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Review

Morphokinetics of In Vitro-Derived Embryos—A Lesson from Human and Bovine Studies

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Abstract: Embryo transfer has become a major method to improve fertility in both humans and cattle. The current review focuses on predicting an embryo with a high developmental competence and high potential to establish pregnancy. One way to evaluate the embryo quality is to assess its morphology. However, this approach provides only limited and inadequate information. Using a time-lapse system allows a continuous monitoring of embryonic development. Here we introduce the main morphokinetic parameters and discuss their potential to predict embryo viability, implantation, pregnancy, and live birth. Special attention is given to the association between the transcript's profile and the embryo developmental potential; normally cleaved embryos differ in their profile from their abnormally cleaved counterpart; synchronously and asynchronously cleaved embryos, which are both defined as suitable for transfer, differ in their transcript profile. Recently, the advancements and wide use of time-lapse systems led to the development of algorithms for embryo selection. This technology is already implanted in human in vitro fertilization units, but it is not yet used for domestic animals. The authors believe that combining information from both the human and bovine morphokinetics might reveal the benefits of using a time-lapse system to select embryos with good potential for transfer.

Keywords: morphokinetic; bovine embryos; embryonic development; time-lapse system; abnormal cleavage

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1. Introduction

The decline in fertility in cattle is well documented for dairy cows [1], mostly due to high milk production and environmental changes. The conception rate from the first insemination has declined from 55.6 to 39.7%, at a derived average rate approaching 1% per year over 20 years [2]. To improve fertility, mostly in cows that are subjected to heat stress, embryo transfer procedures have been used as a routine breeding strategy [3]. For instance, each year, over 2.5 million bovine embryos are transferred in the United States alone [4]. Similar trends have been reported for humans. According to Osterman et al. [5], the fertility rate in the USA, expressed as births per 1000 women, declined from 62% in 2010 to 56% in 2020. A decline in the fertility rates was also reported in most European countries in 2019 [6]. To overcome this problem, assisted reproductive technologies (ART) have been developed. According to the Centers for Disease Control and Prevention (CDC), the main procedures are in vitro fertilization (IVF) and embryo transfer. The Society for Assisted Reproductive Technology (SART) reported that there were 368,502 cycles of oocyte retrieval, freezing, and thawing oocytes for IVF and for transferring frozen embryos in 2021. In the same year, about 91,906 live births were achieved from ART in the United States alone [7]. Taken together, embryo transfer has become a major procedure to improve fertility in both humans and cattle.

However, the efficiency of the embryo transfer procedure has not yet arrived at the optimum. In both human and bovine models, the success rate is relatively low, with ~30%

of blastocyst development and ~30% pregnancy rate per transfer [8]. Some limitations are suggested to underscore the low efficiency of embryo transfer; these limitations are associated with the oocyte source, embryo quality, and the maternal ability to receive the embryo [9]. The current review focuses on predicting those embryos with a high developmental competence and a high potential to establish pregnancy. A successful prediction might increase the proportion of pregnancies and live offspring in ART programs, for both humans and domestic animals. One way to evaluate the embryo quality is to assess its morphology. However, this approach provides only limited and inadequate information, since it cannot identify an embryo with the greatest potential for successful implantation [10,11]. Since a time-lapse system allows continuous monitoring of embryonic development [12–18], additional parameters, classified as morphokinetic parameters, have been introduced [17].

It is worth mentioning that human and bovine embryos differ in many aspects that are associated with embryogenesis. Both human and bovine embryos undergo extensive epigenetic reprogramming but the timing, the extent and the specific regulatory mechanisms express species-specific differences. For instance, in bovine embryos, the embryonic genome activation timing occurs between the 8 to 16 cell stages while that of human embryos between the 4 to 8 cell stages. Nonetheless, the current review introduces the main morphokinetic parameters of in vitro-derived embryos from both human and live-stock models and discusses their potential to predict embryo viability, implantation, pregnancy, and live birth.

2. Embryo Selection Criteria

The developmental stages of the embryo are characterized by specific morphological characteristics that are associated with embryo development and the ability to implant and establish pregnancy [19]. Routinely, embryos are classified as having good, fair, or poor morphologies [19–22]. Therefore, the most used parameter for embryo selection for transfer is the morphological score of the embryo. In humans, the morphological criteria include the pronuclear score post-fertilization [23,24], the number, shape, and evenness of the blastomeres, the degree of the blastomere fragmentation, the presence of vacuoles and multinucleation on day 2 or 3 post-fertilization [25,26], and the morphology of the blastocyst on day 5 or 6 post-fertilization [27]. In bovine, the 7- to 8-day blastocysts are usually frozen and then selected shortly before their transfer, based on the morphological criteria of the thawed blastocysts. The parameters commonly used to evaluate bovine embryo morphology are based on the International Embryo Technology Society guidelines [28]. These parameters include the shape, color, number, and compactness of cells, the size of the perivitelline space, the number of extruded and degenerated cells, and the number and size of the vesicles (Figure 1) [16]. During the early stages of development, from the zygote to the blastocyst stage, the embryo undergoes dynamic and intensive morphological changes [29]. Thus, utilizing only static morphological criteria provides limited and inadequate information to predict the developmental potential of the embryo [10]. Moreover, the morphological appearance of an embryo does not always reflect its physiology. For instance, blastocysts that share a similar morphology score might underlie a different morphokinetic developmental pattern [16] and/or different metabolic activity [29]. Interestingly, embryos with shorter duration timing through the first cleavages, also expressed good morphology [16]. Considering the above, assessing the embryo only by its morphology is limited and subjective [30,31]. To overcome these limitations, a time-lapse system has been suggested as a routine procedure in human IVF laboratories. However, it has not been widely implemented in bovine embryo transfer programs.

