Time-Lapse Embryo culture: A better understanding of embryo development and clinical application

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ABSTRACT
Conventional embryo assessment is performed by removing embryos from incubators at least once a day. However, it is static and limited to specific time points, reducing the amount of information that could potentially be obtained. Fortunately, the time-lapse system is a powerful technology that enables to observe embryo development progression by image acquisition at recurrent time intervals, without interfering in the culture conditions. There are numerous studies that used time-lapse incubators, focusing on embryo kinetics, patient characteristics and clinical outcomes. This review aims to find agreements in the literature concerning embryo kinetics and patient characteristics: age, body mass index, smoking habit, polycystic ovary syndrome and endometriosis; as well as culture conditions that involved culture media and oxygen concentration. Furthermore, they showed differences according to ploidy status, direct/reverse cleavage, gender and the potential association between embryo collapse and clinical outcomes.

Keywords: blastocyst, aneuploidy, embryo culture

INTRODUCTION
Since the beginning of assisted reproduction techniques (ART), morphology has been used as a standard method for embryo selection, generating comparable clinical outcomes among laboratories (Ludwig et al., 2000). Currently, several laboratories are culturing embryos until the blastocyst stage, and the best morphology-embryo is selected for embryo transfer (Scott et al., 2000).

The Time-Lapse (TL) system in ART was first described by Payne et al. (1997), who reported morphokinetic events in early embryo development stages, some of them were: second polar body extrusion, pronuclear formation (maternal and paternal) and cellular cleavage. Morphokinetics enables to compare time frames and dysmorphic events during embryo development (Payne et al., 1997). With all this information, algorithms have been proposed with the aim to increase implantation rates in ART (Sakkas et al., 1998; Gardner et al., 2015).

Embryo culture in TL incubators enabled us to continuously monitor embryo development, without interfering with culture conditions (temperature, gas concentrations and pH); thus, decreasing the negative impact on embryo development (Kahraman et al., 2020) and minimizing the negative effect of standard embryo evaluation under conventional microscopes.

The clinical effectiveness of TL systems is still controversial. A meta-analysis concluded that the TL was associated with a significantly higher ongoing pregnancy, live birth rate and significantly lower early pregnancy loss in comparison to the conventional incubator and morphological selection (Pribenszky et al., 2017); whereas a recent Cochrane review concluded that there are insufficient evidence to support a higher live birth rate when the TL system is used with the aid of software assessment (Armstrong et al., 2018). Furthermore, similar meta-analyses have demonstrated some clinical benefits using TL (Armstrong et al., 2018; Chen et al., 2017). This study aimed to describe how morphokinetic parameters differ according to patient characteristics and culture conditions during embryo development.

PATIENT CHARACTERISTICS AND EMBRYO MORPHOKINETICS

Age
It is widely accepted that older women have diminished ovarian reserve and poor oocyte quality (Navot et al., 1991). Additionally, old patients have higher chances to produce aneuploid embryos, which reduce pregnancy rates (Grøndahl et al., 2017).

Retrospective studies concluded that there are no significant differences in morphokinetic parameters (Tables 1 and 2) during cleavage stages between the embryos from advanced maternal age and those of younger women (Hickman et al., 2013; Silva et al., 2015; Gryshchenko et al., 2014; Warshaviak et al., 2019). By contrast, two retrospective studies reported a significantly faster cleavage in some kinetic parameters in younger patients when compared to older ones (Akarsu et al., 2017; Dal Canto et al., 2021). Nevertheless, these differences are not evident in patients aged ≥ 37 years (Dal Canto et al., 2021).

On the other hand, morphokinetics is faster in later stages (tM, tSB, tB and tHatched) in younger patients (<36 years) than their counterparts; it becomes slower with increasing maternal age (Hickman et al., 2013; Silva et al., 2015).

Body Mass index (BMI)
BMI is defined as the body weight in kilograms divided by the square of height in meters; it is universally expressed in units of kg/m2, used to broadly categorize a person as underweight (<18,5 kg/m2), normal weight (18.5 to 24.5 kg/m2), overweight (25 to 30 kg/m2) or obese (over 30 kg/m2) (WHO, 2020).

Overweight and obesity
Obese women need longer time to become pregnant (Gesink Law et al., 2007; Wise et al., 2010) and their risk of infertility is almost three times higher compared to non-obese women (Zaadstra et al., 1993). Obese women who underwent fertility treatments have lower number of retrieved and mature oocytes, poorer embryo quality (Bellver et al., 2013), lower fertilization (Krizanovská et al., 2002) and increased miscarriage rates (Rittenberg et al., 2011).

