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Review

Time-lapse monitoring technologies for the selection of bovine *in vitro* fertilized embryos with high implantation potential

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Abstract

Over the years, the utilization of *in vitro* fertilization (IVF) in bovine embryo production has increased globally to accelerate the selection of cows with high genetic values. The selection of embryos with high implantation potential is a critical factor in establishing pregnancy. Time-lapse monitoring (TLM) has emerged as a new technique that allows frequent and non-invasive imaging of developing embryos. TLM is considered to have several advantages over the conventional morphological evaluation of embryos, which has been widely used in bovine embryo production. Establishing a novel embryo selection algorithm specifically for bovine IVF embryos is a critical challenge, but information on the association between morphokinetic data obtained using TLM and the implantation potential of embryos is still limited. This review outlines the potential application of TLM technology to improve the fertility of bovine IVF embryos, focusing on the results of human and bovine TLM studies that can be applied to select bovine embryos with high implantation potential. First, the progress of the TLM technology in bovine embryo production is summarized. The association between kinetic and morphological parameters and the developmental and implantation potential of human and bovine embryos is outlined. Finally, the benefits of evaluating blastocyst collapse and re-expansion as indicators of bovine embryo viability and the possible application of TLM to detect chromosomal abnormalities and determine embryo sex will be discussed.

Introduction

Over the past century, significant innovations in reproductive technology have revolutionized dairy and beef cattle production, thereby increasing the profitability of farms [1]. Embryo transfer has become an essential technique in bovine breeding, not only to improve the reproductive efficiency but also to accelerate genetic evolution by increasing the number of cattle with high genetic values [2, 3]. Genomic selection of superior cows has been further accelerated by the combination of embryo transfer and *in vitro* fertilization (IVF) [2, 4-6]. Globally, IVF embryo production has increased significantly over the years, with similar numbers of embryos produced *in vivo* and *in vitro* since 2014 [4]. In contrast, the pregnancy rate after the transfer of IVF embryos has been shown to be lower than that of *in vivo*-produced embryos [7-9]. The selection of embryos with high implantation potential is a critical factor for the establishment of pregnancy [10, 11]. Traditionally, IVF embryos have been selected based on their morphology, developmental rate, and overall appearance at the end of culture [12]. This selection method is widely considered inadequate and subjective because of the possibility of overlooking critical events that are detrimental to embryo survival and the variability between and within observers [13-15]. The frequent evaluation of embryo development is believed to improve implantation rates, but is invasive owing to the frequent handling and exposure to changes in temperature and gas concentration [16, 17]; therefore, developing non-invasive and accurate methods for determining embryo quality is essential.

Time-lapse monitoring (TLM) has emerged as a new technique that allows frequent and non-invasive imaging of developing embryos. It is considered to have several advantages over conventional morphological evaluation of embryos, which has been widely used in bovine embryo production. Continuous observation of embryo development with TLM allows accurate quantification of cellular dynamics and cell cycle length. Several human and bovine studies have suggested that a detailed analysis of the timing and pattern of the first post-fertilization cleavage may allow the selection of embryos with a high implantation potential [11, 18-22]. Furthermore, it has been postulated that TLM can predict the ploidy status of embryos because aneuploid embryos can present morphokinetic differences compared

66 to normal embryos during their development due to aberrant chromosome complement [23, 24]. Since
67 aneuploidy is one of the critical causes of implantation failure and miscarriage [25-27], non-invasive
68 determination of ploidy status using TLM would be beneficial. Clinical studies using human IVF
69 embryos have shown that the evaluation of morphokinetics during the early cleavage stages improves
70 pregnancy outcomes [28-32]. Therefore, applying TLM to the selection of bovine IVF embryos may
71 also improve the pregnancy rate after embryo transfer.

72 Because of these advantages, human laboratories of assisted reproductive technology (ART) have
73 rapidly and globally introduced TLM technology. As a result, considerable morphokinetic data has been
74 collected, and embryo selection algorithms have been gradually established in human ART [33-36],
75 although their application to improve pregnancy outcomes remains uncertain [33, 37-39]. Despite
76 several TLM studies of bovine embryos, information on the association between morphokinetic data
77 obtained using TLM and implantation potential is still limited; therefore, TLM has not yet been applied
78 commercially in bovine embryo production. Few studies have reviewed the findings obtained from time-
79 lapse observations of bovine IVF embryos to improve fertility and production efficiency in cows.
80 Therefore, this review outlines the potential applications of TLM technology to improve the fertility of
81 bovine IVF embryos. This article focuses on the findings of human and bovine TLM studies that can be
82 applied to select bovine embryos with high implantation potential. First, the progress of the TLM
83 technology in bovine embryo production is summarized. Second, the association of kinetic parameters,
84 such as the timing of cleavage, and morphological parameters, mainly focusing on abnormal cleavage
85 obtained using time-lapse observation during the early cleavage stage, with the developmental and
86 implantation potential of human and bovine embryos are outlined. This review describes the benefits of
87 evaluating blastocyst collapse and re-expansion as indicators of viability of bovine embryos. Finally,
88 the possible application of TLM to the detection of chromosomal abnormalities and embryo sexing is
89 discussed.

90

91 **Progress of TLM technology in bovine embryo production**

92 The milestones for the progress of TLM in bovine embryo assessment are summarized in Table 1.
93 Time-lapse embryo observations began with studies using animal embryos produced *in vivo*. In 1929,
94 Lewis and Gregory first applied cinematographic monitoring to the development of rabbit embryos
95 collected from the oviduct, from the one-cell to the hatching stage [40]. In the early 1980s, bovine
96 morulae collected from uterine horns were cultured in flat capillary tubes for five days to continuously
97 monitor blastocyst formation and hatching [41, 42]. These studies revealed that bovine embryos actively
98 hatch by escaping through a slit of the zona pellucida. The TLM of IVF embryos was first performed in
99 1994 using bovine embryos that matured and fertilized *in vitro* [43]. Embryo culture was performed
100 using a 4-well dish covered by an *in vitro* culture chamber and placed on an inverted microscope stage.
101 The development of 130 embryos from the one-cell to the blastocyst stage was monitored for eight days
102 using time-lapse cinematography, during which the timing of cleavage, duration of each cell cycle, and
103 time to the morula/blastocyst stage were investigated. Grisart et al. (1994) revealed that the faster the
104 embryos are cleaved in the early stages, the higher the ability to develop to the morula-blastocyst stage.
105 In addition, the developmental arrest ('lag-phase') occurred at four to eight-cell stages, which is likely
106 to be related to the developmental competence of embryos. In 1997, the first human TLM study using
107 *in vitro*-produced embryos following intracytoplasmic sperm injection demonstrated the precise timing
108 of fertilization, indicated by polar body extrusion and pronuclear formation [44]. In the late 1990s and
109 the 2000s, several studies determined the developmental kinetics of bovine IVF embryos using a
110 cinematographic chamber placed on an inverted microscope [45-51]. These studies provided the basis
111 for time-lapse analysis of bovine IVF embryos by demonstrating that the timing of cleavage and cell
112 cycle length during the early cleavage stage indicates the developmental potential of embryos.
113 Commercial equipment for TLM, in which microscopes and cameras are built inside an incubator,
114 became available and was introduced to many human ART laboratories worldwide around 2008 [34,
115 36]. This has led to several studies on the relationship between morphokinetics and the developmental
116 or implantation potential of *in vitro*-produced embryos, mostly using human embryos. In 2010, Somfai
117 et al. [19] performed the first bovine TLM study using a commercial time-lapse imaging system and

demonstrated that the duration of the cell cycle and cleavage pattern during the early cleavage stage indicate the developmental ability to reach the blastocyst stage. In the same year, Sugimura et al. [52] developed a well-of-the-well system that allowed tracking and time-lapse observation of individual bovine embryos throughout the culture period. These studies provide an essential foundation for the progress of TLM in bovine IVF embryos. Subsequently, several studies have validated the association of morphokinetic and morphological parameters of bovine IVF embryos with developmental ability and implantation potential after transfer [11, 53-58]. However, this information is limited compared to that of humans, and commercial and practical applications have not yet been achieved.

