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

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12 Abstract

13 During preimplantation development, the human embryo forms the blastocyst, the structure 14 enabling uterine implantation. The blastocyst consists of an epithelial envelope, the 15 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 16 17 further development of the human embryo. Furthermore, the morphology of the human embryo 18 is a prime determinant for clinicians to assess the implantation potential of in vitro fertilized 19 human embryos, which constitutes a key aspect of assisted reproduction technology. 20 Therefore, it is crucial to understand how the human embryo builds the blastocyst. As any 21 material, the human embryo changes shape under the action of forces. Here, we review recent 22 advances in our understanding of the mechanical forces shaping the blastocyst. We discuss 23 the cellular processes responsible for generating morphogenetic forces that were studied 24 mostly in the mouse and review the literature on human embryos to see which of them may be 25 conserved. Based on the specific morphological defects commonly observed in clinics during 26 human preimplantation development, we discuss how mechanical forces and their underlying 27 cellular processes may be affected. Together, we propose that bringing tissue mechanics to 28 the clinics will advance our understanding of human preimplantation development, as well as 29 our ability to help infertile couples to have babies.

30 Introduction:

31 In the first days after fertilization, the human embryo needs to acquire implantation 32 competencies [1-4]. The structure enabling the human embryo to implant is called the 33 blastocyst, which has a very characteristic architecture [5,6]. It consists of an epithelium, the 34 trophectoderm (TE), enveloping a fluid-filled lumen, the blastocoel, and a cluster of pluripotent 35 stem cells, the inner-cell mass (ICM). The TE invades the maternal uterus and implants the 36 embryonic tissues deriving from the ICM [7]. Until implantation, the human embryo can develop 37 without intervention from the mother. As a consequence, the self-organisation of the human 38 embryo into the blastocyst can be studied ex vivo and offers unique opportunities to study 39 human embryonic development.

40 To form the blastocyst, the human embryo initially undergoes cleavage divisions, *i.e.* 41 there is no cell growth during the interphase (Fig 1). Essentially, cells halve their volume with 42 each cleavage and cells rearrange themselves to shape the blastocyst without changing the 43 total cellular volume of the embryo, as measured accurately in the mouse [8]. The series of 44 cellular rearrangements sculpting the blastocyst can be broken down into three steps: 45 compaction, internalisation and lumen formation [5]. Compaction is the process by which the 46 loosely attached blastomeres enlarge their cell-cell contacts and reduce their surface exposed 47 to the outside medium [9–12]. Internalisation occurs when a subset of cells become entirely 48 surrounded by neighbouring blastomeres and isolated from the outside medium [13–17]. This 49 differential positioning of cells is a prerequisite to the first lineage specification into ICM for 50 inner cells and TE for those remaining at the embryo surface [18-21]. Finally, the blastocoel 51 appears when TE cells pump fluid, which pushes the ICM into one quadrant of the blastocyst 52 [22-24]. The lumen breaks the radial symmetry of the embryo, which is key to the formation of 53 the axes of symmetry after implantation [23,25,26]. The lumen also serves as a new interface 54 for the differentiation of the ICM into primitive endoderm (PrE) and epiblast (Epi) and of the TE 55 into mural and polar TE (mTE and pTE respectively) [27-30]. Sandwiched between the pTE 56 and PrE, the Epi will provide all cells of the human body while the PrE contributes to most of 57 the yolk sac [31,32]. The pTE is thought to mediate uterine implantation in human whereas 58 this role seems devoted to the mTE in mice [28,29,31,33,34].

59 Since the advent of in vitro culture, the morphology of the human embryo has been 60 observed and described in numerous studies. In fact, together with cell number, the 61 morphology of the human embryo is one of the prime determinant in clinicians assessment of 62 the implantation potential of human embryos [35,36]. For example, poor compaction or slow 63 lumen growth are associated with lower implantation rates [10,37–39]. Therefore, the current 64 and future efforts in developing algorithms able to better predict the implantation potential of 65 human embryos grown in vitro will undoubtedly rely on morphological criteria [40–42].

