupting the TE seal, they may report on a healthy dividing TE [22,116]. Alternatively, if collapses are too frequent, they may reflect a poorly sealed and therefore dysfunctional TE. Finally, reports of human blastocyst with multiple clusters of inner cells suggest that monozygotic twinning may originate from the blastocyst [128]. If conserved in human embryos, hydraulic fracturing of contact between inner cells during blastocoel initiation may be responsible for the formation of multiple ICM. However, the formation of multiple ICM was not observed in the mouse, even when the fracturing or repair mechanisms were genetically manipulated [23]. Further investigations in the adhesive properties of ICM and TE cells will be needed to identify how the ICM could split.

Perspectives

Based on recent studies in mouse and human embryos, we have detailed how key steps of the shaping of the human blastocyst could proceed and fail. Since morphology serves as the basis for selecting the most suitable human embryos during assisted reproduction technology procedures [35,36], we believe there is much to be learned from the mechanisms of blastocyst morphogenesis. Current and future studies are taking exciting directions with the development of artificial intelligence (AI) based image analysis and non-invasive mechanical measurements. AI already shows performances equivalent to or even better than clinicians in predicting implantation rates, based on retrospective studies [40–42]. Most importantly, AI will allow more systematic procedures between clinics, as embryo diagnostic will rely less on the subjectivity of clinician eyes. However, algorithms, especially those based on machine learning, do not necessarily permit knowing the precise criteria used for predicting the implantation potential of embryos. Therefore, we should not rely on AI to uncover mechanistic understanding underlying developmental defects. On the other hand, non-invasive mechanical measurements offer the possibility to better understand the mechanisms for a given morphogenetic defects. For example, micropipette aspiration has been used on mouse and human oocytes and provided improved prediction of developmental potential [129]. Micropipette aspiration was performed through the zona pellucida, a porous glycoprotein shell of high elasticity compared to the oocyte. This aspiration measurement is therefore likely to reflect how porous and elastic the zona pellucida is, rather than probing the mechanics of the oocyte. Nevertheless, similar measurements could reveal how compaction fails in human embryos or even specific blastomeres. Such microaspiration could for example be performed as blastomeres are being remove during preimplantation genetic diagnostics. Together, better understanding the shaping of human blastocyst will improve the success rates of ART

procedures, which become more frequent as the age of first conception keeps on increasing [130].