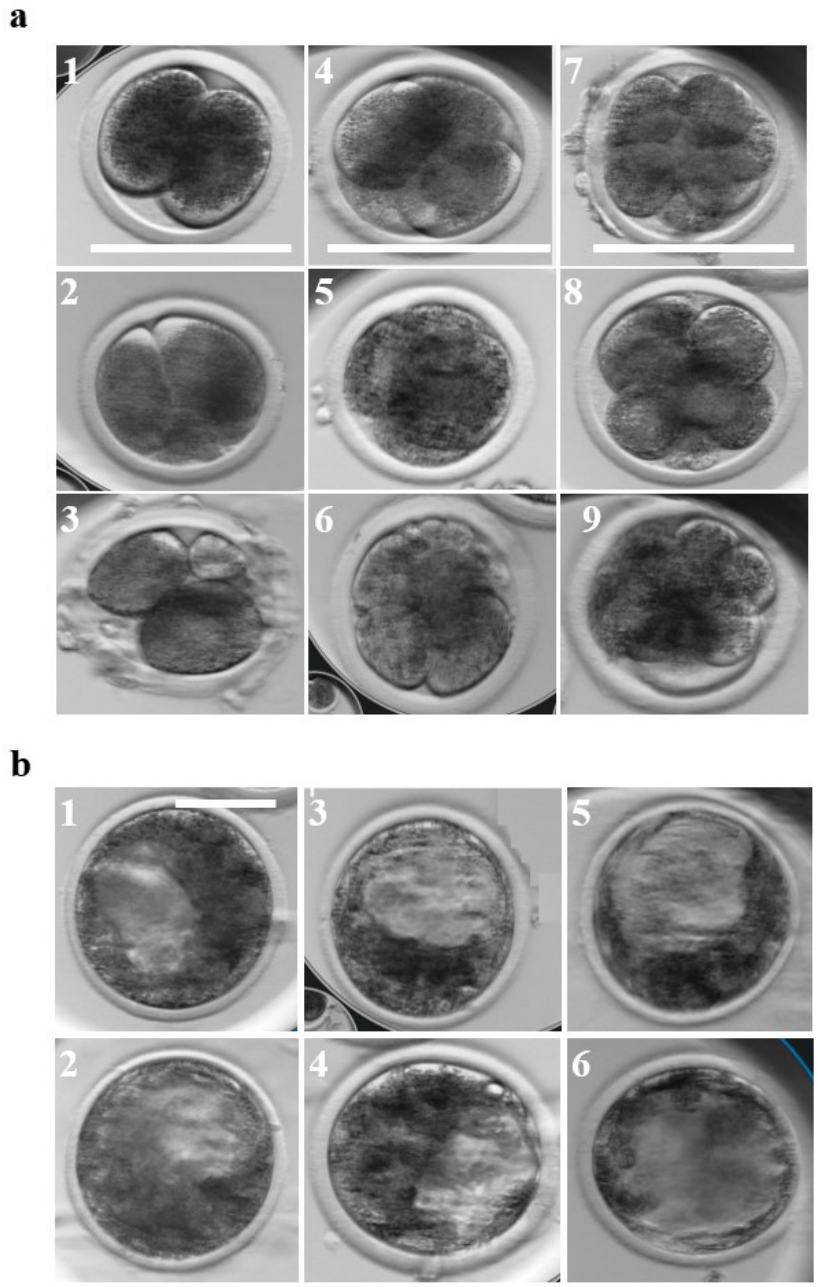

Figure 1. Morphological categories of bovine embryos and blastocysts. (**a**) Representative images of morphological categories of cleavage-stage embryos, a 2-cell-stage embryo with equal-sized blastomeres (1, good morphology); a 2-cell-stage embryo with equally sized blastomeres and up to 20% fragmentation (2, fair morphology); a 2-cell-stage embryo with unequal-sized blastomeres and >50% fragmentation (3, poor morphology); a 4-cell-stage embryo with four equal-sized blastomeres (4, good morphology); a 4-cell-stage embryo with fragmentation, equal-sized blastomeres (5, fair morphology); a 4-cell stage embryo with unequal-sized blastomeres and >50% fragmentation (6, poor morphology); an 8-cell-stage embryo with equal-sized blastomeres (7, good morphology); an 8-cell-stage embryo with unequal-sized blastomeres and minor (<20%) fragmentation (8, fair morphology); and an 8-cell-stage embryo with unequal-sized blastomeres and severe (>50%) fragmentation (9, poor morphology). (**b**) Representative images of blastocysts, a blastocyst with evident inner cell mass (ICM) with many tightly packed cells and many trophoblast (TE) cells forming a cohesive epithelium (1 and 2, good morphology); a blastocyst with a few TE cells forming a loose epithelium (3 and 4, fair morphology); a blastocyst with ICM presenting loosely grouped cells and TE composed of very few cells (5, fair morphology); a blastocyst with ICM presenting loosely grouped cells and TE composed of a few large cells (6, fair morphology), bar = 100 μm. Adopted with permission from Yaacobi-Artzi et al. [16].

3. Embryo Morphokinetics

Embryo kinetics involves several timing intervals. These intervals include the duration (i) between fertilization and the appearance of the pronucleus (i.e., the timing of the first cleavage), (ii) the duration of the disappearance of the pronuclei (PN) after fertilization [32], (iii) the duration of the first cytokinesis [33], (iv) the duration between the first and second mitosis [34], and (v) the duration of the second and third mitosis [15,33]. Additional time intervals include the times of blastulation [35] and blastocyst expansion [36], as well as the hatching time of the blastocyst [37]. Some prominent kinetic features that were found to be associated with embryo developmental competence will be discussed next.