Classical morphological evaluation does not seem to be useful for analyzing the impact of obesity on embryo quality (Bellver et al., 2010; Shah et al., 2011). However, morphokinetic parameters showed differences. A recent study, found that t5 and t8 were delayed in obese and in overweight women in relation to the normal-weight group.
**Table 1. Definition of time-lapse morphokinetic parameters.**

| Morphokinetic variables | Description                                                                 |
|-------------------------|------------------------------------------------------------------------------|
| tPB                     | Appearance of second polar body                                               |
| tPNa                    | Appearance of the first pronuclei                                             |
| tPN                     | Appearance of both pronuclei                                                 |
| tPNF                    | Time when both pronuclei had faded                                            |
| t2                      | Time to 2-cell embryo                                                         |
| t3                      | Time to 3-cell embryo                                                         |
| t4                      | Time to 4-cell embryo                                                         |
| t5                      | Time to 5-cell embryo                                                         |
| t6                      | Time to 6-cell embryo                                                         |
| t7                      | Time to 7-cell embryo                                                         |
| t8                      | Time to 8-cell embryo                                                         |
| t9                      | Time to 9-cell embryo                                                         |
| tSC                     | Time from insemination to initiation of compaction                            |
| tM                      | Time from insemination to formation of a morula, completion of compaction process |
| tSB                     | Time from insemination to star blastulation                                   |
| tB                      | Time form insemination to formation of a full blastocyst                     |
| tEB                     | Time to expanded blastocyst                                                   |
| tH                      | Time to hatching blastocyst, the trophectoderm herniation through the zona pellucida |
| tHatched                | Time to hatched blastocyst, the embryo has full escaped the zona pellucida    |

| Duration variables      | Description                                                                 |
|-------------------------|------------------------------------------------------------------------------|
| cc1                     | Time of first cell cycle (t2- tPB)                                            |
| cc2                     | Time of second cell cycle (t3-t2), from 2 to 3 cells                           |
| cc3                     | Time of third cell cycle (t5-t3), from 3 to 5 cells                            |
| s2                      | Time of synchrony of the second cycle (t4-t3), from 2 to t4 cells              |
| s3                      | Time of synchrony of the third cell cycle (t8-t5), from t5 to 8 cells.         |

(Bartolacci et al., 2019); while another study reported that embryos generated from obese and normal-weight infertile women showed similar cleavage patterns, but both groups were slower in early cleavage stages (t2, t3, t4 and t5) than embryos from fertile donors (control) (Bellver et al., 2013). In contrast, another study reported that higher BMI (>25 kg/m²) was associated with earlier occurrence of tM (Leary et al., 2015) as well as earlier tP, tPN, t2, t4, t6, t7 and t8 and S2 than normal-weight women (Mumusoglu et al., 2017).

**Under weigh**

There is no strong evidence that underweight women are associated with menstrual abnormalities or infertility. However, TL systems have enabled to study the morphokinetics of these patients. A study found that underweight women presented later t4, t5, t6 and t8 cell stages than their normal-weight, overweight and obese counterparts (Lammers et al., 2013). By contrast, a study reported that BMI (low or high) does not impact embryo morphokinetics (Silva et al., 2015).

**Polycystic Ovary Syndrome (PCOS)**

PCOS affects 5-10% of women of reproductive age. It is considered a heterogeneous disorder. It is identified using the Rotterdam criteria, in which two out of three of the following conditions must be met: the presence of oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries.

Morphokinetic parameters in hyper-androgenic PCOS women had a delay in early stages (from tPNF to t8) compared with embryos from non-PCOS regular cycles, but there was no kinetic difference between normo-androgenic PCOS and control women (Wissing et al., 2014). In addition, another report stated that embryos generated from PCOS patients had long developmental timings (tPNF, t2, t3, t4, t5, t6, t7 and t8) compared to normo-ovulatory women (Tabibnejad et al., 2019).

Nevertheless, other studies reported a faster development in PCOS and hyper-androgenic patients when compared to embryos from non-PCOS patients during the cleavage stage (t5, t6, t7, t8, t9 and 4th cleavage division (from 8-cells to 9 or more cells), time to tSC and tM (Sundvall et al., 2015; Chappell et al., 2017; 2020).

On the other hand, a recent paper documented any significant differences in early embryonic development between the PCOS group and control patients. However, we must emphasize that in this study, the embryos were cultured until day 2 (Tam Le et al., 2019).