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Figure Legends

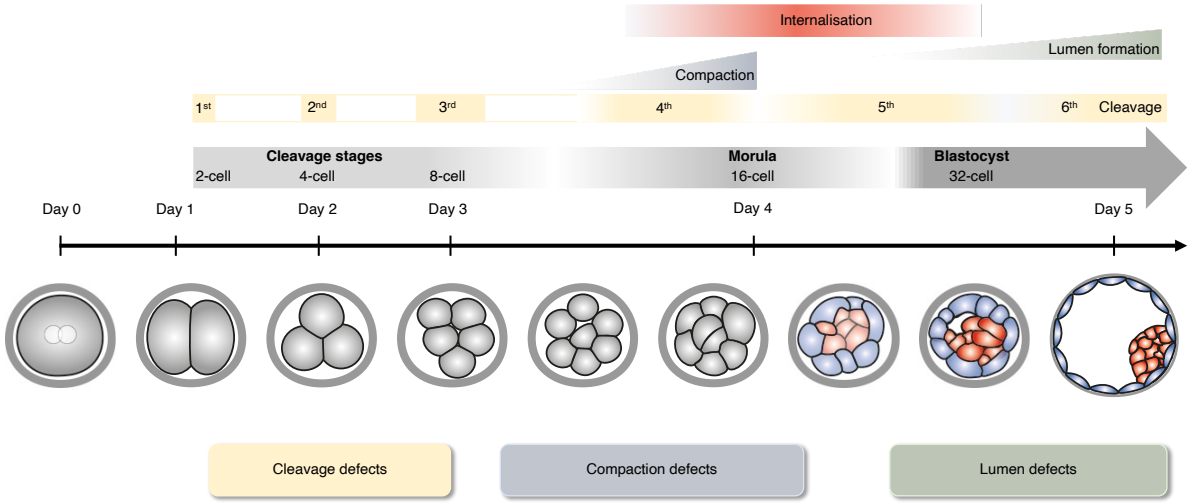


Figure 1: Human blastocyst morphogenesis

Starting from the zygote (Day 0), the human embryo undergoes cleavage divisions, which become progressively less synchronous with successive waves of cleavages, finishing here with the 6th wave on Day 5. During the cleavage stages, there is no morphogenesis. Compaction begins on Day 3, around the time of the 4th wave and ends on Day 4. The morula is complete when the embryo is compacted and contains inner cells (red). At the time of 5th cleavage, the outer cells (blue) pump fluid to inflate a lumen and turn the embryo into the blastocyst. Defective morphologies result from defects in cleavages (see Fig 2), compaction (see Fig 3) and/or lumen formation. Note that developmental time is not represented linearly.

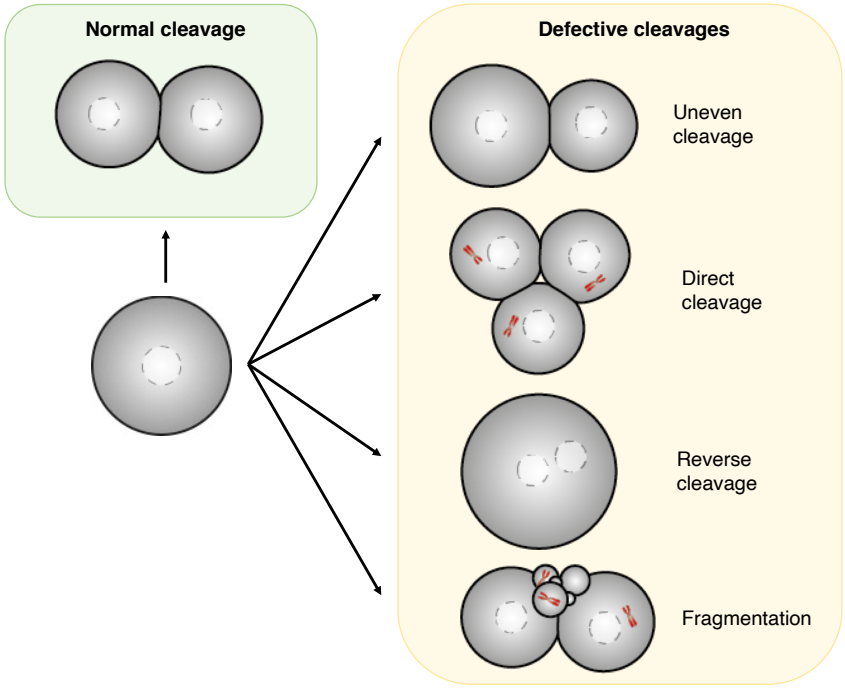


Figure 2: Cleavage defects during human preimplantation development

Normal cleavage division splits the volume of the blastomere in equal parts (top). Cleavage defects can affect the volume of daughter cells in a noticeable way (right). Cytokinesis itself can be asymmetric and produce daughter cells of unequal size. Tripolar mitotic spindles can produce three daughter cells instead of two and result in what is referred to as direct cleavage. This often result in the production of aneuploid cells (marked by the presence of extra chromosomes in red). Cytokinesis or abscission can fail resulting in the fusion of sister cells and the formation of a polyploid or bi-nucleated cell (reverse cleavage). During cytokinesis, cellular fragment frequently form. Fragments waste material and sometimes entrap chromosomes leading to aneuploidy.