Embryo selection by quantitative parameters

Recent information on human embryo selection algorithms has been summarized in several systematic reviews [33, 34, 36, 59]. Several human studies have indicated that blastocyst formation can be predicted by several quantitative parameters, such as the duration of the second cleavage [28, 32, 60], the duration of the second synchronization (time between 3-cells to the 4-cells stage) [31, 32, 61], the time to 2-cell [61, 62], and the time to 5-cells [60, 61]. Similarly, in bovine IVF embryos, it has been shown that the duration of the first, second, and third cell cycles of viable embryos that developed to the compact morula or blastocyst stage were shorter than those of non-viable embryos [19, 48, 63]. Furthermore, embryos that developed slowly during early cleavage showed increased expression of apoptosis- and cellular stress-related genes around the time of genome activation (112 h post-fertilization) compared to fast-developing embryos [63]. These human and bovine findings indicate that quantitative parameters obtained by kinetic analysis with TLM can predict embryo transfer outcomes. Mesenguer et al. (2011) proposed the first embryo selection algorithm as a predictor of implantation after transfer of embryos produced by intracytoplasmic sperm injection [30]. This study presented an optimal range of morphokinetic parameters and showed that embryos that develop too early or too late have a low implantation rate. Subsequently, several studies have reported that implantation potential can be predicted by the duration of the second cleavage [30, 60, 64-66] and times to 5-cells [30, 60, 65, 66],

144 blastulation [60], and expanded blastocysts [67]. In general, early cleaving embryos may have a higher
145 implantation potential [33, 59]. The association between morphokinetic parameters and implantation
146 ability has been demonstrated in bovine IVF embryos and is consistent with human studies [11, 68]. A
147 logistic regression analysis revealed that morphokinetic parameters, such as the timing of the first
148 cleavage (within 27 h post-fertilization), cell number at the end of the first cleavage (2-cell), and cell
149 number at the beginning of the lag phase (6 to16-cell), are indicative of successful pregnancy outcomes
150 after embryo transfer [68]. In these studies, the authors concluded that combining several quantitative
151 and qualitative parameters could successfully predict pregnancy (Table 1).
152
153 **Embryo selection by qualitative parameters**
154 Accumulating evidence suggests that abnormalities in qualitative parameters during the early
155 cleavage stage, such as abnormal cleavage, fragment formation, unequal cleavage, and multinucleation,
156 are useful for de-selecting human and bovine embryos because of their association with poor growth
157 and implantation potential [11, 19, 21, 22, 33, 34, 36, 53, 69, 70]. The most common abnormal cleavage
158 events are reverse cleavage (RC), blastomere fusion post-cleavage [65], and direct cleavage (DC),
159 cleavage of one blastomere into more than three daughter cells (Fig. 1) [21]. Although the mechanisms
160 underlying abnormal cleavage are not fully understood, it has been hypothesized that the causes include
161 sperm quality or DNA damage, chromosomal abnormalities, multipolar spindles, and aberrant
162 centrosomes [19, 22, 24, 71]. The prevalence of RC has been reported to be 0.4%–27.4% [22, 69, 72,
163 73] in humans and 7.6–17.2% in cattle [53, 57]. For DC, the reported prevalence in different studies
164 varies from 4.4–26.1% in humans [30, 69, 73-76] and 14.1–28.7% in cattle [19, 53, 57]. Human studies
165 have shown that abnormal cleavage is associated with reduced blastocyst development, implantation
166 potential, or live birth rate [21, 22, 69, 73-77]. Similarly, our previous study using bovine IVF embryos
167 demonstrated that at eight days post-insemination, embryos presenting RC or DC had a lower proportion
168 of blastocysts with good morphology than normally cleaved embryos, although the overall blastocyst
169 rate was only reduced in the RC groups [53]. On the other hand, some abnormally cleaved embryos

developed into blastocysts of the same quality as normally cleaved embryos (Fig. 2), indicating that conventional morphological embryo selection may result in the transfer of embryos experiencing abnormal cleavage. Morphokinetic evaluation revealed that bovine IVF embryos that presented RC and DC developed more slowly than normally cleaved embryos with prolonged time to first cleavage, onset of lag-phase, morula formation, blastulation, and hatching. The prolonged time to first cleavage in DC embryos was consistent with another bovine TLM study [19]. A recent bovine metabolome analysis revealed differences in several metabolic pathways between normal and DC embryos, mainly involving pyruvic acid, and an increased level of pyruvate acid in DC embryos, possibly indicating a disturbance in the switch from lipid to glucose metabolism [57].

Blastocyst collapse and hatching

Continuous morphological observation of embryos using TLM enabled dynamic investigation of blastocyst collapse, re-expansion, and hatching (Fig. 3). Hatching, a protrusion of blastocysts from the zona pellucida with continuous expansion of the blastocoele, is an essential process for successful implantation. Spontaneous and transient collapse and re-expansion are frequently repeated before hatching, although these processes are not a prerequisite for hatching [70]. Blastocyst collapse occurs due to the loosening of cellular connections in the trophectoderm, causing an efflux of blastocoel fluid and embryo contraction. Subsequently, the gradual accumulation of fluid in the blastocoel via the sodium pump causes the re-expansion of embryos [78, 79]. A previous study using mouse embryos suggested that weak contractions play an essential role in hatching, whereas strong collapse has an inhibitory effect [80]. Human studies have revealed that collapsed blastocysts have a lower implantation rate compared to non-collapsed blastocysts [78, 81]. The mechanisms underlying the detrimental effect of embryo collapse on implantation are primarily unknown but are presumably related to mechanical pressure, damage to the gap junctions in the trophectoderm, or excessive energy consumption for re-expansion, which may negatively affect subsequent embryonic development [82]. Our previous study using bovine IVF embryos demonstrated that RC embryos presented an increased number of blastocyst

