Faster fertilization and cleavage kinetics reflect competence to achieve a live birth after intracytoplasmic sperm injection, but this association fades with maternal age

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Objective: To assess the relationship of early developmental kinetics with competence to provide a live birth and the impact of maternal age in this context.
Design: Retrospective cohort study including 4,915 embryos, of which 1,390 were transferred and provided a clinical outcome paired with morphokinetic data; 168 of them resulted in a live birth (LB), and 1,222 did not (NLB). Early morphokinetic parameters were compared between LB and NLB embryos from patients stratified into two age groups (<37 and ≥37 years), and between embryos at the same competence group from patients aged <37 and ≥37 years. The association of morphokinetic parameters with live birth was tested by univariate and multivariate analyses.
Setting: Fertility clinic.
Patient(s): The study population included 1,066 patients undergoing autologous intracytoplasmic sperm injection cycles with fresh single (SET), double (DET) or triple (TET) embryo transfers on day 2 or 3. Of them, 669 patients produced NLB embryos and 134 produced LB embryos.
Intervention(s): None.
Main Outcome Measure(s): Fertilization and cleavage morphokinetic parameters and live birth.
Result(s): In the total patient population, all morphokinetic parameters were achieved earlier in LB compared with NLB embryos. The same was observed in patients aged <37 years ($P<.015$), but not ≥37 years. Except for the t8 (time at which an 8-blastomere embryo was identified), all morphokinetic parameters were reached earlier in LB embryos from patients aged <37 years compared with LB embryos from patients aged ≥37 years. Univariate analysis revealed that earlier occurrence of all morphokinetic parameters was associated with live birth, although only earlier t2 (time at which two separate and distinct cells were identified) was associated with live birth independently from maternal age in the multivariate analysis.
Conclusion(s): Despite its retrospective nature and performance in a single IVF center, this study presents novel data indicating that embryos competent to provide a live birth display overall faster early developmental kinetics compared with embryos that do not achieve a live birth after transfer, a difference that, however, narrows as maternal age advances. The findings suggest that fertilization and cleavage morphokinetic parameters may constitute valuable references for embryo selection strategies aiming to improve live birth rates, specifically before advanced maternal age while holding limited usefulness in advanced maternal age. (Fertil Steril® 2021;115: 665–72. ©2020 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Time lapse, embryonic morphokinetics, live birth, maternal age, ICSI

Received April 14, 2020; revised June 11, 2020; accepted June 12, 2020; published online September 2, 2020.
M.D.C. has nothing to disclose. A.B. has nothing to disclose. D.T. has nothing to disclose. D.P. has nothing to disclose. M.L. has nothing to disclose. E.D.P. has nothing to disclose. C.B. has nothing to disclose. M.M.R. has nothing to disclose. J.B. has nothing to disclose.
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Fertility and Sterility® Vol. 115, No. 3, March 2021 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2020.06.023

The outcomes of in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) critically depend on the accuracy of selecting the best embryo to be transferred, a choice that has been predominantly based on static morphologic criteria. During the past decade, time-lapse technology has permitted a more detailed evaluation of embryo morphokinetics, although its ability to improve IVF/ICSI clinical success remains to be confirmed (1). A substantial improvement in the accuracy of embryo selection has been projected through the integration of genetic, metabolic, and morphokinetic data by means of artificial intelligence systems (2). The clarification of the potential and limits of morphokinetic information is essential to optimizing the performance of time-lapse based strategies, as well as of developing integrative approaches for embryo selection.

Time-lapse microscopy (TLM) has been applied to assess a variety of morphokinetic parameters from fertilization to blastulation and their association with laboratory and clinical outcomes. Faster developmental kinetics has been associated with higher embryo cell number (3), improved blastocyst formation (4–7) and higher implantation (8, 9), and pregnancy rates (10, 11). Importantly, while these studies provided a basis for the development of TLM-based algorithms for embryo selection reported to improve implantation (11, 12) and pregnancy rates (12, 13), the ability of these strategies to improve live birth rates remains to be clarified (14).