66 Despite the importance of human embryo morphology for its development, we know 67 little of the mechanisms responsible for shaping the human embryo [1–3]. When it comes to 68 shaping any materials, forces are necessarily in action [43,44]. In the past decades, major 69 discoveries regarding the nature of forces that shape animals were made using model 70 organisms such as the worm, fly, fish, frog and, more recently, mouse [11,45-48]. The same 71 forces are most likely responsible for the shaping of the human embryo. Of particular relevance 72 to the shaping of the human blastocyst are surface tension [49-51], adhesive coupling 73 between cells [52-54] and, osmotic pressure [55,56]. In animal cells, surface tension is 74 governed by the contractility of the acto-myosin cortex, a thin layer of cross-linked actin 75 filaments underneath the plasma membrane that is put under tension by the action of non76 muscle myosin II motors [57,58]. The mechanical stresses exerted by the acto-myosin cortex 77 is a prime determinant of cell shape as it drives cell rounding and cytokinetic cleavage in half 78 during cytokinesis, or cell body retraction during migration or blebbing [59-63]. Adhesive 79 coupling between cells resists their separation [23,64,65]. It is mediated by cadherin adhesion 80 molecules and associated proteins, the catenins, which anchor the adhesion complex to the 81 actin cytoskeleton. By doing so, the adhesion complex connects the acto-myosin cortex of 82 contacting cells and transmits its tension throughout the tissue [66,67]. Osmotic pressure 83 dictates the movement of water between sealed compartments such as the outside medium, 84 the cytoplasm and fluid-filled lumens. Osmotic pressure relies on tight junctions, which seal 85 intercellular spaces, and ion pumps, which actively change the osmolarity of sealed 86 compartments [68,69]. Osmotic gradients will then trigger the passive movement of water 87 between compartments through aquaporin water channels [70].

88 In this review, we discuss how learning about the mechanics of tissues can help 89 understanding the shaping of the human blastocyst. We consider how defective morphologies 90 could be explained by aberrant force patterns and point to specific cellular process underlying 91 them. In particular, we examine defects in cleavage divisions, compaction, internalisation and 92 lumen formation. Since defects in the morphology of human embryos is a prime determinant 93 of their health, bringing mechanics to the clinics will be key to improve assisted reproduction 94 technologies. For reviews covering the patterning of blastocyst lineages or general clinical 95 aspects, we recommend alternative reviews [1-4].

96

97 Cleavages

98 Before human morphogenesis begins, cleavage divisions provide the only changes in 99 the morphology of the embryo (Fig 1). The first division takes place after a day and the second 100 half a day later [71,72]. Then, sister blastomeres divide every day in waves of progressively 101 decreasing synchrony (Fig 1). The early cleavages are notoriously error prone [4,73]. They 102 frequently lead to chromosome segregation errors [74] and are at the origin of an uploidy that 103 can lead to developmental arrest [73]. Alternatively, these errors can be corrected by excluding 104 aneuploid cells from the embryonic tissue, which are later found in the placenta [75]. Aneuploid 105 cells can also contribute to the epiblast with or without further consequences on embryonic 106 development [76]. In addition to chromosome mis-segregation, cytokinesis can also be faulty. 107 Cytokinesis should split cells in two equal volumes, forming embryos with even cell number 108 and size. However, this is frequently not the case and has dire consequences for the embryo. 109 Indeed, human embryos with unequally sized blastomeres at the 4- and 8-cell stages were 110 found to have more frequent multinucleation and ploidy issues, which eventually reduces their 111 implantation rate [77–79]. Therefore, unequally sized blastomeres can be used as an indicator 112 of the genome integrity of the embryo and of its health in general.