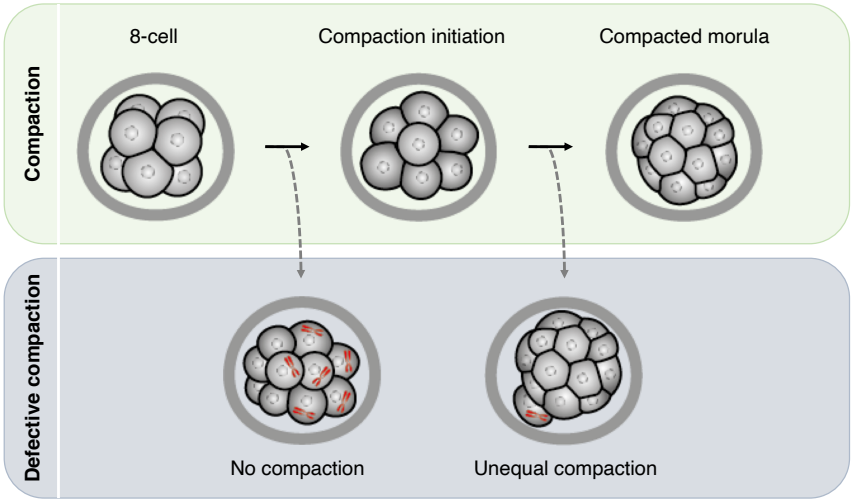
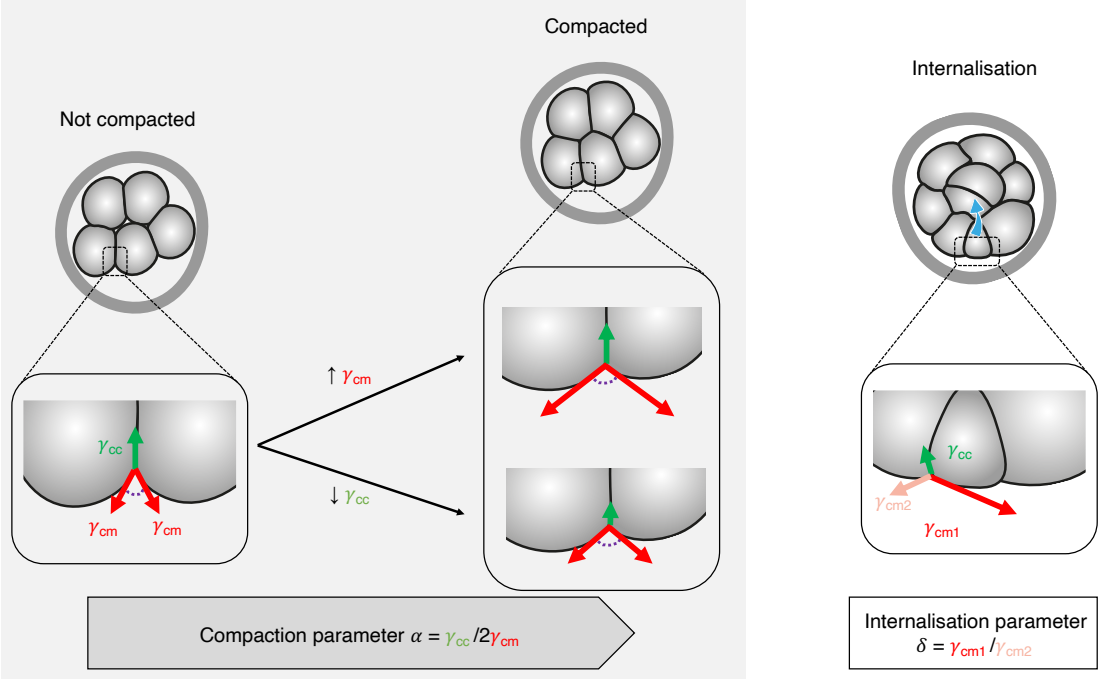