To date, very few studies have investigated the association between embryonic kinetics and competence to provide a live birth, which is still a major gap to prove the usefulness of TLM technology in clinical practice (15). Nevertheless, a recent robust study indicates that blastocyst dynamics, as assessed by blastulation start time and duration, has greater power to predict live birth than classic morphology (16). The predictive value of embryonic morphokinetics may vary across distinct embryo developmental stages, because no association between fertilization or cleavage parameters and live birth rates was observed in another recent study in which, however, a limited number of embryos were assessed (17).

The accuracy of TLM-based embryo selection has been questioned, particularly when externally developed algorithms are used (14, 18). The reasons of such limitation appear to derive from differences between morphokinetic parameters from different clinics (19), which in turn are thought to result mostly from variations in culture conditions and patient profile (20). Among the confounding patient-related factors, maternal age has been suggested to be especially relevant (21, 22). However, the influence of maternal age on embryo morphokinetics has not been fully addressed. An initial study comparing 530 embryos from patients younger than 40 years with 86 embryos from patients older than 40 years found no impact of maternal age on cleavage morphokinetics (23). The same conclusion was reached in a more recent study comparing 611 embryos from patients younger than 38 years with 467 embryos from patients older than 42 years (24). In contrast, in a third study assessing 320 embryos from patients with normal ovarian reserve, earlier tPNf, t2, t3, and t4 (see "Evaluation of Time-Lapse Images and Morphokinetic Parameters" in the Materials and Methods section) were reported in patients aged 20–30 years compared with patients aged 30–40 years (25). Importantly, in all of these previous studies, all embryos, regardless their developmental competence, were allocated to different age groups, making it impossible to isolate the impact of maternal age from that of embryonic quality on morphokinetics. Because a higher percentage of low-quality embryos is observed in advanced maternal age (26), one would naturally expect to observe slower embryonic kinetics in those patients. Therefore, whether maternal age does alter developmental morphokinetics of embryos competent to provide a live birth is still an unsolved question. This clarification is fundamental to elucidate the usefulness of morphokinetic parameters provided by TLM technology for embryo selection in patients at different ages.

In the face of the aforementioned gaps to clarify the clinical value and determine the best use of TLM-derived morphokinetic parameters, we tested the hypotheses that: 1) embryos competent to provide a live birth present faster fertilization and cleavage kinetics; and 2) maternal age affects early developmental morphokinetics and its association with achievement of live birth.

MATERIALS AND METHODS

Patients and Experimental Design

This was a retrospective study, conducted at the Biogenesi Reproductive Medicine Center, Monza, Italy, and all procedures and protocols were approved by the local ethical committee. The study included 1,066 patients undergoing fresh embryo transfer (ET) at the end of ICSI cycles using the patients' own oocytes, performed from July 2014 to 2019. Of these 1,066 patients, 937 (88%) contributed with only one cycle, 118 (11%) with two consecutive cycles, and 11 (1%) with three consecutive cycles. Overall, 4,915 embryos were produced, and 2,093 of them were morphologically selected to be transferred on day 2 or 3 in single (SET), double (DET), or triple (TET) transfers. Of these 2,093 embryos transferred, only those with a known live birth outcome [live birth achieved (LB) vs. live birth not achieved (NLB)] that could be paired with their morphokinetic information were used in the analysis assessing the relationship of embryo morphokinetics with live birth achievement and maternal age (n = 1,390). Accordingly, DET and TET resulting in deliveries with a number of babies smaller than the number of embryos transferred were excluded from this analysis. Embryos transferred in SET resulting in singleton births or in DET resulting in twin births were included in the analysis as LB embryos (n = 168). NLB embryos were those transferred in SET, DET, or TET not achieving a live birth, and included embryos failing to implant (negative β-hCG), embryos with a positive β-hCG followed by a negative clinical pregnancy test, and embryos resulting in an abortion (n = 1,222).