Finally, the current available data concluded that the implantation rate and the clinical pregnancy rate did not differ between PCOS patients and their control counterparts (Wissing et al., 2014; Sundvall et al., 2015; Tabibnejad et al., 2019; Tam Le et al., 2019). Retrospective studies concluded that PCOS patients have a trend towards a lower live birth rate and higher spontaneous abortion rates (Chappell et al., 2017; 2020).
Table 2. Summary of the studies highlighting the morphokinetic variables with statistically significant differences.

| Item | Reference | Type of study | Embryos (n) | Patients (n) | Embryo culture | Day transfer | Morphokinetic parameters | Statistically significant variables |
|------|-----------|---------------|-------------|--------------|----------------|--------------|---------------------------|-----------------------------------|
| Young vs Older | Hickman et al., 2013 | Retrospective | 348 | NA | Blastocyst stage | NA | x | tM, tEB, tHed |
| | Silva et al., 2015 | Retrospective | 288 | 76 | Blastocyst stage | NA | x | tM, tSB, tEB, tEB |
| | Akarsu et al., 2017 | Retrospective | 1144 | 197 | Cleavage stage | d3 | X | tPFN, t2, t3, t4 |
| | Dal Canto et al., 2021 | Retrospective | 4915 | 1066 | Cleavage stage | d2/d3 | X | tPFN, t2, t3, t4, t8 |
| | Gryshchenko et al., 2014 | Prospective | 262 | 616 | Cleavage/Blastocyst stage | d3/d5 | | |
| | Warshaviak et al., 2019 | Retrospective | 1148 | 122 | Cleavage stage | d3 | | |
| BMI | Bartolacci et al., 2019 | Retrospective | 7180 | 1528 | Cleavage/Blastocyst stage | d3/d5 | X | t5, t8 |
| | Belver et al., 2013 | Retrospective | 424 | 89 | Blastocyst stage | d3 | | t2, t3, t4 and t5 |
| | Lammers et al., 2013 | Retrospective | NA | 366 | Cleavage stage | NA | | t4, t5, t8 |
| | Leary et al., 2015 | Retrospective | 155 | 29 | Blastocyst stage | d3/d5 | X | tM |
| | Mumusoglu et al., 2017 | Retrospective | 415 | 103 | Blastocyst stage | NA | X | tPFN, t2, t3, t4, t5, t6, t7, t8, s2 |
| | Silva et al., 2015 | Retrospective | 288 | 76 | Blastocyst stage | NA | X | |
| PCOS vs non PCOS | Chappel et al., 2017 | Retrospective | 1108 | 86 | Blastocyst stage | NA | X | t7, t8, t9, tM |
| | Chappell et al., 2020 | Retrospective | 1618 | 256 | Blastocyst stage | NA | X | t5, t6, t7, t8, t9, tSC |
| | Sundvall et al., 2015 | Prospective | 1388 | 217 | Blastocyst stage | d6 | | tSC, tM and t4-t5 |
| | Tabibnejad et al., 2019 | Prospective | 547 | 100 | Cleavage stage | d3 | | |
| | Tam Le et al., 2019 | Prospective | 851 | 106 | Cleavage stage | d2 | | |
| | Wissering et al., 2014 | Prospective | 347 | 71 | Blastocyst stage | d2 | | tPFN, t2, t3, t4, t7 |
| Endometriosis | Boynukalin et al., 2019 | Retrospective | 439 | NA | Cleavage stage | NA | X | tPB, tPFN, cc1, s2 |
| | Freis et al., 2018 | Retrospective | 477 | 168 | Blastocyst stage | NA | X | |
| | Schenk et al., 2019 | Retrospective | 1148 | 163 | NA | d3/d4/d5 | X | s2, t9 |
| Smoking habitus | Fréour et al., 2013 | Retrospective | 485 | 135 | Cleavage/Blastocyst stage | d3/d5 | | |
| | Siristatidis et al., 2015 | Prospective | NA | 239 | Cleavage stage | d2/d3 | X | t3, t4, t5 |
| Single vs. sequential media | Basile et al., 2013 | Prospective | 532 | 75 | Cleavage stage | d3 | | cc2, S2 |
| | Ciray et al., 2012 | Prospective | 319 | 51 | Blastocyst stage | d3/4/5 | | |
| | Costa-Borges et al., 2016 | Prospective | 628 | 59 | Blastocyst stage | d5 | | |
| | Hardarson et al., 2015 | Prospective | 1356 | 128 | Blastocyst stage | d3/5/6 | | t7, t8, cc2 |
| VF vs. ICSI | Bodri et al., 2015 | Retrospective | 1285 | 209 | Blastocyst stage | d5 | | tBE |
| | Cruz et al., 2013 | Retrospective | 1203 | 178 | Blastocyst stage | d3/5 | | |
| | Dal Canto et al., 2012 | Retrospective | 459 | NA | Blastocyst stage | d3/5 | X | t2, t3 |
| | Kim et al., 2017 | Cohort study | 1830 | NA | Blastocyst stage | NA | X | tPFN, t1, t2, t3, t4, t5, t6 |
| | Lemmen et al., 2008 | Cohort study | 102 | NA | Cleavage stage | d2 | | t2 |
| Oxygen 5% vs. 20% | Kirkegaard et al., 2013a | Retrospective | 363 | 84 | Blastocyst stage | NA | X | s3 |