196 collapses or re-expansions and lower hatchability than normally cleaved embryos [53] (Table 1). In
197 contrast, the hatching rate was reduced in DC embryos compared to embryos with normal cleavage,
198 without an increase in the number of collapses before hatching. Therefore, although reduced hatchability
199 in embryos presenting abnormal cleavage suggests impaired implantation ability, different mechanisms
200 may be involved in reducing hatchability in RC and DC embryos. Although the mechanism of impaired
201 hatchability in embryos with abnormal cleavage has not yet been fully elucidated, it is hypothesized that
202 the reduced embryo viability caused by poor oocyte or sperm quality or unsuitable culture conditions
203 may be involved. Therefore, it is recommended to prioritize the transfer of embryos that do not show
204 strong blastocyst collapse to increase the implantation rate in bovine embryo transfer.
205
206 **Prediction of embryo chromosome status using TLM**
207 Both quantitative and qualitative evaluations of embryos during the early developmental stage may
208 be applicable to detect aneuploidy. A recent systematic review in humans reported that the times to 8-
209 cells, 9-cells, blastulation, and expanded blastocyst were prolonged in aneuploid embryos, indicating
210 that these morphokinetic variables have prognostic potential [24]. Similarly, a bovine IVF study
211 revealed that slowly cleaving embryos had an increased proportion of chromosomal abnormalities
212 compared with rapidly cleaving embryos [11]. Therefore, morphokinetic evaluation can be applied to
213 select bovine embryos with high implantation potential.
214 Fragmentation, abnormal cleavage, contraction, and multinucleation have been postulated as
215 qualitative parameters for screening embryo ploidy status. A human meta-analysis showed that RC is
216 associated with euploidy, whereas DC is not associated with chromosomal abnormality [24]. However,
217 the authors cautioned that further validation is needed because RC is frequently associated with
218 compromised embryo quality and inferior implantation rates [24]. Bovine studies have demonstrated
219 that RC and DC embryos have an increased proportion of blastomeres with abnormal chromosomes, as
220 determined by karyotyping [19, 53]. Therefore, the association between abnormal cleavage and embryo
221 ploidy requires further elucidation in large-scale studies. Human studies have shown that embryos

222 presenting abnormal cleavage, which presumably have abnormal chromosomes, were able to develop
223 into euploid blastocysts [73, 74], suggesting that abnormal cleavage may have a potential role in the
224 process of "self-correction" to avoid an aberrant chromosomal complement. This is supported by human
225 and bovine studies revealing that abnormal cleavages are associated with partial compaction, in which
226 embryos exclude several blastomeres at the morula stage [55, 70, 74, 83]. These excluded blastomeres
227 showed significantly higher abnormal chromosome content than the corresponding embryos.
228 Furthermore, the incidence of RC and DC was higher in embryos with partial compaction than in fully
229 compacted embryos, indicating the possible role of partial compaction in rescuing chromosomal
230 aberrations associated with abnormal cleavage [55, 74, 83].
231 Recent bovine studies have demonstrated that live-cell imaging using confocal laser microscopy
232 enables long-term and noninvasive observation of embryo chromosomal dynamics, including
233 chromosomal segregation, which is thought to be a critical indicator of embryo viability [84, 85]. The
234 authors reported that abnormal chromosome segregation was associated with delayed first cleavage and
235 reduced developmental potential, with a much lower blastocyst formation rate than in normal embryos.
236 Furthermore, the dynamics of pronuclei in bovine embryos, which have been considered difficult to
237 observe owing to the lipid-rich dark cytoplasm, could be observed using live-cell imaging, and multiple
238 pronuclei were correlated with DC [84]. More practically, analysis of pronuclear number and dynamics
239 has also been achieved by combining lipid removal from embryos by centrifugation with time-lapse
240 observation [56]. These novel techniques can potentially provide new predictive tools for implantation
241 of bovine IVF embryos.
242
243 **Possible application of TLM for embryo sexing**
244 In dairy and beef cattle breeding, pre-selection of calf sex has significant economic advantages and
245 a major impact on management strategies. Sex-sorted semen has been used for *in vitro* embryo
246 production to control the sex of offspring, significantly improve breeding programs, and shorten
247 generation intervals. *In vitro* production of female embryos using X-sorted semen has been shown to

reduce the cost of producing female offspring in dairy cows [5]. Despite these advantages, many studies have reported that using sex-selected semen in IVF reduces embryonic viability and pregnancy rates [54, 86-93]. Our recent TLM study that determined the developmental kinetics of bovine IVF embryos revealed that embryos fertilized with X-sorted semen developed slower than embryos fertilized with unsorted semen [54]. Furthermore, the incidence of RC during the early cleavage stage increased in embryos produced with X-sorted semen, whereas the incidence of DC did not differ between the groups. These findings indicate that slower growth and high prevalence of abnormal cleavage are likely to contribute to the low viability and implantation potential of embryos produced with sex-sorted semen. Therefore, TLM may improve the implantation potential of IVF embryos derived from sex-sorted semen by de-selecting the embryos that exhibit abnormal developmental kinetics.

Several bovine and human studies have attempted to determine the association of time-lapse parameters with embryo sex, with contradictory results. In early bovine studies without TLM, some have reported that early developing embryos were more prone to be male, suggesting that male embryos are likely to develop faster than female embryos [94, 95]. In contrast, other studies failed to find any effect of embryo sex on the timing of bovine embryo cleavage [96-98]. Consequently, although morphokinetic evaluations using TLM have been applied to verify the developmental differences in bovine male and female embryos, the effect of embryo sex on cleavage timing remains controversial. Holm et al. (1998) and Sugimura et al. (2012) failed to find any relationship between developmental kinetics and bovine embryo sex [11, 45]. On the other hand, Peippo et al. (2001) reported that bovine embryo sex is related to time-lapse parameters during the early cleavage stage, which are affected by glucose concentration in the culture medium [47]. When glucose was supplemented into the culture media, male embryos cleaved faster than female embryos, whereas in the absence of glucose, female embryos cleaved faster than male embryos [47]. This is supported by early bovine and human studies suggesting distinct metabolic demands and utilization efficiencies between male and female embryos [99, 100]. These findings suggest that one reason for the discrepancy in the association of morphokinetic parameters with embryo sex between studies may be variations in culture conditions among laboratories.

Therefore, further large-scale studies are necessary to determine the possible application of TLM for pre-selection of embryos of the required sex.

Future perspectives

This review outlines the application of TLM technology as a novel method for selecting embryos to achieve improved pregnancy outcomes in bovine IVF embryo transfer. TLM enables the prediction of the growth and developmental potential of the bovine embryo through morphokinetic parameters, such as the timing of cleavage and morphological dynamics, to detect abnormal cleavage patterns or blastocyst collapse. Furthermore, the possible utilization of TLM to detect chromosomal status or embryo sex would be of practical value in breeding and management strategies. However, very few trials have attempted to transfer TLM embryos into cattle to assess implantation ability, and the usefulness of time-lapse analysis of bovine embryos has rarely been validated. Only one large-scale study has determined the pregnancy rate after the transfer of embryos, which were continuously monitored using time-lapse cinematography [11]. The developmental kinetics of embryos vary greatly among laboratories and culture conditions; therefore, the establishment of bovine embryo selection criteria that can be applied in various culture systems is a critical issue to be resolved for the commercial utilization of TLM for bovine embryo production. Another issue for the practical application of TLM in bovine embryos is the enormous amount of time required to analyze the images. Quantitative and qualitative parameters during the early cleavage stage or contraction and re-expansion of embryos during the blastocyst stage are likely to contribute to the implantation ability of embryos; therefore, an intensive analysis focusing on several critical points may solve this issue. Alternatively, automatic image analysis using artificial intelligence (AI) is an attractive new technology. The usefulness of embryo analysis platforms based on automated algorithms, such as EEVA^{TM}, is under investigation in human ART laboratories [28, 101]. The use of machine learning for embryo selection requires solving problems, such as database quality improvement and the difficulty of making clinical decisions during the learning phase. However, the potential application of AI technology as a predictive tool is a promising approach

for the commercial application of TLM for bovine IVF embryos, where large-scale embryo production is desired. It is evident that the global agricultural industry will benefit from incorporating novel technologies into its management practices to achieve sustainability of livestock production worldwide. The application of TLM in bovine embryo production to improve the pregnancy outcomes of IVF embryos is a key innovation for supporting a growing world population. Therefore, establishing an embryo selection algorithm based on quantitative and qualitative parameters obtained using TLM for embryos, specifically for bovine IVF embryos, is a critical challenge.

Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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References

1. **Moore SG, Hasler JF.** A 100-Year Review: Reproductive technologies in dairy science. *J Dairy Sci* 2017; **100**: 10314-10331.
2. **Hansen PJ.** Current and future assisted reproductive technologies for mammalian farm animals. *Adv Exp Med Biol* 2014; **752**: 1-22.
3. **Hasler JF.** Forty years of embryo transfer in cattle: A review focusing on the journal Theriogenology, the growth of the industry in North America, and personal reminisces. *Theriogenology* 2014; **81**: 152-169.

4. **Blondin P.** Logistics of large scale commercial IVF embryo production. *Reprod Fertil Dev* 2017; **29**: 32-36.
5. **Kaniyamattam K, Block J, Hansen PJ, De Vries A.** Economic and genetic performance of various combinations of in vitro-produced embryo transfers and artificial insemination in a dairy herd. *J Dairy Sci* 2018; **101**: 1540-1553.
6. **Weigel KA, Hoffman PC, Herring W, Lawlor TJ.** Potential gains in lifetime net merit from genomic testing of cows, heifers, and calves on commercial dairy farms. *J Dairy Sci* 2012; **95**: 2215-2225.
7. **Farin PW, Slenning BD, Britt JH.** Estimates of pregnancy outcomes based on selection of bovine embryos produced in vivo or in vitro. *Theriogenology* 1999; **52**: 659-670.
8. **Siqueira LG, Torres CA, Souza ED, Monteiro PL, Jr., Arashiro EK, Camargo LS, Fernandes CA, Viana JH.** Pregnancy rates and corpus luteum-related factors affecting pregnancy establishment in bovine recipients synchronized for fixed-time embryo transfer. *Theriogenology* 2009; **72**: 949-958.
9. **Pontes J, Nonato-Junior I, Sanches B, Ereno-Junior J, Uvo S, Barreiros T, Oliveira J, Hasler J, Seneda M.** Comparison of embryo yield and pregnancy rate between in vivo and in vitro methods in the same Nelore (Bos indicus) donor cows. *Theriogenology* 2009; **71**: 690-697.
10. **Farin PW, Slenning BD, Britt JH.** Estimates of pregnancy outcomes based on selection of bovine embryos produced in vivo or in vitro. *Theriogenology* 1999; **52**: 659-670.
11. **Sugimura S, Akai T, Hashiyada Y, Somfai T, Inaba Y, Hirayama M, Yamanouchi T, Matsuda H, Kobayashi S, Aikawa Y, Ohtake M, Kobayashi E, Konishi K, Imai K.** Promising System for Selecting Healthy In Vitro–Fertilized Embryos in Cattle. *PLoS One* 2012; **7**: e36627.
12. **IETS.** Manual of the international embryo transfer society. *In:* Stringfellow DA, Seidel SM (eds.). The Society; 1998.
13. **Paternot G, Wetzels AM, Thonon F, Vansteenbrugge A, Willemen D, Devroe J, Debrock S, D'Hooghe TM, Spiessens C.** Intra- and interobserver analysis in the morphological assessment of early stage embryos during an IVF procedure: a multicentre study. *Reprod Biol Endocrinol* 2011; **9**: 127.
14. **Farin P, Britt J, Shaw D, Slenning B.** Agreement among evaluators of bovine embryos produced in vivo or in vitro. *Theriogenology* 1995; **44**: 339-349.
15. **Ruiz de Assín R, Clavero A, Gonzalvo MC, Ramírez JP, Zamora S, Fernández A, Martínez L, Castilla JA.** Comparison of methods to determine the assigned value in an external quality control programme for embryo evaluation. *Reprod Biomed Online* 2009; **19**: 824-829.
16. **Sciorio R, Smith GD.** Embryo culture at a reduced oxygen concentration of 5%: a mini review. *Zygote* 2019; **27**: 355-361.
17. **Walters EA, Brown JL, Krisher R, Voelkel S, Swain JE.** Impact of a controlled culture temperature gradient on mouse embryo development and morphokinetics. *Reprod Biomed Online* 2020; **40**: 494-499.

18. **Kirkegaard K, Kesmodel US, Hindkjær JJ, Ingerslev HJ.** Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. *Hum Reprod* 2013; **28**: 2643-2651.
19. **Somfai T, Inaba Y, Aikawa Y, Ohtake M, Kobayashi S, Konishi K, Imai K.** Relationship between the length of cell cycles, cleavage pattern and developmental competence in bovine embryos generated by in vitro fertilization or parthenogenesis. *J Reprod Dev* 2010; **56**: 200-207.
20. **Mandawala AA, Harvey SC, Roy TK, Fowler KE.** Time-lapse embryo imaging and morphokinetic profiling: Towards a general characterisation of embryogenesis. *Anim Reprod Sci* 2016; **174**: 2-10.
21. **Rubio I, Kuhlmann R, Agerholm I, Kirk J, Herrero J, Escribá M-J, Bellver J, Meseguer M.** Limited implantation success of direct-cleaved human zygotes: a time-lapse study. *Fertil Steril* 2012; **98**: 1458-1463.
22. **Liu Y, Chapple V, Roberts P, Matson P.** Prevalence, consequence, and significance of reverse cleavage by human embryos viewed with the use of the Embryoscope time-lapse video system. *Fertil Steril* 2014; **102**: 1295-1300.
23. **Campbell A, Fishel S, Bowman N, Duffy S, Sedler M, Hickman CF.** Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics. *Reprod Biomed Online* 2013; **26**: 477-485.
24. **Bamford T, Barrie A, Montgomery S, Dhillon-Smith R, Campbell A, Easter C, Coomarasamy A.** Morphological and morphokinetic associations with aneuploidy: a systematic review and meta-analysis. *Hum Reprod Update* 2022; **28**: 656-686.
25. **Hassold T, Hunt P.** To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet* 2001; **2**: 280-291.
26. **Rajagopalan H, Lengauer C.** Aneuploidy and cancer. *Nature* 2004; **432**: 338-341.
27. **Fragouli E, Alfarawati S, Spath K, Jaroudi S, Sarasa J, Enciso M, Wells D.** The origin and impact of embryonic aneuploidy. *Hum Genet* 2013; **132**: 1001-1013.
28. **Wong CC, Loewke KE, Bossert NL, Behr B, De Jonge CJ, Baer TM, Reijo Pera RA.** Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. *Nat Biotechnol* 2010; **28**: 1115-1121.
29. **Chen AA, Tan L, Suraj V, Reijo Pera R, Shen S.** Biomarkers identified with time-lapse imaging: discovery, validation, and practical application. *Fertil Steril* 2013; **99**: 1035-1043.
30. **Meseguer M, Herrero J, Tejera A, Hilligsøe KM, Ramsing NB, Remohí J.** The use of morphokinetics as a predictor of embryo implantation. *Hum Reprod* 2011; **26**: 2658-2671.
31. **Kirkegaard K, Hindkjaer JJ, Grøndahl ML, Kesmodel US, Ingerslev HJ.** A randomized clinical trial comparing embryo culture in a conventional incubator with a time-lapse incubator. *J Assist Reprod Genet* 2012; **29**: 565-572.
32. **Conaghan J, Chen AA, Willman SP, Ivani K, Chenette PE, Boostanfar R, Baker VL, Adamson GD, Abusief ME, Gvakharia M, Loewke KE, Shen S.** Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicenter trial. *Fertil Steril* 2013; **100**: 412-419.