113 Unequal blastomere sizes can result from distinct events (Fig 2). Cytokinesis itself can 114 produce sister cells with uneven sizes. Skewing the cleavage furrow during cytokinesis, for 115 example by contractility unbalance, will result in uneven cleavage [62]. Alternatively, 116 blastomeres can undergo "reverse cleavages", fusing sister cells back together after the 117 cleavage furrow completed the separation of the cellular volumes of the sister cells [72,80]. In 118 addition to reducing the total expected number of cell, this results in the formation of polyploid 119 blastomeres with twice the size of their neighbouring cells, which can easily be detected. 120 Incomplete cytokinesis could result from defects in abscission, the process sealing apart the 121 cytoplasm of sister cells and leading to the formation of the midbody [81]. Abscission requires 122 the coordination of complex cellular machineries and often fails when chromosomes are mis-123 segregated. Often referred to as "direct cleavage", blastomeres can cleave into 3 similarly 124 sized daughter cells instead of 2. After the first and second cleavages, embryos can show 3 125 cells and 5 cells respectively [72,82]. This increases the number of expected cells and results 126 in the formation of blastomeres that are 1/3rd smaller than expected, which can be difficult to 127 notice with insufficient resolution. Direct cleavages are associated with fewer embryos 128 reaching blastocyst stage and reduced implantation rate [82]. Nevertheless, blastocysts 129 produced after direct cleavages can show normal ploidy, suggesting some repairing 130 mechanism, possibly involving the exclusion of defective cells [72,83]. Such abnormal divisions 131 can result from multipolar spindle and supernumerary centrioles caused by defective sperms 132 [84,85]. Finally, human blastomeres often produce cellular fragments during cytokinesis, which 133 wastes cellular material [73,86]. Importantly, fragments sometimes entrap chromosomes, 134 making their cell of origin an euploid [73,87]. Unsurprisingly, fragmentation is associated with 135 poor prognosis for implantation [88,89]. Fragmentation is observed frequently in human 136 embryos but rarely in mice, which makes the process difficult to study. Why human 137 blastomeres produce so many fragments during cytokinesis is unclear. Blocking the actin and 138 microtubule cytoskeletons reduces fragmentation, suggesting that these generate the forces 139 driving fragmentation [90]. Cellular fragments have been compared to blebs due to their 140 spherical shape. However, blebs are short-lived (around a minute), while fragment are stable 141 over tens of hours [60]. Blebs could persists for longer time if contractility would be hyper-142 activated [91]. However, there is not enough information available on fragment dynamics to 143 understand how they form and persist. Alternatively, membrane threads connecting the zona 144 pellucida to blastomeres were proposed to pull on blastomeres during cytokinesis [92].

146 **Compaction**

145

147 Starting as early as during the 8-cell stage, compaction is the first morphogenetic 148 process associated with the formation of the blastocyst (Fig 1). During this process, 149 blastomeres get closer together, forming a tighter structure (Fig 3). This developmentally 150 regulated adhesion process requires the calcium-dependent cell-cell adhesion molecule CDH1 151 (formerly known as E-cadherin or uvomorulin) [90,93–95]. Removing extracellular calcium 152 prevents CDH1 binding and causes embryos to de-compact [96–98]. The compaction process 153 was long thought to be driven by increased adhesion of cells via modifications of the CDH1-154 dependent adhesion machinery [1,99]. However, mechanical measurements in the mouse 155 embryo revealed that the forces driving compaction are located at the embryo surface rather 156 than at cell-cell contacts [11]. Micropipette aspiration of mouse blastomeres throughout 157 compaction uncovers raising tensions at the surface of the embryo as a result from the action 158 of the actomyosin cortex (Toolbox 1). Cells literally pull themselves together using their 159 intracellular muscles. The adhesion molecule CDH1 enables compaction by anchoring the 160 actin cytoskeleton of contacting cells so that they can effectively pull onto each other [67]. 161 CDH1 also provides local signals lowering contractility at cell-cell contacts and effectively 162 relaxing them [100]. Whether such mechanism is also responsible for human compaction 163 remains to be tested. Identifying the nature of the forces driving human embryo compaction 164 would help understanding what causes this process to fail in some instances.

165 Clinical studies found that compaction can be defective in several different ways (Fig 166 3). Compaction can be globally reduced with all blastomeres simply failing to grow their cell-167 cell contacts, which is associated with fewer embryos reaching blastocyst stage and lower 168 implantation rates [38,101]. Several cellular processes could be at fault, such as cell adhesion 169 or contractility. Micropipette aspiration on embryos failing to compact would narrow down the 170 options. Low tensions at the embryo surface would point towards contractility problems, such 171 as in mouse mutants for myosin paralogs [16,102]. High tensions at the embryo surface would 172 instead suggest defective adhesion, such as in mouse mutants for cadherin adhesion 173 molecules [11].

174 Alternatively, compaction can be delayed. While a minority of embryo start compaction 175 as early as during the 8-cell stage, the majority of human embryos begin compaction during 176 the 4th wave of cleavages, with at least 9 blastomeres and most finish compaction during the 177 16-cell stage [10]. Embryos compacting less than 80 h after fertilization show higher rate of 178 implantation and live birth than embryos compacting after the 80 h mark [12,101,103]. What 179 controls the timing of compaction remains unclear though. In the mouse, activation of PKC can 180 induce a premature (and transient) compaction [104,105], which has been proposed to 181 promote contractility at the embryo surface [106].