3.1. Cleavage Timing

The timing of the first cleavage is the most accepted morphokinematic parameter, and it is suggested to be a good predictor of the developmental competence of embryos [11,13–15,38–44]. Gendelman et al. [45] found that the first embryonic division in bovine embryos can classified into two waves, i.e., early cleavage that occurred in the timeframe of 18–25 h post-fertilization (hpf) and late cleavage that occurred between 26 and 42 hpf. In agreement, it was reported that zygotes cleaved by 30 hpf have the highest potential to develop into blastocysts, whereas very late-cleaved embryos (>42 hpf) rarely reach the blastocyst stage, suggesting that developmental potential diminishes with longer durations from insemination until the initial cleavage [40,46].

Data from human studies suggest that the earlier cleaved embryos are of better quality and have greater chances of developing into blastocysts. An embryo is defined as either an early- or late-cleaved embryo if the embryo is cleaved within 25.9 hpf or later, respectively [34]. A 3 h shortened interval from fertilization to the first cleavage resulted in a higher proportion of embryos that developed to the blastocyst stage [47]. Following embryo transfer, a higher implantation and pregnancy rate was recorded with early-cleaved rather than late-cleaved human embryos; the early-cleaved embryos were associated with a reduced abortion rate [43,48–51].

However, the association between early cleavage and the implantation potential of bovine embryos is not yet clear. Previous studies reported that the pregnancy rate following bovine embryo transfer did not differ between early- vs. late-cleaved embryos [13,46]. On the other hand, early-cleaved embryos have significantly higher cell numbers and better viability and embryo morphology [39,40,42,43,52–54]. Moreover, Sugimura et al. [14] reported that late-cleaved embryos had a higher incidence of abnormal chromosomes and were identified as mixoploid more often than early-cleaved embryos.

Some attempts to explain the differences between the early- vs. late-cleaved embryos arose. The differences between the lipid concentration in early- vs. late-cleaved embryos have been pointed out. A higher lipid concentration was reported in the early-cleaved compared with the late-cleaved bovine embryos, which was associated with embryonic metabolism [55]. Similarly, it was found that bovine early-cleaved embryos express a different metabolomic pattern and transcript abundance compared with late-cleaved embryos [56]. Moreover, another explanation was introduced based on the differences in the expression of some fundamental genes. The early-cleaved bovine embryos are characterized by a differential expression of the *GDF9*, *POU5F1*, and *GAPDH* genes relative to the late-cleaved ones [45]. The timing of the first cleavage correlates with the expression of the *IFN-tau* and *IGF2R* genes [14], which are known to be involved in pregnancy reorganization and placentation [57]. A differential gene expression has been reported for blastocysts that developed from early- vs. late-cleaved bovine embryos [55,56,58,59]. An earlier study by Brevini et al. [60] found a variation in the polyadenylation patterns' pre-cleavage, with distinct changes in the delayed cleaved embryos. These changes included genes that are related to early differentiation, compaction, cavitation, energy metabolism, RNA processing, and stress response. With the evoking of cutting-edge technologies, an omics analysis, mainly a microarray, indicates more profound changes in the transcriptomic profile of early- vs. late-cleaved embryos. Orozco-Lucero et al. [61] identified 774 and

594 differentially expressed genes in early- and late-cleaved 2-cell embryos, respectively. The most profound biological functions associated with these alterations were cell cycle, DNA damage response, gene expression, RNA processing, and protein degradation. Song et al. [62] reported the differential expression of cell-cycle-related genes (*TFDP1*, *CDKN2*, and *CCCND3*) between early- and late-cleaved bovine embryos. A porcine study revealed 3077 differentially expressed genes between early- vs. late-cleaved porcine embryos [59]. In support, Song et al. [62] performed RNA sequencing and identified 71 differentially expressed genes from porcine blastocysts derived from early- and late-cleaved embryos, involved in pathways related to the proteasome, DNA repair, cell cycle arrest, autophagy, and apoptosis. Moreover, alteration in the methylation status has also been suggested. This included identifying 11,584 differently methylated regions between early- vs. late-cleaved bovine embryos, clustered by the means of biological processes that relate to cell survival/differentiation and energy/lipid metabolism [63]. Taken together, it is becoming clear that the lengthy time from the zygote to the first cleavage (i.e., early vs. late cleavage) is associated with the transcriptome profile of the formed blastocysts and with its developmental competence.

Not only has the timing of the first cleavage been found to be associated with embryonic development, but also the time of the second and third cleavages. For instance, the later stages of human embryo development can predict embryo implantation, in particular, the duration of the second cleavage and the time that the embryo developed to five cells [15,35,64,65]. The "lag phase", a temporary developmental arrest between the fourth and the fifth division, has been suggested as a good parameter to predict the development potential of an embryo [66]. It was reported that the "lag-phase" in the later cleavages is associated with the ability of the embryo to reach the blastocyst stage [66]. Similarly, the synchrony of the second and third cell divisions, combined with morphologic exclusion criteria, are correlated with embryo implantation [15]. Recently we reported that embryos that exhibited rapid developmental progress through the two first cleavages, expressed by a shorter interval from the first to the second cleavage, and from the second to the third cleavage, were further developed to the blastocyst stage (Figure 2a) [16]. Furthermore, the cleaved embryos that developed to the blastocyst stage differed in their morphology, as recorded in the first, second, and third cleavages, compared with those that did not develop into blastocysts (Figure 2b) [16]. Although not significant in our study, the duration through the third cleavage in embryos that further developed to the blastocyst stage was shorter than those that were arrested and did not develop further. In agreement, other studies reported that first, second, and third cleavages of embryos that developed to the compact morula or blastocyst stage were earlier than in those embryos that did not develop further [44,56,67]. In addition, Holm et al. [67] demonstrated that bovine embryos that develop to morula or blastocyst stages underwent their first three cleavages approximately 1–2 h earlier than did embryos that arrested at earlier developmental stages.