Note: NA: not available; d2/3/5: day 2/3/5; other abbreviations in Table 1.
**Endometriosis**

Endometriosis is defined as the presence of endometrial glands and stroma cells growing outside the uterus (Bunney & Giudice, 2012). Endometriosis affects 10-15% of all women in reproductive age (Giudice & Kao, 2004). It is associated with chronic pelvic pain and infertility (Parasar et al., 2017), and it is subdivided into four categories: stage I (minimal), stage II (mild), stage III (moderate) and stage IV (severe) (Johnson et al., 2017). One study reported that endometriosis influences morphokinetic parameters without considering the severity of the disease; especially in early embryo stages (Freis et al., 2018). However, a deeper analysis associated with the endometriosis stages reported that patients with severe endometriosis reached t9 faster than patients with minimal or moderate endometriosis, as well as the control group (patients without endometriosis). Moreover, the synchrony of the two blastomere divisions within the second cell cycle (s2) was faster in the endometriosis group than in women without endometriosis (Schenk et al., 2019).

On the other hand, a recent study concluded that embryos from endometriotic patients had different kinetics during cleavage stages, because they had a shorter cc1 and longer tpB, tpNa; as well as s2 than control group patients (Boynukalin et al., 2019).

**Smoking**

Its negative impact on fertility has been widely described in women and men, affecting gamete quality and embryo development (Waylen et al., 2009). Smoker women have a higher risk of fertilization failure and lower implantation rates (Gruber et al., 2008). Furthermore, smoking causes obstetric complications, such as miscarriages, preterm births and premature membrane rupture (Levis et al., 2014).

We know that smoking affects at least one of the embryological events in the early cleavage stage of development (Siristatidis et al., 2015). A study found that embryos from smokers are significantly slower during cleavage stages (t3, t4 and t5), than those from nonsmokers; curiously, there are no kinetic differences between embryos from smokers and nonsmokers that stopped their development (Freour et al., 2013).

**CULTURE MEDIA: SINGLE AND SEQUENTIAL MEDIA**

Embryos can be cultured in uninterrupted culture using a single medium throughout the 5/6 days of culture or sequential media, where two media with different composition are used sequentially (Costa-Borges et al., 2016).

Studies with oocytes from donors and patients have not reported differences in morphokinetic parameters between single step media and sequential media in embryo cultures until days 3 or 5/6 (Basile et al., 2013; Costa-Borges et al., 2016).

On the other hand, a study using autologous oocytes suggested that embryos cultured in a single media have faster development from TPNF until t5 than those cultured in sequential media (Ciray et al., 2012); while a multicenter clinical trial reported a delay in t7, t8 in embryos cultured in single media (Hardarson et al., 2015). Interestingly, besides the differences in morphokinetics, the studies did not find differences in implantation rate, ongoing pregnancy rates and live birth between the groups (Ciray et al., 2012; Basile et al., 2013; Hardarson et al., 2015; Costa-Borges et al., 2016). However, single media simplifies the logistics of an IVF laboratory, and the embryos can be assessed without disturbing culture conditions through the time-lapse system.

**FERTILIZATION TECHNIQUE**

Oocytes are inseminated using conventional in vitro fertilization (IVF) or Intracytoplasmic sperm injection (ICSI). When IVF is performed, some sperm selection processes are maintained in vitro. These processes are sperm penetration of the cumulus cells, zona pellucida interaction and fusion of the oolemma, whereas during ICSI a single sperm is injected into a mature oocyte.

These two processes are different, and it is not possible to determine exactly when fertilization occurs in IVF. Some time-lapse studies reported a faster development in embryos generated by ICSI than in IVF (Levem et al., 2008; Dal Canto et al., 2012; Kim et al., 2017). However, it is a consensus that TPNF can be used as starting point when IVF or ICSI are used as a fertilization method (Cruz et al., 2013; Bodri et al., 2015).