182 Finally, compaction can be unequal (also referred to as partial compaction), with some 183 blastomeres forming a compact mass while other cells appear excluded from this group (Fig 184 3). Excluded cells may later on contribute to the TE or be excluded entirely from the blastocyst. 185 The time of exclusion can occur before or after compaction is complete, with higher survival 186 rates for embryos excluding cells during the compaction process [9]. Interestingly, excluded 187 cells tend to be an euploid while the rest of the embryo seems euploid [72]. This indicates that 188 exclusion of cells during compaction could serve as a repair mechanism ensuring that 189 defective cells do not participate to the blastocyst or to the embryonic tissues. Indeed, cells of 190 the placenta show a high rate of an uploidy and have long been thought to act as a sink 191 eliminating defective cells from embryonic tissues [75]. However, the mechanism underlying 192 unequal compaction and exclusion of defective cells remain unknown. Again, excluded cells 193 could have lower tension at their surfaces or higher tensions at their cell-cell contacts, which, 194 if measured, would point at the underlying cellular process at fault, contractility and/or adhesion 195 (Toolbox 1).

196

197 Internalisation of the ICM precursors

198 The positioning of cells within the human embryo away from the cell-medium interface 199 is a critical morphogenetic step for the formation of the TE and ICM lineages (Fig 1). 200 Experimental manipulation of cell position reveals that outside cells from early human 201 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 202 203 definitely set in the late blastocyst stage [20,31,107]. Until then, the position of cells within the 204 human embryo will guide their differentiation into either TE or ICM. How do blastomeres adopt 205 different positions within the human embryo in the first place?

206 Regardless of the molecular and cellular mechanisms that may control cell 207 internalisation, with sufficient cleavages, blastomeres could end up on the inside of the embryo 208 for geometrical reasons only [108]. Indeed, the packing of spherical objects into a sphere would 209 necessarily position cells on the inside when they become sufficiently small as compared to 210 the size of the embryo. If human embryos would be relying on geometrical packing only, then 211 reducing the embryo size would prevent the formation of an ICM. Dissociating human embryos 212 at the 4-cell stage reveals that 4-cell stage blastomeres are able to form smaller blastocyst 213 [21]. Quarter embryos compact, grow their lumen and contain inner cells expressing the ICM

214 marker NANOG. This implies that mechanisms other than geometrical packing drive cell 215 internalisation in human embryos.

216 In the mouse, at least two distinct mechanisms can internalise cells: oriented cell 217 divisions and contractility-meditated cell sorting. Oriented cell division consists in aligning the 218 mitotic spindle perpendicularly to the embryo surface so that one daughter cell is pushed 219 inward after cytokinesis [15,109,110]. Cell sorting occurs during the interphase and relies on 220 differences in surface tensions between cells, which, if sufficiently high, allow the strongest 221 cells to pull their way inside the embryo [16,17]. Both positioning mechanisms rely on the 222 establishment of apicobasal polarity with the apical domain facing the cell-medium interface 223 [13–16]. Division orientation is guided by the tethering of one of the spindle pole to the apical 224 domain [15]. This also facilitates the asymmetric inheritance of the apical domain, including 225 sub-apical components such as intermediate filaments when present [111]. The asymmetric 226 division of apical components, such as the apical kinases PRKCz and PRKCi (also known as 227 aPKCι and aPKCζ) that downregulate actomyosin contractility, among sister cells is key to 228 generate cell populations with different mechanical properties and drive cell sorting (Toolbox 229 1) [16,112]. Last but not least, apicobasal polarity governs the positional signals guiding the 230 differentiation of TE and ICM lineages (Toolbox 2). Apical signals promote TE lineages by 231 enforcing the nuclear localisation of the co-transcriptional activator YAP while cell-cell contacts 232 favour its cytoplasmic degradation [112,113]. Therefore, the apical domain is a master 233 regulator of lineage positioning and specification in the mouse embryo.