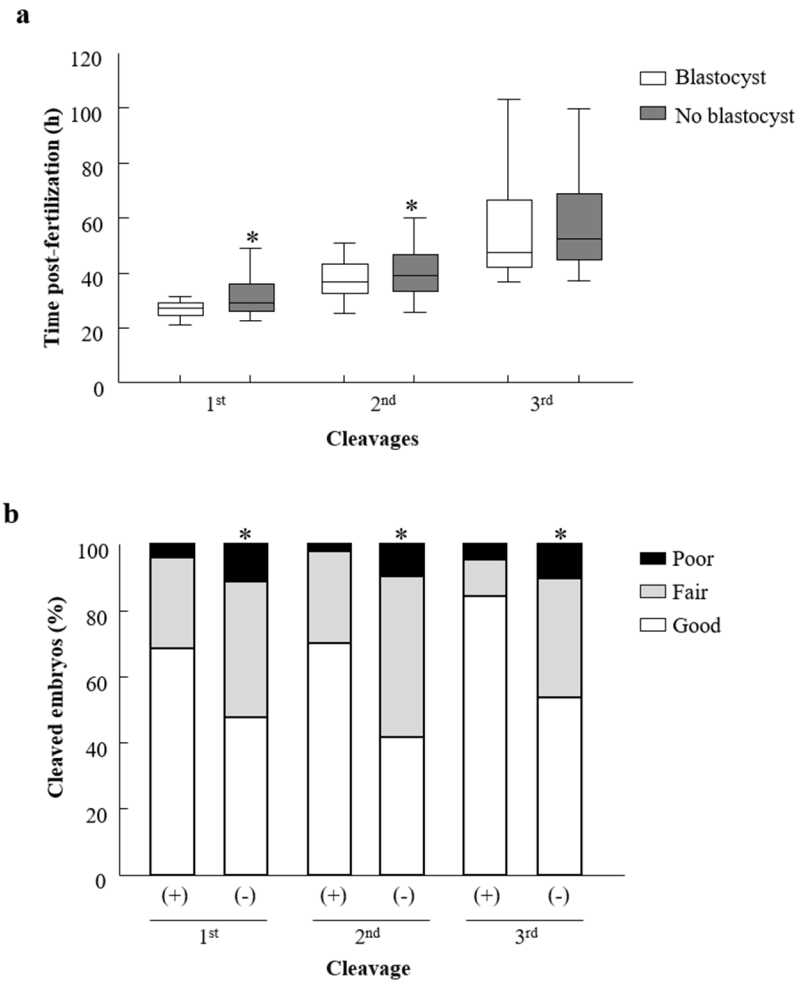

Figure 2. Association between first cleavage timing and blastocyst formation. Cumulus-oocyte complexes (COCs) were matured in vitro and fertilized. The resulting embryos were individually cultured for 190 h in an incubator equipped with a time-lapse monitoring system, and images were captured every 5 min to track the embryonic development. The timing of the 1st, 2nd, and 3rd divisions post-fertilization were recorded for each embryo. (**a**) The data presented includes the cleavage timings post-fertilization of synchronously cleaved embryos that successfully developed to the blastocyst stage and those that did not. The box plots contain the middle 50% of the values and the median (horizontal line) and the whiskers depict the maximum and minimum values for each group. The number represents the median value for each developmental stage, * indicates statistically significant differences between experimental groups ($p < 0.05$), $n = 383$ embryos. (**b**) Distribution of normal synchronously cleaved embryos that developed (+) or did not (-) to the blastocyst stage into the different morphological categories (good, fair, and poor). A chi-squared test, followed by Fisher's exact test, was used for pair comparison within each cleavage. * $p < 0.05$; $n = 431$ embryos. Adopted with permission from Yaacobi-Artzi et al. [16].

3.2. Division Patterns

After fertilization, the zygote undergoes a series of mitotic divisions, resulting in the formation of smaller nucleated cells, the blastomeres. Culturing embryos in a time-lapse system enables their classification into two distinct division patterns: those embryos that exhibit either normal or abnormal division at any time point from fertilization to the time of embryo compaction. A normal cleavage pattern is mainly characterized by a division of zygote and/or blastomeres into equally sized blastomeres. Embryos of normal cleavage can be categorized into two subgroups: those embryos that divide synchronously and those that exhibit at least one asynchronous event [16]. Synchronously cleaved embryos are characterized by synchronous divisions from 2 blastomeres into 4, 8, and 16 blastomeres.

Asynchronously cleaved embryos are characterized by at least one asynchronous cleavage resulting in embryos with 3, 5, 6, 7, 10, or 12 blastomeres. Studies in both humans and bovines identified several abnormal division patterns through early embryonic development (Figure 3a) [16]. Abnormally cleaved embryos include the following: (1) direct cleavage, in which a single blastomere cleaves into more than two daughter blastomeres [68,69], (2) unequal cleavage, i.e., producing asymmetrically sized blastomeres [14], and (3) reverse cleavage, manifested by a reduced number of blastomeres, most likely due to blastomere fusion or cytokinesis failure [70].