When TPNF was used as a starting point, there were any observed differences in the cleavage stage (early and late) between the IVF and the ICSI techniques (Cruz et al., 2013; Bodri et al., 2015), although one of them reported a faster development in the blastocyst stage in IVF- fertilized embryos (Bodri et al., 2015). This could be explained because when IVF is performed the semen characteristics are superior to those in ICSI (Cruz et al., 2013; Bodri et al., 2015). Under this evidence, confounding factors should also be considered when both techniques are compared.

**OXYGEN CONCENTRATION AND UNINTERRUPTED CULTURE CONDITIONS**

Oxygen concentration differs in the female reproductive tract, and it decreases from the fallopian tubes towards the uterus. The concentration ranges from 5-7% to 2% (Ng et al., 2018). Oxygen can influence embryo development. It is well documented that embryos cultured under lower oxygen concentration (5%) have more cleavage, good quality and more embryos are cryopreserved, which improved embryo implantation compared to those embryos cultured under higher oxygen concentration (20%) (Sciorio & Smith, 2019; Van Montfoort et al., 2020). Similar results were also found in the TL-system. A study reported a low quality of embryos, a lower number of utilized embryos and delays in s3 when the embryos were cultured in high-oxygen concentration (20%) (Kaleas et al., 2020).

Additionally, in the TL-system, the embryos do not need to be removed from the incubator, this minimizes environmental disturbances caused by bench-top incubators. Interestingly, a study reported a higher live birth rate in embryos cultured in the TL-system, when compared to embryos cultured in high-quality K-systems, both using 5% O2 (Kaleas et al., 2020).

**COLLAPSE**

Collapse is a separation of all or part of the trophoderm (TE) cells from the zona pellucida (ZP) during blastocyst growth, the cells could be separated; when more than 50% form the inner side of the ZP (collapse or strong contraction) or less than 50% (weak contraction) (Marcos et al., 2015). Blastocyst collapse is independent of the insemination technique (Sciorio et al. 2020a,b) and maternal age (Bodri et al., 2016; Gazzo et al., 2020). Whereas the relationship between collapse and embryo quality is arguable, because a study did not find a relation (Marcos et al., 2015), while two studies proposed a higher incidence of blastocyst collapse in poor quality embryos (Bodri et al., 2016; Sciorio et al. 2020b).
Embryos at the blastocyst stage could collapse at least one time. However, not all the blastocyst collapses (Marcos et al., 2015; Bodri et al., 2016; Viñals Gonzalez et al., 2018; Sciorio et al., 2020a;b). Retrospective studies in TL showed that embryos that collapse during their development have lower implantation and pregnancy rates, especially if they were multiple when compared to blastocysts that did not display this event, although there are no relations between collapse duration and decreased implantation rates (Marcos et al., 2015; Bodri et al., 2016; Viñals Gonzalez et al., 2018; Gazzo et al., 2020; Sciorio et al., 2020a;b).

Embryo collapse and aneuploidy has also been investigated. It is reported that collapses were predominantly present in aneuploid embryos; moreover, embryos affected with monosomies have lower numbers of collapses when compared with trisomy or complex aneuploidies (Viñals Gonzalez et al., 2018). Even if an euploid embryo has collapses, it has a lower live-birth rate (Harton et al., 2016).

According to morphokinetic parameters, embryos without collapse had a slow development during early stages (t2,t3,t4 and tB) compared to those blastocyst which underwent blastocyst collapse (Marcos et al., 2015); while other studies reported that collapsed embryos took longer to reach the blastocyst stage (Gazzo et al., 2020), and a delayed development during the blastocyst stage (Bodri et al., 2016).

RE-EXPANSION IN THAWED EMBRYOS
Freezing embryos is a common practice. It enables a single embryo transfer, genetics test or freeze all strategy for better endometrium preparation. During vitrification processes, embryos are dehydrated through the addition of cryoprotectants and, consequently, they shrunk. Immediately after warming, the embryos look collapsed, and need more time to recover their initial volume (re-expansion).

Blastocyst quality evaluation is difficult after the warming process. For that reason, post-warmed culture helps assess the vitrified/warmed blastocyst (Zhao et al., 2019). A study using the TL-system reported that re-expansion started as early as 10 minutes and can complete the re-expansion in 2 hours after warming (Ebner et al., 2017).