33. **Kaser DJ, Racowsky C.** Clinical outcomes following selection of human preimplantation embryos with time-lapse monitoring: a systematic review. *Hum Reprod Update* 2014; **20**: 617-631.
34. **Gallego RD, Remohí J, Meseguer M.** Time-lapse imaging: the state of the art. *Biol Reprod* 2019; **101**: 1146-1154.
35. **Sciorio R, Meseguer M.** Focus on time-lapse analysis: blastocyst collapse and morphometric assessment as new features of embryo viability. *Reprod Biomed Online* 2021; **43**: 821-832.
36. **Bolton VN, Leary C, Harbottle S, Cutting R, Harper JC.** How should we choose the 'best' embryo? A commentary on behalf of the British Fertility Society and the Association of Clinical Embryologists. *Hum Fertil* 2015; **18**: 156-164.
37. **Racowsky C, Martins WP.** Effectiveness and safety of time-lapse imaging for embryo culture and selection: it is still too early for any conclusions? *Fertil Steril* 2017; **108**: 450-452.
38. **Paulson RJ, Reichman DE, Zaninovic N, Goodman LR, Racowsky C.** Time-lapse imaging: clearly useful to both laboratory personnel and patient outcomes versus just because we can doesn't mean we should. *Fertil Steril* 2018; **109**: 584-591.
39. **Bhide P, Maheshwari A, Cutting R, Seenan S, Patel A, Khan K, Homburg R.** Time lapse imaging: is it time to incorporate this technology into routine clinical practice? *Hum Fertil* 2017; **20**: 74-79.
40. **Lewis WH, Gregory PW.** Cinematographs of Living Developing Rabbit-Eggs. *Science* 1929; **69**: 226-229.
41. **Massip A, Mulnard J.** Time-lapse cinematographic analysis of hatching of normal and frozen-thawed cow blastocysts. *J Reprod Fertil* 1980; **58**: 475-478.
42. **Massip A, Mulnard J, Vanderzwalmen P, Hanzen C, Ectors F.** The behaviour of cow blastocyst in vitro: cinematographic and morphometric analysis. *J Anat* 1982; **134**: 399-405.
43. **Grisart B, Massip A, Dessy F.** Cinematographic analysis of bovine embryo development in serum-free oviduct-conditioned medium. *J Reprod Fertil* 1994; **101**: 257-264.
44. **Payne D, Flaherty SP, Barry MF, Matthews CD.** Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. *Hum Reprod* 1997; **12**: 532-541.
45. **Holm P, Shukri NN, Vajta G, Booth P, Bendixen C, Callesen H.** Developmental kinetics of the first cell cycles of bovine in vitro produced embryos in relation to their in vitro viability and sex. *Theriogenology* 1998; **50**: 1285-1299.
46. **Yoshioka K, Suzuki C, Iwamura S.** Effects of activin A and follistatin on developmental kinetics of bovine embryos: cinematographic analysis in a chemically defined medium. *J Reprod Fertil* 2000; **118**: 119-125.
47. **Peippo J, Kurkilahti M, Bredbacka P.** Developmental kinetics of in vitro produced bovine embryos: the effect of sex, glucose and exposure to time-lapse environment. *Zygote* 2001; **9**: 105-113.

48. **Holm P, Booth PJ, Callesen H.** Kinetics of early in vitro development of bovine in vivo- and in vitro-derived zygotes produced and/or cultured in chemically defined or serum-containing media. *Reproduction* 2002; **123**: 553-565.
49. **Majerus V, Lequarré AS, Ferguson EM, Kaidi S, Massip A, Dessy F, Donnay I.** Characterization of embryos derived from calf oocytes: kinetics of cleavage, cell allocation to inner cell mass, and trophectoderm and lipid metabolism. *Mol Reprod Dev* 2000; **57**: 346-352.
50. **Alomar M, Tasiaux H, Remacle S, George F, Paul D, Donnay I.** Kinetics of fertilization and development, and sex ratio of bovine embryos produced using the semen of different bulls. *Anim Reprod Sci* 2008; **107**: 48-61.
51. **Lequarre AS, Marchandise J, Moreau B, Massip A, Donnay I.** Cell cycle duration at the time of maternal zygotic transition for in vitro produced bovine embryos: effect of oxygen tension and transcription inhibition. *Biol Reprod* 2003; **69**: 1707-1713.
52. **Sugimura S, Akai T, Somfai T, Hirayama M, Aikawa Y, Ohtake M, Hattori H, Kobayashi S, Hashiyada Y, Konishi K, Imai K.** Time-lapse cinematography-compatible polystyrene-based microwell culture system: a novel tool for tracking the development of individual bovine embryos. *Biol Reprod* 2010; **83**: 970-978.
53. **Magata F, Ideta A, Okubo H, Matsuda F, Urakawa M, Oono Y.** Growth potential of bovine embryos presenting abnormal cleavage observed through time lapse cinematography. *Theriogenology* 2019; **133**: 119-124.
54. **Magata F, Urakawa M, Matsuda F, Oono Y.** Developmental kinetics and viability of bovine embryos produced in vitro with sex-sorted semen. *Theriogenology* 2021; **161**: 243-251.
55. **Nagai H, Okada M, Nagai Y, Sakuraba Y, Okae H, Suzuki R, Sugimura S.** Abnormal cleavage is involved in the self-correction of bovine preimplantation embryos. *Biochem Biophys Res Commun* 2021; **562**: 76-82.
56. **Suzuki R, Okada M, Nagai H, Kobayashi J, Sugimura S.** Morphokinetic analysis of pronuclei using time-lapse cinematography in bovine zygotes. *Theriogenology* 2021; **166**: 55-63.
57. **Lechniak D, Sell-Kubiak E, Warzych E.** The metabolic profile of bovine blastocysts is affected by in vitro culture system and the pattern of first zygotic cleavage. *Theriogenology* 2022; **188**: 43-51.
58. **Yaacobi-Artzi S, Kalo D, Roth Z.** Association between the morphokinetics of in-vitro-derived bovine embryos and the transcriptomic profile of the derived blastocysts. *PLoS One* 2022; **17**: e0276642.
59. **Liu Y, Qi F, Matson P, Morbeck DE, Mol BW, Zhao S, Afnan M.** Between-laboratory reproducibility of time-lapse embryo selection using qualitative and quantitative parameters: a systematic review and meta-analysis. *J Assist Reprod Genet* 2020; **37**: 1295-1302.
60. **Goodman LR, Goldberg J, Falcone T, Austin C, Desai N.** Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? A randomized controlled trial. *Fertil Steril* 2016; **105**: 275-285.e210.