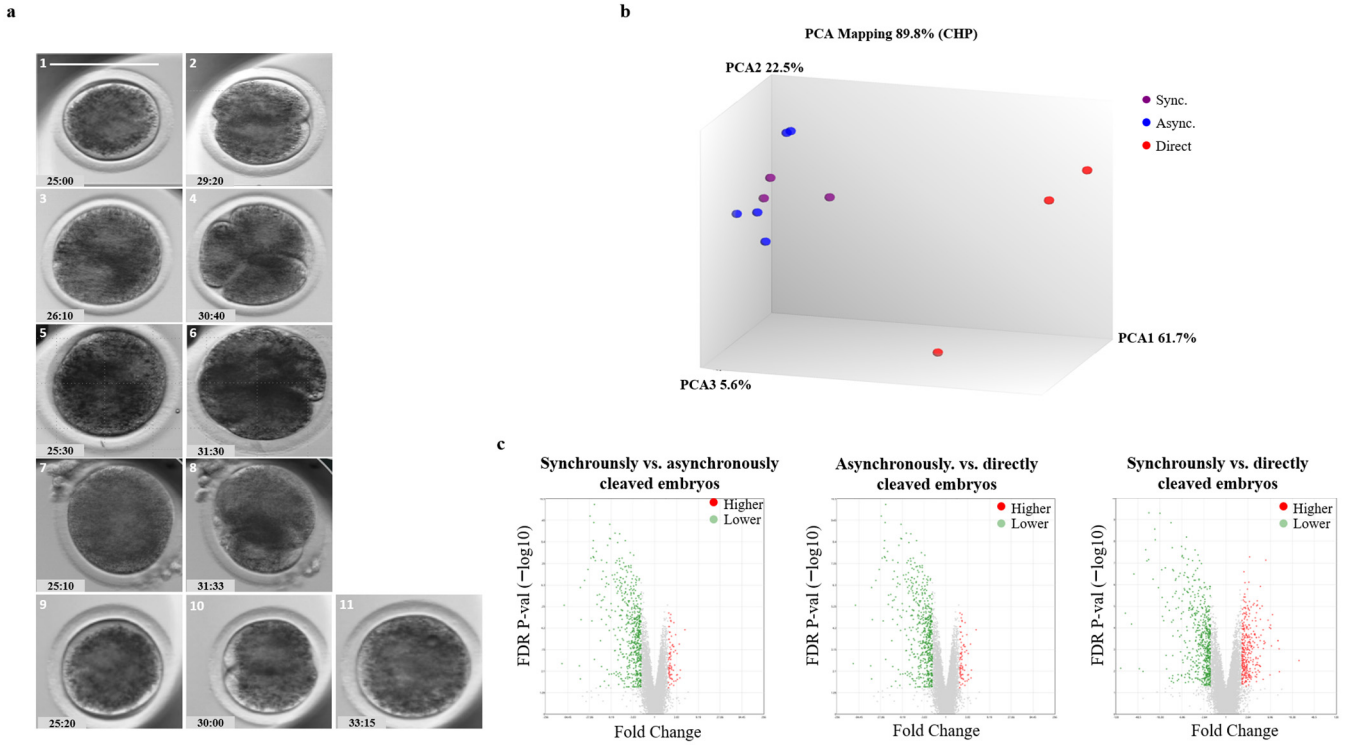

Figure 3. Normal and abnormal cleavage pattern. Cumulus–oocyte complexes (COCs) were matured in vitro, and fertilized, and the resulting embryos were individually cultured for 190 h in an incubator equipped with a time-lapse monitoring system, capturing images every 5 min to track the embryonic development. The developed blastocysts were collected (n = 4 blastocysts per sample) and subjected to RNA extraction, followed by microarray analysis. (**a**) Presented are representative images of the morphological categories of embryos at the first cleavage: normally cleaved embryos are characterized by two equal-sized blastomeres (putative zygote after fertilization) (1), after normal cleavage (2); directly cleaved embryos are characterized by the transition from a 1 cell (3; putative zygote post-fertilization) directly into a 3-cell-stage with 3 blastomeres (4); or direct cleavage from 1 cell to 4 blastomeres (5 and 6); an unequally cleaved embryo is characterized by the transition from 1 cell into two blastomeres of unequal size (7 and 8); a reverse-cleaved embryo is characterized by the transition from 1 cell into two blastomeres (9 and 10) and then reduced number of blastomeres after merging to 1 cell (11). The precise time (h:min) post-fertilization is presented in the lower-left corner of the images. Scale bar = 100 µm. (**b**) Principal component analysis (PCA) plots of blastocysts taken from asynchronously cleaved embryos (5 samples; blue), synchronously cleaved embryos (3 samples; purple), and from directly cleaved embryos (3 samples; red). Each symbol represents one replicate. (**c**) Volcano plots are presented to visualize the differential gene expression patterns between groups. The values plotted on the x- and y-axes represent the average normalized signal values of each group (log2-scaled). For each gene, the p-value was plotted against the fold change. Vertical bars represent statistical significance and dots represent the genes that were higher expressed (red) or lower expressed (green). Adopted with permission from Yaacobi-Artzi et al. [16].