234 How much of this mechanism is conserved in human embryos is still unclear. Recently, 235 the presence and function of the core components of the apical machinery controlling TE fate 236 were confirmed in human and cow embryos [20]. Apical markers such as PRKCz, PARD6B or 237 AMOT are observed at the apical domain of human embryos (Toolbox 2). The accumulation 238 of PARD6B also seems to occur about 90 h post fertilization [114]. The human embryo then 239 consists of at least 16 cells and compaction has completed [10,12,103]. This is later than in 240 the mouse, which compacts and polarises during the 8-cell stage [11,20]. Similarly to the 241 mouse [112], inhibition of PRKCz using a chemical inhibitor or TRIM-AWAY, which targets 242 proteins to the proteasome using antibodies [115], impacts lineage specification in human 243 embryos [20]. When apical signals are reduced, YAP nuclear localisation is compromised and 244 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, 245 246 whether human embryos also rely on apicobasal polarity to positions inner and outer cells 247 remains to be determined.

248

249 At that point, the human embryo forms what is called the morula (Fig 1). The morula is 250 compact, surface cells have an apical domain facing the outside medium, which initiate their 251 TE differentiation, while inner cells do not have apical material and start adopting an ICM fate. 252 Importantly, compaction is functionally independent from polarisation (and cell internalisation). 253 This is best illustrated with mouse mutant embryos in which the processes can be impaired 254 selectively. Lacking essential apical kinases, *Prkcz;Prkci* mutants fail to form normal apical 255 domains, which prevents the correct specification of TE and ICM lineages but show a normal, 256 or even enhanced, compaction [112]. On the other hand, Myh9 or Myh9;Myh10 mutants fail to 257 compact due to the absence of sufficient actomyosin contractility but polarise correctly and 258 form ICM and TE lineages in the correct proportions [102]. Therefore, despite their apparent 259 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.

264

265 Lumen formation

266 Apicobasal polarisation is also key for the formation of the blastocoel, the first 267 mammalian lumen, which is the last morphogenetic step shaping the blastocyst (Fig 1). Fluid 268 accumulates within the morula and inflates the embryo to almost 10 times its original volume 269 [39]. Fluid is pumped into the intercellular space through the polarised surface epithelium while 270 inner cells cluster into a bud against the epithelium. This bud forms the embryonic pole of the 271 blastocyst, where the human embryo will mediate the uterine implantation [28,29,33,34]. In the 272 expanded blastocyst, surface cells are now differentiated into TE and inner cells into ICM, 273 which further specifies into PrE and Epi as the blastocyst prepares for implantation.

274 The formation of the blastocoel relies on the tight sealing of the TE, polarised transport 275 and the ordered detachment of the ICM into one pole. Recent studies in the mouse provided 276 mechanistic details on how these architectural changes proceed. Initially forming a domain in 277 the centre of the cell-medium interface of surface cells, the apical domain expands [15]. When 278 the apical domain hits the apical edge of cell-cell junctions, tight junctions seal the TE [24]. 279 The expansion of the apical domain relies on coordinated cytoskeletal actions of the 280 microtubules signalling to exclude acto-myosin from the apical domain, as observed in PRKC 281 mutants [16,24]. This sealing can be challenged during cell divisions, which requires cells to 282 round up and pull on their neighbouring cells, putting junctions under mechanical stress 283 [22,116]. Therefore, tight junctions must reinforce themselves to prevent the embryo from 284 collapsing under the increasing pressure of the blastocoel and during the waves of divisions 285 of the TE. Polarised transport occurs through the cells rather than via junctions, as suggested 286 by mouse mutants that, after failing all successive divisions, form a single-celled embryo, which 287 nevertheless initiates blastocoel formation [102]. Indeed, as long as osmolytes transporters, 288 such as the Na/K pump, and aquaporins are polarised along the apicobasal axis, blastomeres 289 pump fluid from their apical to their basolateral compartment. This basolateral compartment is 290 enriched in adhesion molecules, such as CDH1, and mechanically opposes detachment and 291 fluid accumulation [23]. However, the fluid pressure, between 5-10 times higher than that of 292 cells, is large enough to fracture cell-cell contacts. This includes contacts between ICM cells, 293 which are transiently broken [23]. Contacts then repair themselves thanks to the action of their 294 actomyosin cytoskeleton [117]. Interestingly, patterning the contractility of cells is sufficient to 295 direct the positioning of the lumen and ICM, effectively dictating the first axis of symmetry of 296 the mammalian embryo, which guides its implantation.