61. **Milewski R, Kuć P, Kuczyńska A, Stankiewicz B, Łukaszuk K, Kuczyński W.** A predictive model for blastocyst formation based on morphokinetic parameters in time-lapse monitoring of embryo development. *J Assist Reprod Genet* 2015; **32**: 571-579.
62. **Chamayou S, Patrizio P, Storaci G, Tomaselli V, Alecci C, Ragolia C, Crescenzo C, Guglielmino A.** The use of morphokinetic parameters to select all embryos with full capacity to implant. *J Assist Reprod Genet* 2013; **30**: 703-710.
63. **Silva T, Santos EC, Annes K, Soares CA, Leite RF, Lima CB, Milazzotto MP.** Morphokinetic-related response to stress in individually cultured bovine embryos. *Theriogenology* 2016; **86**: 1308-1317.
64. **VerMilyea MD, Tan L, Anthony JT, Conaghan J, Ivani K, Gvakharia M, Boostanfar R, Baker VL, Suraj V, Chen AA, Mainigi M, Coutifaris C, Shen S.** Computer-automated time-lapse analysis results correlate with embryo implantation and clinical pregnancy: a blinded, multi-centre study. *Reprod Biomed Online* 2014; **29**: 729-736.
65. **Liu Y, Chapple V, Feenan K, Roberts P, Matson P.** Time-lapse deselection model for human day 3 in vitro fertilization embryos: the combination of qualitative and quantitative measures of embryo growth. *Fertil Steril* 2016; **105**: 656-662.e651.
66. **Basile N, Vime P, Florensa M, Aparicio Ruiz B, García Velasco JA, Remohí J, Meseguer M.** The use of morphokinetics as a predictor of implantation: a multicentric study to define and validate an algorithm for embryo selection. *Hum Reprod* 2015; **30**: 276-283.
67. **Motato Y, de los Santos MJ, Escriba MJ, Ruiz BA, Remohí J, Meseguer M.** Morphokinetic analysis and embryonic prediction for blastocyst formation through an integrated time-lapse system. *Fertil Steril* 2016; **105**: 376-384.e379.
68. **Sugimura S, Akai T, Imai K.** Selection of viable in vitro-fertilized bovine embryos using time-lapse monitoring in microwell culture dishes. *J Reprod Dev* 2017; **63**: 353-357.
69. **Barrie A, Homburg R, McDowell G, Brown J, Kingsland C, Troup S.** Preliminary investigation of the prevalence and implantation potential of abnormal embryonic phenotypes assessed using time-lapse imaging. *Reprod Biomed Online* 2017; **34**: 455-462.
70. **Coticchio G, Barrie A, Lagalla C, Borini A, Fishel S, Griffin D, Campbell A.** Plasticity of the human preimplantation embryo: developmental dogmas, variations on themes and self-correction. *Hum Reprod Update* 2021; **27**: 848-865.
71. **Kalatova B, Jesenska R, Hlinka D, Dudas M.** Tripolar mitosis in human cells and embryos: occurrence, pathophysiology and medical implications. *Acta Histochem* 2015; **117**: 111-125.
72. **Ozbek IY, Mumusoglu S, Polat M, Bozdag G, Sokmensuer LK, Yarali H.** Comparison of single euploid blastocyst transfer cycle outcome derived from embryos with normal or abnormal cleavage patterns. *Reprod Biomed Online* 2021; **42**: 892-900.
73. **Desai N, Goldberg JM, Austin C, Falcone T.** Are cleavage anomalies, multinucleation, or specific cell cycle kinetics observed with time-lapse imaging predictive of embryo developmental capacity or ploidy? *Fertil Steril* 2018; **109**: 665-674.

74. **Lagalla C, Tarozzi N, Sciajno R, Wells D, Di Santo M, Nadalini M, Distratis V, Borini A.** Embryos with morphokinetic abnormalities may develop into euploid blastocysts. *Reprod Biomed Online* 2017; **34**: 137-146.

75. **Zhan Q, Ye Z, Clarke R, Rosenwaks Z, Zaninovic N.** Direct Unequal Cleavages: Embryo Developmental Competence, Genetic Constitution and Clinical Outcome. *PLoS One* 2016; **11**: e0166398.

76. **Wirka KA, Chen AA, Conaghan J, Ivani K, Gvakharia M, Behr B, Suraj V, Tan L, Shen S.** Atypical embryo phenotypes identified by time-lapse microscopy: high prevalence and association with embryo development. *Fertil Steril* 2014; **101**: 1637-1648. e1635.

77. **Azzarello A, Hoest T, Hay-Schmidt A, Mikkelsen AL.** Live birth potential of good morphology and vitrified blastocysts presenting abnormal cell divisions. *Reprod Biol* 2017; **17**: 144-150.

78. **Marcos J, Pérez-Albalá S, Mifsud A, Molla M, Landeras J, Meseguer M.** Collapse of blastocysts is strongly related to lower implantation success: a time-lapse study. *Hum Reprod* 2015; **30**: 2501-2508.

79. **Baltz JM, Smith SS, Biggers JD, Lechene C.** Intracellular ion concentrations and their maintennance by Na+/K+ -ATPase in preimplantation mouse embroys. *Zygote* 1997; **5**: 1-9.

80. **Niimura S.** Time-Lapse Videomicrographic Analyses of Contractions in Mouse Blastocysts. *J Reprod Dev* 2003; **49**: 413-423.

81. **Sciorio R, Saura RH, Thong KJ, Algam ME, Pickering SJ, Meseguer M.** Blastocyst collapse as an embryo marker of low implantation potential: a time-lapse multicentre study. *Zygote* 2020; **28**: 139-147.

82. **Togashi K, Kumagai J, Sato E, Shirasawa H, Shimoda Y, Makino K, Sato W, Kumazawa Y, Omori Y, Terada Y.** Dysfunction in gap junction intercellular communication induces aberrant behavior of the inner cell mass and frequent collapses of expanded blastocysts in mouse embryos. *J Assist Reprod Genet* 2015; **32**: 969-976.

83. **Lagalla C, Coticchio G, Sciajno R, Tarozzi N, Zacà C, Borini A.** Alternative patterns of partial embryo compaction: prevalence, morphokinetic history and possible implications. *Reprod Biomed Online* 2020; **40**: 347-354.

84. **Yao T, Suzuki R, Furuta N, Suzuki Y, Kabe K, Tokoro M, Sugawara A, Yajima A, Nagasawa T, Matoba S, Yamagata K, Sugimura S.** Live-cell imaging of nuclear-chromosomal dynamics in bovine in vitro fertilised embryos. *Sci Rep* 2018; **8**: 7460.

85. **Yao T, Ueda A, Khurchabilig A, Mashiko D, Tokoro M, Nagai H, Sho T, Matoba S, Yamagata K, Sugimura S.** Micronucleus formation during early cleavage division is a potential hallmark of preimplantation embryonic loss in cattle. *Biochem Biophys Res Commun* 2022; **617**: 25-32.

86. **Palma GA, Olivier NS, Neumüller C, Sinowatz F.** Effects of Sex-sorted Spermatozoa on the Efficiency of in vitro Fertilization and Ultrastructure of in vitro Produced Bovine Blastocysts. *Anat Histol Embryol* 2008; **37**: 67-73.