In considering the proportion of cleaved embryos into normal or abnormal cleavage patterns, usually, the embryos represent a normal cleavage pattern. A recent study in bovine reported that 68.5% of in vitro-derived embryos were normally cleaved and 31.6% were abnormally cleaved, with a relatively high proportion of direct (18.9%), unequal (9.3%), and reverse first-cleavage (2.6%) embryos [16]. However, Lechniak et al. [71] reported that only 37.7% of the bovine embryos underwent normal first cleavage and about 62.3% were abnormal; of the latter, 28.7% of the bovine embryos were directly cleaved and 7.6% were reversed cleaved. The resulting outcome of the normally cleaved embryos is more likely to present a better developmental competence, with a higher chance of developing into the blastocyst stage. Meseguer et al. [15] found that out of 247 human-transferred embryos, 19.4% embryos exhibited one of the following cleavage anomalies: direct first cleavage, uneven blastomere size at the 2-cell stage, or multinucleation at the 4-cell stage [15]. Studies in humans reported that 9.8% of in vitro-derived embryos were direct-first cleaved embryos [68] and 17.7% of human embryos showed a reverse cleavage [72]. Another study in humans, which focused on the first three cleavages reported that only 27.8% of the embryos had normal cleavage and the others cleaved abnormally [73]. In that respect, both human and bovine studies reported that abnormal cleavage is associated with a low blastocyst formation rate [16,18,68,74] and with reduced implantation competence [13,15,68,73,75]. In addition, normally derived blastocysts were also found to differ in their transcriptomic profile relative to their abnormal counterpart (Figure 3b) [16,33]. This point is further discussed next.

3.2.1. Synchronous vs. Asynchronous Cleavage

Cell division may not always occur in perfect synchronization, resulting in asynchronously cleaved embryos with three or five cells [76]. A recent study in bovine reported that about 60.2% of the embryos were synchronously cleaved and about 39.7% were asynchronously cleaved. The proportion of cleaved embryos that developed further to the blastocyst stage was higher for the asynchronously cleaved relative to the synchronously cleaved embryos [16]. Furthermore, a high correlation between the number of asynchronous events during embryo development and the probability of developing to the blastocyst stage was recorded [16]. Similarly, studies in humans have categorized the cleaved embryos into either synchronous or asynchronous patterns [25,77,78]. To date, the blastocysts that developed from synchronously or asynchronously cleaved embryos are both considered normal and suitable for transfer [79]. Wiener-Megnazi et al. [78] showed that synchronously cleaved embryos have a higher survival rate relative to the asynchronously cleaved ones, expressed by a high proportion of embryos with a good morphological grade following cryopreservation. However, no difference was found in their capacity to implant, establish pregnancy, or forming a live birth. In contrast, Yakovenko et al. [77] reported that asynchronously cleaved embryos express a higher proportion of aneuploid embryos relative to the synchronously cleaved embryos and concluded that the latter is more suitable for implantation. Another study in humans suggested that the asynchronous 5-cell stage can be used as a predictive parameter correlated with implantation [15]. A recent study in bovine revealed that the synchronously cleaved embryos differ in their transcriptome profile from that of the asynchronously cleaved embryos, especially regarding metabolism and apoptosis. It was shown that the *GSTM1* and *GSTM3* genes, which are associated with the metabolic pathways, were lower in blastocysts that developed asynchronously compared with synchronously cleaved embryos [16]. In addition, a differential expression of apoptosis genes (*HSPA1A*, *EDA*, and *CTH*) was noted in blastocysts that developed from synchronously vs. asynchronously cleaved embryos. Nevertheless, the implications of the differences in the expressed genes are not clearly understood.

Taken together, blastocysts that developed from synchronously and asynchronously cleaved embryos are both considered normal and suitable for transfer, in both humans and bovine. Currently, the competence of asynchronously vs. synchronously cleaved

embryos to implant and establish pregnancy remains an open question and warrants further investigation.

3.2.2. Direct Cleavage

Special attention should be given to the directly cleaved embryos, in which a single blastomere cleaves into more than two daughter blastomeres [68,69]. This subgroup of embryos can potentially develop to the blastocyst stage and might be chosen for transfer. Previous studies in bovine reported that the proportion of directly cleaved embryos that progressed to the blastocyst stage was lower than the normally cleaved embryos [71,80]. Another bovine study reported that about 12.4% of the directly cleaved embryos progressed further to the blastocyst stage, with a similar morphological score relative to those that developed from normally cleaved embryos [16]. In light of these findings, embryo transfer programs based solely on morphological evaluation rather than utilizing a time-lapse system can potentially select blastocysts that were derived from directly cleaved embryos, and unintentionally lower the chance of implantation. A pioneering human study, which involved more than 41,000 embryos, reported that within the directly cleaved embryos, the proportion of live embryos decreased as the development progressed [81]. Similarly, Lagalla et al. [82] reported that about 21.6% of the directly or reverse-cleaved human embryos developed further into blastocysts, whereas most of these embryos were arrested at earlier stages, suggesting that directly cleaved embryos are of low developmental competence. In addition, directly cleaved embryos exhibited a lower hatching rate than did embryos with normal cleavage, as was found in bovine [80].

Studies in both humans and bovine suggest that direct cleavage is not only associated with low potential to develop into blastocysts—it is also associated with low potential to establish pregnancy. Previous studies in humans reported that blastocysts that developed from directly cleaved embryos exhibited lower implantation rates, by about 10-30% than those embryos that developed from normally cleaved embryos [68,70,74,75]. This was also true for bovine embryos; directly cleaved bovine embryos are associated with reduced competence to develop and establish pregnancy [13,14,18]. For instance, the transfer of bovine blastocysts with normal cleavage resulted in a higher pregnancy rate relative to the transfer of directly cleaved blastocysts (66.7% vs. 28.6%, respectively) [13]. In contrast, Somfai et al. [44] reported that both directly and normally cleaved bovine embryos have a similar potential to develop into blastocysts. Recently, a human study reported that not only the implantation rate of directly cleaved embryos was lower than that of the normally cleaved embryos, but also the clinical pregnancy and live birth rates [83]. A former study even reported that the transfer of directly first-cleaved embryos did not result in live births [68].