Figure 3: Compaction defects in human embryos

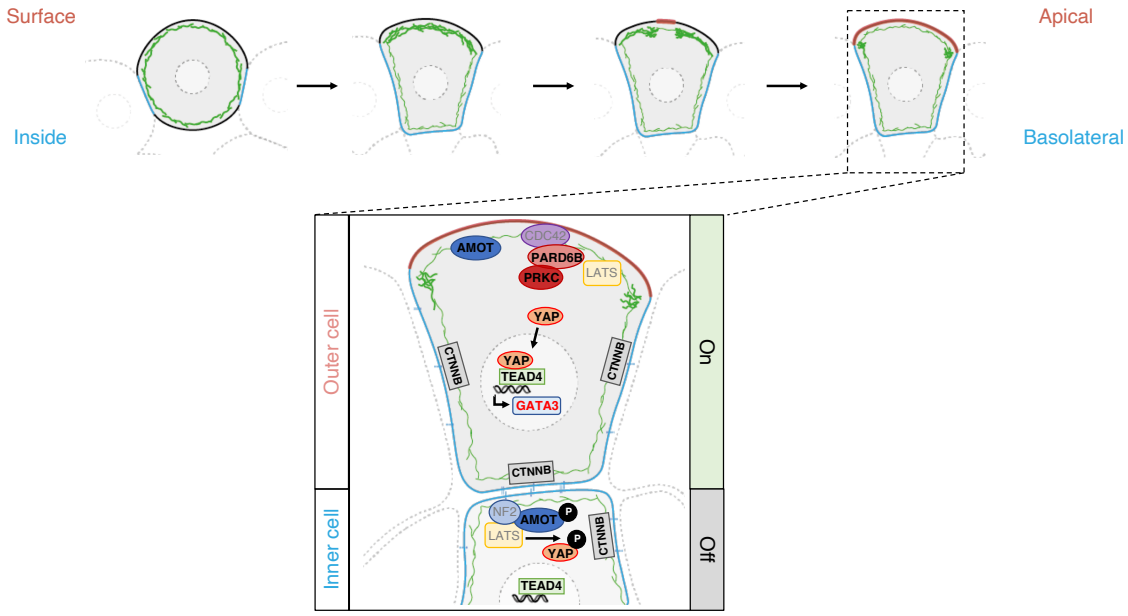
During normal compaction (top), the human embryo increases the area of contact between cells and reduces the surface exposed to the outside medium to form the compacted morula. Compaction can fail completely or be weak (bottom left). Compaction can also occur in subset of blastomeres only, excluding some blastomeres (bottom right). Blastomeres excluded from unequally compacted morula can be aneuploid. Excluded blastomeres could contribute to the TE and therefore placental tissues or be excluded from the blastocyst entirely.



Toolbox 1: *Surface tensions during compaction and cell internalisation*

Comparing the human embryo to contacting soap bubbles provides a general theoretical framework explaining compaction and cell internalisation [11,16,108]. *Blastomeres are given a surface tension at their cell-medium (γcm) and cell-cell interfaces (γcc). Following the Young-Dupré equation, the level of compaction is simply given by the ratio α of tension between cell-cell and cell-medium interfaces (γcc/2γcm). The surface tension of blastomeres can be calculated using the Young-Laplace equation, which relates the surface tension, pressure and curvature of liquid-like materials. Micropipette aspiration can then be used to determine the pressure inside blastomeres* [11,131]. *This approach revealed that, during compaction of the mouse embryo, γcm doubles while γcm decreases by a third. Using the absolute values of surface tensions further uncovers that ¾ of mouse compaction results from the changes in surface tension γcm and ¼ from the relaxation of cell-cell contacts* [11].

Considering the ratio of surface tensions at cell-medium interfaces of individual blastomeres δ = γcm1/γcm2, internalisation occurs when cell 1 grows its tension above a threshold value. This threshold is set at δ > 1 + 2α, and therefore depends the level of compaction [16]. *Interestingly, differences in size have no effect on internalisation in theory. For the mouse embryo, the internalisation threshold is at 1.5, i.e. cells need to grow their tension 50% higher than that of their neighboring cells to pull their way inside the embryo.*



Toolbox 2: De novo apicobasal polarisation and TE-ICM specification

During preimplantation development, blastomeres form de novo a domain of apical material (red) at the surface of the embryo, away from cell-cell contacts (basolateral interfaces in blue). The apical domain forms in the center of the contact free surface and signals to the actomyosin cortex (green) to deplete it locally. The apical domain progressively expands until it reaches the apical edge of the cell junctions. The apical domain contains molecules such as CDC42, PARD6B or PRKC that prevent the LATS kinase from phosphorylating the co-transcriptional activator YAP. YAP can shuttle to the nucleus to interact with the transcription factor TEAD4 and activate the expression of TE specific genes such as GATA3. Without an apical domain, inner cells show less YAP in their nucleus as the LATS kinase, with the help of NF2 and AMOT localised at cell-cell contacts, can phosphorylate YAP and target it to the proteasome. The presence of some of the signalling molecules involved in the subcellular localisation of YAP has been confirmed in human embryos (labelled in bold font), while other have only been studied in mouse embryos.

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