87. **Bermejo-Álvarez P, Rizos D, Rath D, Lonergan P, Gutiérrez-Adán A.** Can Bovine In Vitro-Matured Oocytes Selectively Process X- or Y-Sorted Sperm Differentially?1. *Biol Reprod* 2008; **79**: 594-597.
88. **Lu KH, Cran DG, Seidel GE.** In vitro fertilization with flow-cytometrically-sorted bovine sperm. *Theriogenology* 1999; **52**: 1393-1405.
89. **Morton KM, Herrmann D, Sieg B, Struckmann C, Maxwell WMC, Rath D, Evans G, Lucas-Hahn A, Niemann H, Wrenzycki C.** Altered mRNA expression patterns in bovine blastocysts after fertilisation in vitro using flow-cytometrically sex-sorted sperm. *Mol Reprod Dev* 2007; **74**: 931-940.
90. **Bermejo-Álvarez P, Lonergan P, Rath D, Gutiérrez-Adan A, Rizos D.** Developmental kinetics and gene expression in male and female bovine embryos produced in vitro with sex-sorted spermatozoa. *Reprod Fertil Dev* 2010; **22**: 426-436.
91. **Mikkola M, Andersson M, Taponen J.** Transfer of cattle embryos produced with sex-sorted semen results in impaired pregnancy rate and increased male calf mortality. *Theriogenology* 2015; **84**: 1118-1122.
92. **Wilson RD, Fricke PM, Leibfried-Rutledge ML, Rutledge JJ, Penfield CMS, Weigel KA.** In vitro production of bovine embryos using sex-sorted sperm. *Theriogenology* 2006; **65**: 1007-1015.
93. **Trigal B, Gómez E, Caamaño JN, Muñoz M, Moreno J, Carrocera S, Martín D, Diez C.** In vitro and in vivo quality of bovine embryos in vitro produced with sex-sorted sperm. *Theriogenology* 2012; **78**: 1465-1475.
94. **Yadav BR, King WA, Betteridge KJ.** Relationships between the completion of first cleavage and the chromosomal complement, sex, and developmental rates of bovine embryos generated in vitro. *Mol Reprod Dev* 1993; **36**: 434-439.
95. **Avery B, Madison V, Greve T.** Sex and development in bovine in-vitro fertilized embryos. *Theriogenology* 1991; **35**: 953-963.
96. **Grisart B, Massip A, Collette L, Dessy F.** The sex ratio of bovine embryos produced in vitro in serum-free oviduct cell-conditioned medium is not altered. *Theriogenology* 1995; **43**: 1097-1106.
97. **King WA, Yadav BR, Xu KP, Picard L, Sirard MA, Verini Supplizi A, Betteridge KJ.** The sex ratios of bovine embryos produced in vivo and in vitro. *Theriogenology* 1991; **36**: 779-788.
98. **Lonergan P, Khatir H, Piumi F, Rieger D, Humblot P, Boland MP.** Effect of time interval from insemination to first cleavage on the developmental characteristics, sex ratio and pregnancy rate after transfer of bovine embryos. *J Reprod Fertil* 1999; **117**: 159-167.
99. **Tiffin GJ, Rieger D, Betteridge KJ, Yadav BR, King WA.** Glucose and glutamine metabolism in pre-attachment cattle embryos in relation to sex and stage of development. *J Reprod Fertil* 1991; **93**: 125-132.
100. **Ray PF, Conaghan J, Winston RM, Handyside AH.** Increased number of cells and metabolic activity in male human preimplantation embryos following in vitro fertilization. *J Reprod Fertil* 1995; **104**: 165-171.

101. **Aparicio-Ruiz B, Basile N, Pérez Albalá S, Bronet F, Remohí J, Meseguer M.** Automatic time-lapse instrument is superior to single-point morphology observation for selecting viable embryos: retrospective study in oocyte donation. *Fertil Steril* 2016; **106**: 1379-1385.e1310.

606 **Table 1.** Milestones for the progress of time-lapse monitoring (TLM) of bovine embryos.

607

Authors	Species	Monitoring system	Embryo monitoring	Embryo production	Start point	End point	Culture period	Main findings
Lewis and Gregory (1929) [40]	Rabbit	Glass slides sealed with paraffin	No information	Collected from the oviduct	One-cell	Hatching	No information	The cleavage and hatching of mammalian embryos were observed for the first time
Massip and Mulnard (1980) [41]	Bovine	Flat capillary tube	No information	Collected from the uterine horns	Morulae and frozen-thawed blastocysts	Hatching	5 days	Embryos were hatched actively by escaping through a slit of the zona pellucida
Grisart et al. (1994) [43]	Bovine	Four-well dish covered by incubation chamber	50 embryos per a droplet	IVF	One-cell	Blastocyst	8 days	The kinetics of early cleavage and the occurrence of a lag-phase were related to the developmental ability to morulae-blastocysts
Somfai et al. (2010) [19]	Bovine	Commercial equipment	15 to 25 embryos per a droplet	IVF	One-cell	Blastocyst	175 h	The duration of the cell cycle and the cleavage pattern during the early cleavage stage indicate the blastulation ability
Sugimura et al. (2010) [52]	Bovine	Commercial equipment	Individual	IVF	One-cell	Blastocyst Pregnancy rate	168 h	The well-of-the-well system that allows tracking and TLM of individual embryos was developed
Sugimura et al. (2012) [11]	Bovine	Commercial equipment	Individual	IVF	One-cell	Blastocyst Pregnancy rate	168 h	A combination of several quantitative and qualitative parameters can successfully predict pregnancy
Yao et al. (2018) [84]	Bovine	A confocal laser microscope with a stable incubation chamber	Individual	IVF	One-cell	Blastocyst	8 days	Multiple pronuclei were observed using 3D live-cell imaging and associated with abnormal cleavage

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609

610 **Figure legends**

611 **Fig. 1.** Morphological classification of embryos at the first cleavage. (A) The cleavage of one
612 blastomere into two blastomeres of the same size, without fragmentation (normal cleavage). (B) The
613 blastomere fusion post cleavage (reverse cleavage). (C) The cleavage of one blastomere into more
614 than three daughter cells (direct cleavage). Numbers in the upper-left corner represent time (h: min)
615 after fertilization (the beginning of incubation with sperm = 0:00). Scale bars = 50 μm. Modified from
616 Magata et al. (2019) [53].

617

618 **Fig. 2.** Photomicrographs of representative blastocysts with good morphology at 8 days post *in vitro*
619 fertilization. Blastocyst presented (A) normal cleavage and (B) reverse cleavage during the first
620 cleavage. Notably, some abnormally cleaving embryos developed into blastocysts of the same quality
621 as normally cleaving embryos. Scale bars = 50 μm.

622

623 **Fig. 3.** Photomicrograph of representative blastocyst (A) before collapse and blastocyst presenting (B)
624 collapse, (C) re-expansion, and (D) hatching. Numbers in the upper-left corner represent time (h: min)
625 after fertilization (the beginning of incubation with sperm = 0:00). Scale bars = 50 μm.

626

627 **Fig. 4.** Time-lapse monitoring of bovine embryos has revealed quantitative and qualitative parameters
628 that may be associated with successful blastocyst formation and pregnancy. hpi, hours post-
629 insemination; RC, reverse cleavage; DC, direct cleavage.