Although the mechanism underlying direct cleavage is unclear, it can be suggested that transcript alterations are involved in the mechanism that underlies this pattern of cleavage. An association between directly cleaved embryos and chromosome abnormalities has been reported in both human and bovine studies [14,44,68,80]. A microarray study in bovine revealed differentially expressed genes in blastocysts that developed from normally, synchronously, or asynchronously cleaved embryos (895 and 643, respectively), relative to those that developed from directly cleaved embryos (Figure 3c) [16]. Functional annotation analysis indicated that some of the differential genes were associated with the cell cycle, which is a suggested mechanism that underlies the phenomenon of direct cleavage, a specific case of abnormal cleavage [16]. A metabolome analysis in bovine revealed differences between normally and directly cleaved embryos in several metabolic pathways [71]. In particular, an elevated level of pyruvate acid was recorded in directly cleaved embryos, possibly due to a disturbance in the switch from a lipid to a glucose metabolism. Another possible mechanism is alterations in the cell-cycle-related genes; such an alteration can lead to chromosome abnormality and aneuploidy. In support of this assumption, studies in bovine reported that directly cleaved embryos exhibited a higher proportion of aneuploid embryos or embryos with abnormal chromosome numbers [44,80]. Nevertheless, previous

studies in humans reported that directly cleaved embryos could develop into chromosomally normal blastocysts [82,84], presumably by excluding the abnormal cells during the compaction process as a "self-correction" mechanism that eliminates chromosomally abnormal cells within the embryo [68]. The phenomenon of "self-correction" was also reported for directly cleaved bovine embryos [69]. Nevertheless, associating abnormal morphokinetics with "self-correction" mechanism needs further examination.

3.2.3. Unequal Cleavage

A division is defined as unequal cleavage when a blastomere cleaves into asymmetrical sister blastomeres [14]. Among the abnormal cleavage patterns, the unequal one has been less studied, most likely because these embryos are of low developmental competence. A previous study in bovine reported that the proportion of unequal first cleavage embryos was 9.3% of the total embryos cleaved and 21.3% of the abnormally cleaved embryos [16]. Hardarson et al. [25] reported that unequally cleaved human embryos present a significantly low implantation rate (23.9 and 36.4%, respectively), as well as a low pregnancy rate (37.6 and 52.9%, respectively) compared with normally cleaved embryos. A study in humans revealed that of the 132 clinical pregnancies, only 12 (9%) resulted from 2-cell unequal embryos, but no delivery outcome was recorded [85]. A study in mice found that among the early-cleaved embryos, the prevalence of unequal division was 13.0% and that these embryos yielded a higher proportion of embryos with multinucleated blastomeres than did the normally cleaved embryos [86]. However, the mechanism that leads to unequal cleavage is not yet clear. A recent study in porcine examined the proteomic quantification of blastomeres isolated from normally and unequally cleaved 2-cell-stage embryos [87]. It was found that the blastomere's developmental ability is associated with its size; a higher cleavage rate was recorded in the large relative to the small blastomeres. The proteomic analysis revealed differentially expressed proteins between the large- vs. the medium- and the small-sized blastomeres, respectively. In particular, the differentially expressed proteins between the large- vs. the small-size blastomeres are involved in RNA binding; and are found in actin cytoskeletal tissue, such as DDX1 (ATP-dependent RNA helicase DDX1) and ACTB (actin beta), respectively [87]. A study in humans reported that unequally cleaved embryos expressed a high proportion of blastomeres with numerous chromosomal aberrations, compared with those isolated from normally cleaved embryos (29.4 vs. 8.5%, respectively) [25]. A study in mice reported that unequally sized blastomeres were correlated with multinucleation [86], presumably due to an impaired migration of chromosomes through the mitotic anaphase [88,89]. A study in porcine in which embryos were produced by cloning and parthenogenetic activation resulted in a higher proportion of unequally cleaved embryos [90]. In the latter study, an uneven distribution of organelles (mitochondria and lipid droplets), along with a lower proportion of smaller blastomeres, was observed. Interestingly, the subsequent division of the two sister blastomeres was asynchronized [90]. A recent study in porcine revealed 216 differentially expressed genes among the unequally and normally 2-cell-stage porcine embryos; the genes were mostly related to the regulation of metabolic processes [91]. Although not clear enough, the mechanisms that underlie the unequal division seem to involve alterations in metabolism and energy regulation as well as chromosomal aberrations.

3.2.4. Reverse Cleavage

Another form of abnormal cleavage is reverse cleavage, which is manifested by a reduced number of blastomeres through the division process, most likely due to blastomere fusion or cytokinesis failure [70]. A few studies reported that reverse-cleaved embryos have a low potential to develop into blastocysts [72,82], whereas others reported no differences [80,92]. On the other hand, Yaacobi-Artzi et al. [16] reported that none of the embryos that exhibited reverse cleavage developed further into blastocysts, suggesting that this pattern is a determinate pattern for embryonic development, at least in bovine. In accordance, Liu et al. [72] reported that about 9% of reverse-cleaved human embryos

developed to the blastocyst stage. Moreover, the proportion of reverse-cleaved embryos that reached the 6-cell stage or beyond, by day 3 post-fertilization, was low relative to the normally cleaved embryos (47.7 vs. 71.7%, respectively). On the other hand, Desai et al. [92] showed that approximately 40% of the reverse-cleaved human embryos continued to the blastocyst stage. Similarly, a study in bovine showed that the incidence of reverse cleavage at the first cell division was 17.2%, with no difference in the proportion of embryos that developed into blastocysts, compared with normally cleaved embryos [80]. However, the hatchability of blastocysts that developed from reverse-cleaved embryos was low and was associated with a low proportion of diploid blastocysts.