To test the hypothesis that faster embryo kinetics is associated with competence to provide a live birth, morphokinetic parameters (described below in "Evaluation of Time-Lapse Images and Morphokinetic Parameters") of LB embryos were compared with those of NLB embryos. To test the hypothesis that maternal age affects embryo developmental kinetics, morphokinetic parameters of embryos at the same outcome category (LB vs. NLB) were compared between

patients aged <37 and ≥37 years. In addition, morphokinetic parameters were compared between embryos from the different outcome categories (LB vs. NLB) within each age group (<37 vs. ≥37 years). The age of 37 years was defined as the cutoff age because, apart from dividing the study population evenly into two age groups, it represents the moment after which there is a marked drop in oocyte and embryo quality (27), as well as in live birth rates, as indicated by a recent study assessing 181,523 patients undergoing IVF with autologous fresh cycles (28). Finally, we investigated the impact of maternal age on the association between early morphokinetic parameters and live birth achievement by univariate and multivariate logistic analyses. In those analyses, we tested the hypothesis that morphokinetic parameters are associated with live birth achievement on an individual basis (univariate analysis), but not independently from maternal age (multivariate analysis).

Ovarian Stimulation

Ovarian stimulation and pituitary down-regulation were induced with the use of rFSH (Puregon; Merck Sharp & Dohme; or Gonal-F; Merck) and GnRH antagonist (Ganirelix; Merck Sharp & Dohme), respectively. Hormone doses were decided and adjusted considering patients' characteristics and treatment response. Oocyte maturation was triggered with the use of 10,000 IU highly purified hCG (Ovitrelle Merck) 36 hours before oocyte pickup, when at least three follicles ≥17–18 mm were first detected by means of ultrasound monitoring.

ICSI and Embryo Culture

ICSI and embryo culture were carried out according to conventional methodology as previously described (29). Briefly, after oocyte collection, cumulus-oocyte complexes were cultured in fertilization medium (Sequential Fert; Origio). Semen samples were prepared with the use of discontinuous gradients (47.5% and 90%) of Sil-Select (Ferti-Pro; Beemen), and spermatozoa were washed and resuspended in IVF Sperm Preparation Medium (Origio). Cumulus cells were removed ~2–3 hours after collection by means of brief exposure to human recombinant hyaluronidase (80 IU/mL; ICSI Cumulase; Origio), followed by mechanical action with the use of denuding plastic pipettes. Immediately after ICSI, microinjected oocytes were transferred to Embryoslide Culture slides (Vitrolife; Göteborg), into 30-μL microdrops of culture medium covered with paraffin oil (Origio). Embryos were cultured in an integrated embryo culture TLM system (EmbryoScope; Vitrolife) (4), in which image acquisition was programmed for every 10 minutes at seven different focal planes for each embryo. All embryos were cultured in the same TLM device at identical culture conditions.

Evaluation of Time-Lapse Images and Morphokinetic Parameters

All embryos participating in this study were assessed by TLM during the entire culture period to determine the exact time, in hours after ICSI, of the developmental events listed below, with the use of the EmbryoViewer image analysis software (Unisense FertiliTech). Cleavage time was considered to be the moment at which cell division was completed and the two originating cells were entirely segregated and invested by their own cytoplasmic membranes. The morphokinetic parameters assessed in the present study were: tPNf, time of pronuclear fading (embryos with asynchronous disappearance of pronuclei were excluded from the analysis); t2, time at which two separate and distinct cells were identified; t3, time at which a 3-blastomere embryo was identified; t4, time at which a 4-blastomere embryo was identified; t5, time at which a 5-blastomere embryo was identified; t8, time at which an 8-blastomere embryo was identified. Morphokinetic parameters tPNf and t2 were annotated for all embryos participating in the study. However, owing to performance of ET before their occurrence, t3, t4, t5, and t8 could not be annotated for all embryos. Annotation of morphokinetic parameters was performed by five senior embryologists each with >5 years of laboratory experience with equivalent participation in terms of work hours in the laboratory routine.

Embryo Scoring, Selection, and Transfer

Embryo morphology was evaluated on day 2 and day 3 with the use of digital images, in accordance with the Istanbul Consensus (ALPHA Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology, 2011), considering number of blastomeres, blastomere symmetry, fragmentation degree (fragments defined as nonnuclear membrane-bound extracellular cytoplasmic structures, and fragmentation degree reflecting the percentage of the total embryo volume occupied by fragments), and presence of multinucleated blastomeres. Embryo selection for transfer was based entirely on static morphologic criteria, except for the exclusion of embryos showing abnormal divisions such as direct unequal cleavage and reverse cleavage.