Embryos without re-expansion after the warming process have a significantly lower implantation rate when compared with the completely or partially re-expanded blastocyst (Desai et al., 2016; Coello et al., 2017; Ebner et al., 2017). In addition, after warming, the embryos can also have collapses, even more than once. However, it does not seem to affect implantation (Coello et al., 2017; Ebner et al., 2017). Shorter-duration re-expansion (from start to complete re-expansion) has been documented in those blastocysts that result in pregnancy in comparison to non-pregnant women (Ebner et al., 2017).

Furthermore, a predictive implantation model that evaluated morphology in post-warmed blastocyst has been proposed. The embryos were subdivided into four categories from A to D. If the maximum area values were >14,597mm², the blastocysts were categorized as A or B, depending on whether the initial area values were >9,900mm² or < 9,900mm², respectively. Similarly, if the maximum area values were < 14,597mm², the blastocysts were categorized as C or D, depending on the initial area. They found that implantation was 47.3%, 43.7%, 32.8% and 14.2% for A to D, respectively (Coello et al., 2017).

ATYPICAL DYNAMIC EMBRYO BEHAVIORS RELATED TO LOWER IMPLANTATION
Atypical dynamic embryo behaviors could happen during their early development, and are not maternal-age dependent (Athyade Wirka et al., 2014; Zhan et al., 2016; Yang et al., 2018). Embryos with abnormal divisions are more likely to arrest their development than embryos with normal divisions, especially during their first stages of development (Lagalia et al., 2017). Moreover, their embryo potential and implantation rates are adversely affected (Meseguer et al., 2011; Hlinka et al., 2012; Hur et al., 2018; Yang et al., 2018). These abnormal behaviors can only be visualized through continuous observation and subsequent time-lapse analysis.

Although, several atypical dynamic embryo behaviors have been described, two have shown to play a major role: Direct Cleavage (DC) and Reverse Cleavage (RC).

Direct Cleavage is defined as the second cell cycle being shorter than 5 hours, or more than 2 cells originated from a single cell division event. DC can occur during cleavage and their frequency is higher during the first cleaving periods (Fan et al., 2016); moreover, it could be present more than once (Zhan et al., 2016). It is noteworthy that DC in embryos, especially at earlier stages, strongly correlates with impaired blastocyst formation, implantation and clinical outcome (Rubio et al., 2012; Athyade Wirka et al., 2014; Fan et al., 2016; Zhan et al., 2016).

DC embryos are more often found during IVF cycles than ICSI cycles (Zhan et al., 2016), at higher percentages when testicular and epididymal sperm are used (Kahraman et al., 2020; Zhan et al., 2016). Additionally, DC embryos have 2.5 to 3.1 fold higher likelihood of a multinucleation incidence (Zhan et al., 2016).

Likewise, two retrospective studies suggest that DC embryos can undergo self-correction mechanisms while excluding some cells (with higher incidence of aneuploidies) during the compaction process. Therefore, DC embryos showed a similar euploid rate when compared to non-DC blastocysts on day 5 (Zhan et al., 2016). Nevertheless, this mechanism is significantly lower in older patients (>39 years) (Lagalia et al., 2017).

Reverse Cleavage is defined as either blastomeres rejoining after complete separation, or the blastomere fails to separate. It could occur during the cleavage stage, with major incidence during the third cycle of the mitotic division (5 to 8 cells) (Liu et al., 2014). The mean time of occurrence reported was 46.07h (Quera et al., 2014) and between 24 to 136h (Hickman et al., 2013).

In addition, RC affect the blastocyst stage development (Desai et al., 2018). Embryos with RC have significantly lower good-quality embryos on day 3 (Liu et al., 2014), and consequently a lower blastocyst formation rate (Yang et al., 2018). Furthermore, RC embryos implanted less than their counterparts (Liu et al., 2014), especially if two or more abnormal cleavages were present in a single embryo, increasing the likelihood of the embryo being aneuploid (Desai et al., 2018).

PLOIDY
A meta-analysis concluded that Time-lapse embryo monitoring may help predict the ploidy of embryos (Swain, 2013), even though other studies proposed that morphokinetics are not enough (Reignier et al., 2018); but it provides valuable information for embryo selection (Zaninovic et al., 2017).