It should be pointed out that although some studies reported a similar developmental potential between reversed and normally cleaved embryos, the blastocysts that developed differ in quality. For example, studies in bovine and humans reported that a higher proportion of chromosomally abnormal blastocysts developed from reverse-cleaved embryos [44,82]. In addition, a study in humans reported that blastocysts that developed from reverse-cleaved embryos have a lower morphology score of the inner cell mass but with no significant difference in the trophectoderm cells' morphology score [93]. In support, a study in bovine showed that the proportion of blastocysts with good morphology was significantly lower in embryos presenting reverse cleavage compared with normally cleaved embryos (11.1 vs. 39.6%, respectively) [80]. Moreover, Yang et al. [74] reported that reversed cleaved human embryos that were transferred on day 3 post-fertilization did not establish any implantation or pregnancy. Similarly, Liu et al. [72] reported that none of the blastocysts that developed from reverse-cleaved embryos and that were transferred were successfully implanted. Interestingly, the mechanism of "self-correction" is also suggested in the case of reverse cleavage, as was found in bovine for both reverse and direct cleavages [69]. Overall, although the use of a time-lapse system opens a chance to detect reverse cleavage. Studying the mechanism underlies the reverse cleavage is highly challenging.

4. Algorithms, Machine Learning, and Artificial Intelligence (AI) Are Used to Predict Embryo Developmental Competence

The advancements and wide use of time-lapse systems in human IVF led to the development of embryo selection algorithms. Meseguer et al. [15] proposed an algorithm model for classifying embryos into 10 categories based on the morphological and morphokinetic characteristics that were associated with their implantation rates. Wong et al. [33] developed an algorithm, based on the duration of the three first divisions, to predict human blastocyst formation. Liu et al. [65] used the combination of the morphological score on day 3, morphokinetic parameters, and cleavage patterns to categorize embryos into seven grades of implantation potential. Petersen et al. [94] evaluated the morphokinetic data of 11,218 embryos cultured to day 5 as well as information extracted from a database of known implantation data resulting from the transfer of 3275 embryos on day 3. This algorithm, known as implantation data score (KIDScore), is based on six annotations: the time two pronuclei appeared, the time that pronuclei faded, the time of 2-cell formation, the time of 3-cell formation, the time of 5-cell formation, and the time of 8-cell formation. The algorithm was found to predict the potential of the cleaved embryo to form a blastocyst, as well as the blastocyst quality and its implantation potential [94]. However, none of the developed algorithms have achieved widespread adoption for universal application [15,33,64]. In bovine, using a logistic regression model, Sugimura et al. [14] examined the association between the developmental competence of bovine embryos and various morphokinetic parameters including the timing of the first cleavage and the number of blastomeres at the end of the first cleavage. Yang et al. [95] suggested that both morphological and morphokinetic parameters such as above 50% fragmentation, the pattern of the first cleavage (direct, reverse, or delayed cleavages), and the timing of the first and second cleavages can be used to predict blastocyst formation. Huayhua et al. [96] generated a model that enables the prediction of bovine embryo viability, based on the timing of blastulation (less than 155 h post-fertilization) and the blastocyst diameter (>180 μm).

Recently, numerous artificial intelligence (AI) models were integrated into human IVF clinics as a tool for embryo assessment before transferring them [97,98]. These models leverage time-lapse data and employ machine learning techniques. Several human studies reported that using AI models to choose an embryo is highly correlated with implantation, ongoing pregnancy, miscarriage, live birth, and even euploid rates [99–102]. It is therefore suggested that introducing AI into clinics can replace the subjective assessment of the embryologist. Moreover, it might enhance work efficiency and provide a standard procedure across laboratories [103]. In addition, the advancement of AI might be able to integrate the morphokinetic data with known genetic and/or metabolic information [104]. Nevertheless, utilizing AI models to predict the developmental competence of bovine embryos is limited. A previous study in bovine showed that using AI can be better for assessing variables regarding day 7 bovine blastocyst quality than utilizing experienced embryologists, with an accuracy of 76.4% [105]. An algorithm to classify bovine embryos in accordance with IETS grades has been recently designed [106]. Turki and Wei Z. [107] designed machine learning algorithms for successful pregnancies, with better prediction than using the conventional approaches. Taken together, although there are ongoing attempts to develop an AI model for embryo evaluation in bovine, the application of AI is still limited in the bovine model but holds promise for improving the success rates in the future.

5. Summary

Using a time-lapse system enables one to characterize the embryo morphokinetics and associate it with its developmental competence. Cumulative data from human and animal models suggest that abnormally cleaved embryos have a lower competence to develop into blastocysts than the normally cleaved embryos. Moreover, the pattern of the cleaved embryo has been found to be associated with the transcript profile, i.e., differentially expressed genes were recorded for blastocysts that developed from normally relative to directly cleaved embryo. For a better understanding the mechanisms that underlie the abnormal division, further studies should examine the transcriptome profile of the cleaved embryos at earlier stages of embryonic development. Interestingly, blastocysts that developed from synchronously or asynchronously cleaved embryos differ in their competence to further develop into blastocysts; these embryos also differ in their transcriptome profile. To date, both subgroups of embryos are defined as normal and are considered suitable for transfer in IVF programs. However, whether these embryos have the same potential to establish pregnancy and to develop into live and healthy offspring is not yet clear.

While human and bovine embryos differ in many embryogenesis aspects, the current review points out that in both species the morphokinetics at the early stages of development is associated with the embryo developmental potential and can serve as a valuable predictor for further development into term. The authors believe that combining information from both human and bovine morphokinetics might reveal the benefits of using a time-lapse system to select embryos with good potential for transfer.

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