630

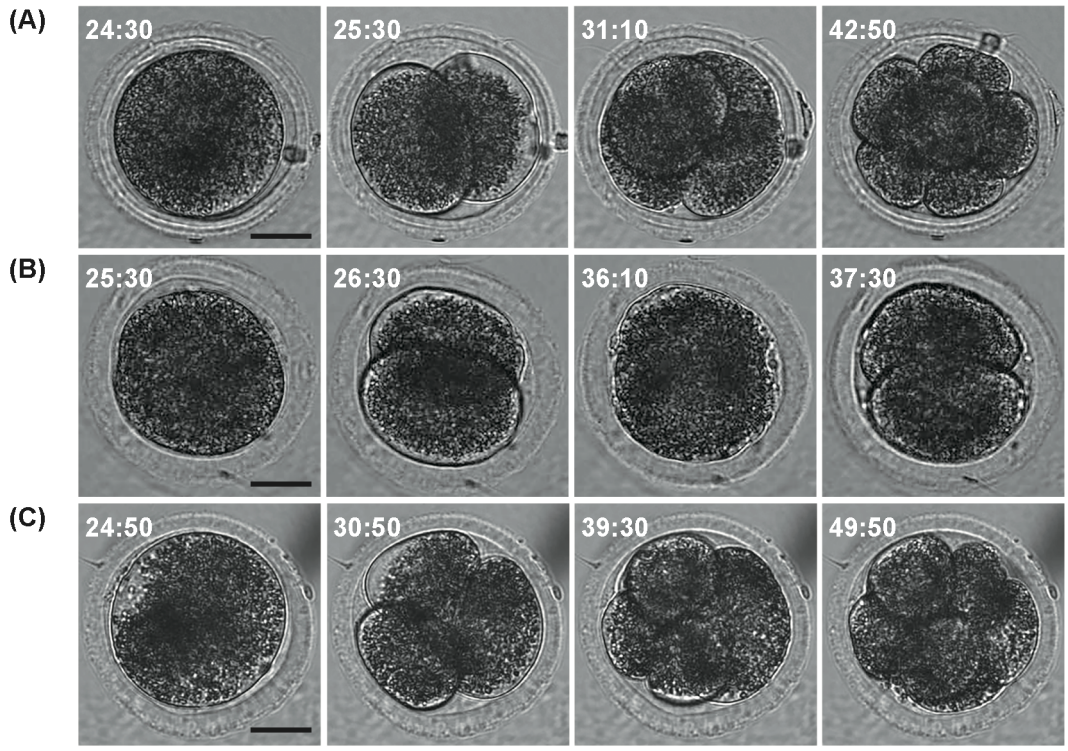


Fig. 1. Morphological classification of embryos at the first cleavage. (A) The cleavage of one blastomere into two blastomeres of the same size, without fragmentation (normal cleavage). (B) The blastomere fusion post cleavage (reverse cleavage). (C) The cleavage of one blastomere into more than three daughter cells (direct cleavage). Numbers in the upper-left corner represent time (h: min) after fertilization (the beginning of incubation with sperm = 0:00). Scale bars = 50μm. Modified from Magata et al. (2019) [53].

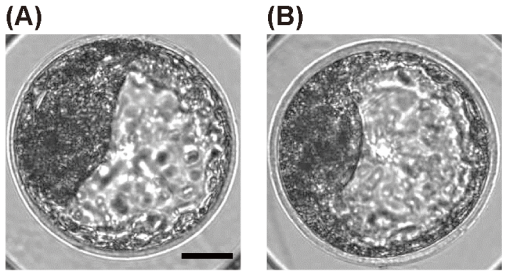
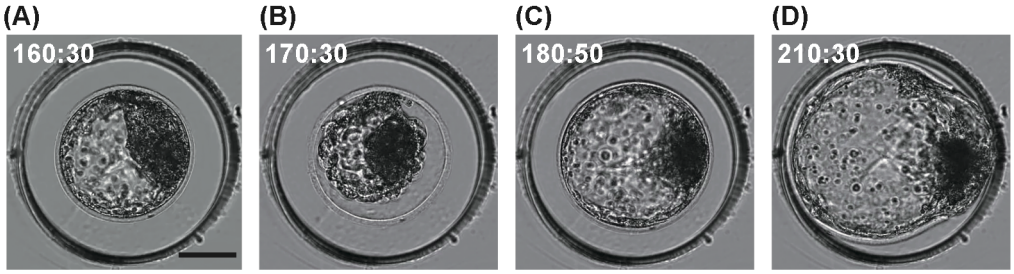


Fig. 2. Photomicrographs of representative blastocysts with good morphology at 8 days post in vitro fertilization. Blastocyst presented (A) normal cleavage and (B) reverse cleavage during the first cleavage. Notably, some abnormally cleaving embryos developed into blastocysts of the same quality as normally cleaving embryos. Scale bars = 50 μm.



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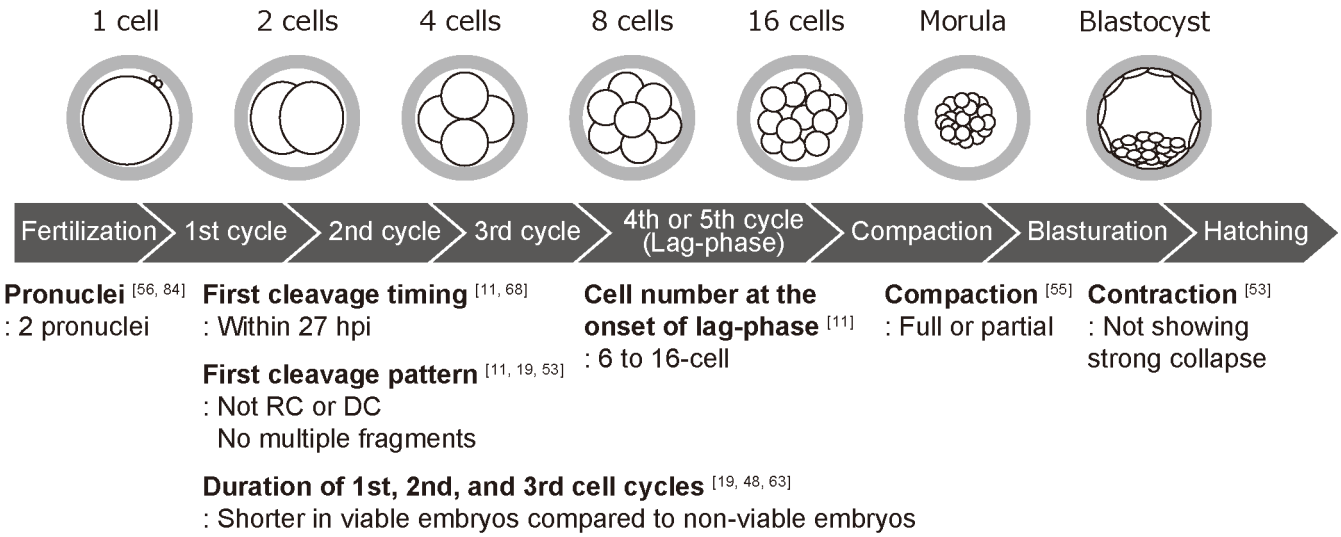

Fig. 4. Time-lapse monitoring of bovine embryos has revealed quantitative and qualitative parameters that may be associated with successful blastocyst formation and pregnancy. hpi, hours post-insemination; RC, reverse cleavage; DC, direct cleavage.