Some studies did not find differences during the cleavage and blastocyst stages of embryo development between euploids and aneuploids (Stevens et al., 2012; Semeniuk et al., 2013; Yang et al., 2014) (Table 3). Conversely, others have reported a development delay in aneuploid embryos during cleavage and blastocyst stages (Table 3) (Davies et al., 2012; Campbell et al., 2013; Vera-Rodriguez et al., 2015; Chawla et al., 2015; Mumusoglu et al., 2017; Zhang et al., 2017; Huang et al., 2019).
| Item | Reference | Type of study | Embryos (n) | Patients (n) | Embryo culture | Day transfer | Morphokinetic parameters significant difference | Statistically significant variables |
|------|-----------|---------------|-------------|--------------|----------------|--------------|-----------------------------------------------|---------------------------------|
|      |           |               |             |              |                |              |                                               |                                 |
| Euploid vs Aneuploid | | | | | | | | |
| Amir et al., 2019 | cohort study | 270 | NA | Blastocyst stage | FISH | x | t4, tSB, tPNf |
| Basile et al., 2014 | Retrospective | 504 | 125 | Blastocyst stage | aCGH | x | t5-t2, cc3 |
| Bayram et al., 2012 | Prospective | 122 | 17 | Cleavage stage | FISH | x | s3 |
| Campbell et al., 2013 | Retrospective | 195 | 25 | Blastocyst stage | aCGH | x | tSC, tSB, tB |
| Chavez et al., 2012 | Cohort studies | 53 | NA | Cleavage stage | aCGH | x | from t1 to t4 |
| Chawla et al., 2015 | Retrospective | 496 | 132 | Blastocyst stage | aCGH | x | tPNf, t2, t5, cc2, cc3, t5-t2 |
| Davies et al., 2012 | Retrospective | 62 | NA | NA | aCGH | x | t2, t3, s2 |
| Del Carmen Nogales et al., 2017 | Retrospective | 485 | 112 | Blastocyst stage | aCGH | x | t3, t5, cc2, cc3, s2, t5-t2 |
| Hong et al., 2013 | Prospective | 307 | 24 | Blastocyst stage | qPCR | x | tEB and from t5 to tSB |
| Huang et al., 2019 | Retrospective | 188 | 34 | Cleavage stage | aCGH | x | tBE |
| Kahraman et al., 2020 | Retrospective | 741 | 279 | Blastocyst stage | aCGH | x | t2, t3, t4, t5, t6, t7, t8, t9, tM, tSB, tB |
| Kramer et al., 2014 | Retrospective | 149 | 25 | Blastocyst stage | aCGH | x | |
| Melzer et al., 2013 | Prospective | | | | | | |
| Minasi et al., 2016 | Retrospective | 928 | 454 | Blastocyst stage | aCGH | x | t4, s2, tB, tEB, tHed |
| Mumusoglu et al., 2017 | Retrospective | 415 | 103 | Blastocyst stage | aCGH | x | t9, tM, tSB, tB, tEB |
| Patel et al., 2016 | Retrospective | 167 | 26 | Blastocyst stage | aCGH | x | |
| Rienzi et al., 2015 | cohort study | 455 | 138 | Blastocyst stage | aCGH | x | |
| Semeniuk et al., 2013 | retrospective | 76 | NA | Blastocyst stage | aCGH | x | |
| Stevens et al., 2012 | Cohort studies | 53 | 35 | Blastocyst stage | qPCR | x | |
| Vera-Rodriguez et al., 2015 | Prospective | 85 | NA | Cleavage stage | aCGH | x | tPNf, t2, s2 |
| Yang et al., 2014 | prospective | 498 | 138 | Blastocyst stage | aCGH | x | |
| Zhang et al., 2017 | Retrospective | 256 | 75 | Blastocyst stage | aCGH | x | |
| Male vs Female | | | | | | | | |
| Bodri et al., 2016 | Retrospective | 291 | NA | Blastocyst stage | NA | x | t8, tM, tB |
| Bronet et al., 2015 | Retrospective | 327 | 93 | Blastocyst stage | aCGH | x | s2, tM |
| Huang & Jin, 2017 | Retrospective | 174 | 134 | NA | NA | x | t3, t4, cc2 |
| Huang et al., 2019 | Retrospective | 308 | 228 | Cleavage stage | NA | x | t3, t4, t5, cc2 |
| Melzer et al., 2013 | Prospective | 213 | 20 | Blastocyst stage | aCGH | x | t4 |
| Serdarogullari et al., 2014 | Retrospective | 177 | 139 | NA | NA | x | |
| Zeyad et al., 2018 | cohort study | 416 | 120 | Blastocyst stage | FISH | x | tPN, tPNf |

Note: NA: not available; d2/3/5: day 2/3/5; FISH: fluorescence in situ hybridization; aCGH: array comparative genomic hybridization; qPCR: quantitative polymerase chain reaction; other abbreviations in Table 1.
Similarly, a prospective study reported that chromosomally normal embryos display strict and tightly clustered cell cycle parameters up to the 4-cell stage (Chavez et al., 2012). Other studies found shorter stages in euploid embryos than aneuploid embryos: in s3 (Bayram et al., 2012), a shorter compaction (Melzer et al., 2013) and early cavitation from first cytokinesis and from t5 (Hong et al., 2013), a shorter cleavage s2 and t4 as well during the blastocyst stage t5, tEB and tHed (Minasi et al., 2016), a faster development from t2 to blastocyst stage; however, these differences are not present in severe forms of male infertility (less than 1x10^6 spermatozoa/ml) (Kahraman et al., 2020).