297 How much of these mechanisms are conserved in human embryos is unclear. The 298 presence of key components from the tight junctions and fluid pumping machineries are likely 299 [19,118–121]. More generally, the differentiation of the TE into a functional epithelium is 300 required for the integrity of the human blastocoel, which fails when key transcription factor 301 POU5F1 is mutated [19]. If conserved in human embryos, these mechanisms could have 302 important clinical implications for better assessing embryos. The rate of blastocyst expansion 303 is thought to provide information on its implantation potential, with implanting embryos showing 304 higher expansion rates than non-implanting embryos [37,39]. Interestingly, the expansion rate 305 is also an indicator of the ploidy state of the embryo, with aneuploid embryos inflating slower

306 than euploid ones [122]. Aneuploidies seem to be more frequent in the TE directly lying over 307 the ICM, the polar TE, which attaches to the uterus [123]. The reason for the slower expansion 308 rate could be linked to leaky TE, for example as a result of poor sealing of tight junctions or 309 could be due to weaker polarised transport. Non-invasive methods allowing measuring the 310 sealing and transport of human embryos would be very helpful to assess these properties and 311 better understand the causes underlying slower expansion. Fluorescence based assays can 312 be used to detect leakage [22,68]. Measuring volume changes after osmotic shocks would 313 allow assessing the transport properties [124]. Besides, analysing blastocyst growth rates with 314 higher temporal resolution could give more information on sudden collapses [55,116]. Several 315 studies investigated the relationship between collapses and implantation rates, with 316 sometimes contradictory conclusions [125-127]. As collapses may result from cell divisions 317 transiently disrupting the TE seal, they may report on a healthy dividing TE [22,116]. 318 Alternatively, if collapses are too frequent, they may reflect a poorly sealed and therefore 319 dysfunctional TE. Finally, reports of human blastocyst with multiple clusters of inner cells 320 suggest that monozygotic twinning may originate from the blastocyst [128]. If conserved in 321 human embryos, hydraulic fracturing of contact between inner cells during blastocoel initiation 322 may be responsible for the formation of multiple ICM. However, the formation of multiple ICM 323 was not observed in the mouse, even when the fracturing or repair mechanisms were 324 genetically manipulated [23]. Further investigations in the adhesive properties of ICM and TE 325 cells will be needed to identify how the ICM could split.

326

327 **Perspectives**

328 Based on recent studies in mouse and human embryos, we have detailed how key 329 steps of the shaping of the human blastocyst could proceed and fail. Since morphology serves 330 as the basis for selecting the most suitable human embryos during assisted reproduction 331 technology procedures [35,36], we believe there is much to be learned from the mechanisms 332 of blastocyst morphogenesis. Current and future studies are taking exciting directions with the 333 development of artificial intelligence (AI) based image analysis and non-invasive mechanical 334 measurements. All already shows performances equivalent to or even better than clinicians in 335 predicting implantation rates, based on retrospective studies [40-42]. Most importantly, AI will 336 allow more systematic procedures between clinics, as embryo diagnostic will rely less on the 337 subjectivity of clinician eyes. However, algorithms, especially those based on machine 338 learning, do not necessarily permit knowing the precise criteria used for predicting the 339 implantation potential of embryos. Therefore, we should not rely on AI to uncover mechanistic 340 understanding underlying developmental defects. On the other hand, non-invasive mechanical 341 measurements offer the possibility to better understand the mechanisms for a given 342 morphogenetic defects. For example, micropipette aspiration has been used on mouse and 343 human oocytes and provided improved prediction of developmental potential [129]. 344 Micropipette aspiration was performed through the zona pellucida, a porous glycoprotein shell 345 of high elasticity compared to the oocyte. This aspiration measurement is therefore likely to 346 reflect how porous and elastic the zona pellucida is, rather than probing the mechanics of the 347 oocyte. Nevertheless, similar measurements could reveal how compaction fails in human 348 embryos or even specific blastomeres. Such microaspiration could for example be performed 349 as blastomeres are being remove during preimplantation genetic diagnostics. Together, better 350 understanding the shaping of human blastocyst will improve the success rates of ART

- 351 procedures, which become more frequent as the age of first conception keeps on increasing
- 352 [130].