In addition, a retrospective study reported that embryos with trisomy showed very similar kinetics to those of normal embryos, whereas embryos with monosomies fall between complex and trisomy embryos (Del Carmen Nogales et al., 2017). Furthermore, embryos with complex chromosomal abnormalities have the shortest division times (t3, t5, cc2, cc3, s2 and t5-t2), which is strongly associated with t3 and t5-t2 (time interval between the 2- and the 5-cell stage) (Del Carmen Nogales et al., 2017). Moreover, a study reported that unbalanced chromosomal translocation embryos showed a delay of t4, tSB and s2, and embryos with balanced translocation did not; even a delay in tPNF was seen in embryos with nonviable unbalanced chromosomal translocation, when compared to potentially viable embryos (Amir et al., 2019).

Likewise, there are models proposed to identify embryos more likely to be euploid, based on variables. Some studies have identified that t5-t2, cc3, tSB and tB are good predictor variables (Basile et al., 2014; Campbell et al., 2013); others have been more specific, a cc3 (t5-t3) > 10.00h and t5-t2 > 20.00h (Chawla et al., 2015), and blastocyst initiation (tSB>96.2h); progression to expanded blastocyst (tSB>166h) and tEB- tSB > 13h in aneuploid embryos (Desai et al., 2018) are morphokinetic parameters associated with aneuploidy.

On the other hand, other studies demonstrated that the models described above were unable to discriminate between euploid and aneuploid embryos (Kramer et al., 2014; Rienzi et al., 2015; Patel et al., 2016; Zhang et al., 2017).

**Sex**
A single study with 177 embryos reported that female embryos showed earlier cavitation than male embryos, but it did not reach statistical significance (Serdarogullari et al., 2014). However, studies with aCGH analysis found a faster development in male embryos. A single study found that male embryos tend towards a faster progression to t4 than female embryos, with 213 embryos included (Melzer et al., 2013); while a study with 327 embryos also found a faster development in male embryos. Hence, they proposed an algorithm based on s2 and tM that permits identifying embryos with higher probability of being female (Bronet et al., 2015). By contrast, a study evaluated 416 embryos through the FISH technique, and they reported that tPNF is significantly faster in female than male embryos; whereas tPNF was significantly faster in male embryos; and the blastulation rate was significantly higher in female embryos (Zeyad et al., 2018).

As described above, a retrospective study based on 81 live births reported a significantly slower development for tB in male embryos than in female embryos (Boadi et al., 2016). Meanwhile, retrospective studies reported t3, t4 and cc2 earlier in male embryos than those of female embryos (Huang & Jin, 2017); as well as t3, t4 and cc2 (Huang et al., 2019). However, only t3 (<14h) was correlated with live birth sex (Huang et al., 2019).

**CONCLUSIONS**
Time Lapse provides valuable information and enormous potential to enhance our understanding of embryo development. Considerable knowledge has been accumulated and describes the morphokinetic dynamics during different stages in human embryos. It has been demonstrated that some patient characteristics and culture conditions modified this development pattern. There is a consensus that embryos generated from advanced maternal age have a slow development. Moreover, the current available data about smoking and high-oxygen concentration cultures are scarce, but it also demonstrated a slow development in both groups. On the contrary, any difference in morphokinetics has been described when IVF or ICSI was used as the fertilization method. Similar observations were found when the embryos were culture in single or sequential media.

On the other hand, the limited number of studies, the considerable differences in the study designs and patients' characteristics like BMI, PCOS, endometriosis, ploidy and gender made it difficult to draw a conclusion. Given this inconsistency and lack of evidence, more prospective studies and further randomized clinical trials are needed.

The association between morphokinetics and ART outcome has important implications in clinical results; where two morphokinetic events have the potential to predict embryo implantation: blastocyst collapse-re-expansion and direct-reverse cleavage seems to have a strong impact on clinical outcomes.

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**Authors’ contributions**
SM; draft the manuscript. LG; draft and review the manuscript. LNH; review the manuscript.

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**CONFLICT OF INTEREST**
The authors declare no conflict of interest.
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