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364 Figure Legends



365 366

367 Figure 1: Human blastocyst morphogenesis

368 Starting from the zygote (Day 0), the human embryo undergoes cleavage divisions, which 369 become progressively less synchronous with successive waves of cleavages, finishing here

370 with the 6th wave on Day 5. During the cleavage stages, there is no morphogenesis.

371 Compaction begins on Day 3, around the time of the 4th wave and ends on Day 4. The morula

372 is complete when the embryo is compacted and contains inner cells (red). At the time of 5th

373 cleavage, the outer cells (blue) pump fluid to inflate a lumen and turn the embryo into the

374 blastocyst. Defective morphologies result from defects in cleavages (see Fig 2), compaction

375 (see Fig 3) and/or lumen formation. Note that developmental time is not represented linearly.



376 377

378 Figure 2: Cleavage defects during human preimplantation development

379 Normal cleavage division splits the volume of the blastomere in equal parts (top). Cleavage

380 defects can affect the volume of daughter cells in a noticeable way (right). Cytokinesis itself

381 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

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

385 and the formation of a polyploid or bi-nucleated cell (reverse cleavage). During cytokinesis,

386 cellular fragment frequently form. Fragments waste material and sometimes entrap

387 chromosomes leading to aneuploidy.



388 389

- 390 Figure 3: Compaction defects in human embryos
- 391 During normal compaction (top), the human embryo increases the area of contact between
- 392 cells and reduces the surface exposed to the outside medium to form the compacted morula.
- 393 Compaction can fail completely or be weak (bottom left). Compaction can also occur in subset
- 394 of blastomeres only, excluding some blastomeres (bottom right). Blastomeres excluded from
- 395 unequally compacted morula can be aneuploid. Excluded blastomeres could contribute to the
- 396 TE and therefore placental tissues or be excluded from the blastocyst entirely.



397 398

399 Toolbox 1: Surface tensions during compaction and cell internalisation

400 Comparing the human embryo to contacting soap bubbles provides a general theoretical 401 framework explaining compaction and cell internalisation [11,16,108]. Blastomeres are given 402 a surface tension at their cell-medium (ycm) and cell-cell interfaces (ycc). Following the Young-403 Dupré equation, the level of compaction is simply given by the ratio a of tension between cell-404 cell and cell-medium interfaces (ycc/2ycm). The surface tension of blastomeres can be 405 calculated using the Young-Laplace equation, which relates the surface tension, pressure and 406 curvature of liquid-like materials. Micropipette aspiration can then be used to determine the 407 pressure inside blastomeres [11,131]. This approach revealed that, during compaction of the 408 mouse embryo, ycm doubles while ycm decreases by a third. Using the absolute values of 409 surface tensions further uncovers that 3/4 of mouse compaction results from the changes in 410 surface tension ycm and 1/4 from the relaxation of cell-cell contacts [11].

411 Considering the ratio of surface tensions at cell-medium interfaces of individual blastomeres δ 412 = $\gamma cm1/\gamma cm2$, internalisation occurs when cell 1 grows its tension above a threshold value.

413 This threshold is set at $\delta > 1 + 2\alpha$, and therefore depends the level of compaction [16].

414 Interestingly, differences in size have no effect on internalisation in theory. For the mouse

415 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.



- 417 418
- 419 Toolbox 2: De novo apicobasal polarisation and TE-ICM specification
- 420 During preimplantation development, blastomeres form de novo a domain of apical material
- 421 (red) at the surface of the embryo, away from cell-cell contacts (basolateral interfaces in blue).
- 422 The apical domain forms in the center of the contact free surface and signals to the actomyosin
- 423 cortex (green) to deplete it locally. The apical domain progressively expands until it reaches
- 424 the apical edge of the cell junctions. The apical domain contains molecules such as CDC42,
- 425 PARD6B or PRKC that prevent the LATS kinase from phosphorylating the co-transcriptional
- 426 activator YAP. YAP can shuttle to the nucleus to interact with the transcription factor TEAD4
- 427 and activate the expression of TE specific genes such as GATA3. Without an apical domain,
- 428 inner cells show less YAP in their nucleus as the LATS kinase, with the help of NF2 and AMOT 429
- localised at cell-cell contacts, can phosphorylate YAP and target it to the proteasome. The
- 430 presence of some of the signalling molecules involved in the subcellular localisation of YAP
- 431 has been confirmed in human embryos (labelled in bold font), while other have only been
- 432 studied in mouse embryos.

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