Embryo transfer strategy was based on maternal age, couple history, number of embryos available, and embryo quality. Fresh single, double, or triple ET were performed 2 or 3 days after fertilization with the use of abdominal ultrasound guidance, in accordance with the American Society for Reproductive Medicine guidelines (Practice Committee of the American Society for Reproductive Medicine, 2013). Because day 2 and day 3 ETs have consistently provided similar results in our clinic, ET day was defined according to the laboratory organizational logistics. Remaining viable embryos were frozen for potential future transfers. Embryo implantation was diagnosed with the use of a β-hCG test 12 days after ET, and clinical pregnancy was diagnosed by an ultrasound examination at the seventh week after ET.

Statistical Analysis

Absolute frequencies and percentages were used to describe categoric items, and mean values and standard deviations were used for continuous characteristics. The chi-square test was used to compare the distribution of infertility causes between patients providing a live birth (LB) or not (NLB). The Wilcoxon sum rank test and the Fisher exact test were used

TABLE 1

Baseline clinical characteristics for all patients, patients providing embryos that were transferred but did not result in a live birth, and patients achieving a live birth.

Characteristic	Total	NLB	LB
No. of patients	1,066	669	134
Maternal age, y	37.70 ± 4.13	38.08 ± 4.30[a]	34.69 ± 3.68[a]
Maternal BMI, kg/m^2	22.56 ± 3.31	22.67 ± 3.47	22.84 ± 3.34
Number of oocytes retrieved	9.13 ± 4.18	8.70 ± 4.14[a]	10.45 ± 4.76[a]
Causes of infertility[b]			
Endometriosis	48 (4.50)	41 (6.13)	5 (3.70)
Polycystic ovarian syndrome	10 (0.94)	6 (0.89)	5 (3.70)
Tubal factor	70 (6.57)	34 (5.08)	7 (5.19)
Reduced ovarian reserve	62 (5.82)	21 (3.14)	12 (8.89)
Male factor	367 (34.42)	254 (37.97)	44 (32.59)
Mixed	201 (18.86)	113 (16.89)	28 (20.74)
Unexplained infertility	308 (28.89)	200 (29.90)	34 (25.19)

Note: Values are presented as mean ± standard deviation or n (%), unless stated otherwise. BMI = body mass index; LB = live birth; NLB = did not result in live birth.
[a] Differences ($P<.0001$) between patients achieving a LB and those producing embryos that were transferred but did not result in a live birth.
[b] Different distribution of infertility causes between NLB and LB ($P<.003$).

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to assess differences between morphokinetic parameters from experimental groups varying in embryo competence or maternal age. Univariate and multivariate logistic analysis was used to test the association of morphokinetic parameters and maternal variables (age <37 or ≥ 37 years and body mass index [BMI]) with live birth. The statistical analysis was performed with the use of Stata Software 9.0 (Stata Corp.), and a level of $P<.05$ was adopted for significance.

RESULTS

Patient Characteristics and General Outcomes

This study includes data from 1,066 patients that underwent single or consecutive ICSI cycles with the use of the patients' own oocytes, performed from July 2014 to 2019. These 1,066 patients produced 4,915 embryos, 2,093 of which were transferred in 1,206 ETs: 404 SETs (33.5% of total ETs, 392 patients, 404 embryos), 642 DETs (53.2% of total ET, 530 patients, 1,284 embryos), and 160 TETs (13.3% of total ETs, 144 patients, 480 embryos). Of these 1,206 transfers, 1,001 (83% of total ETs, 871 patients, 1,730 embryos) were performed on day 3 and 205 (17% of total ET, 195 patients, 363 embryos) on day 2. Overall clinical pregnancy and live birth rates per transfer were 27% (325/1,206) and 21.7% (262/1,206), respectively.

Of 2,093 embryos transferred, 1,390 provided a live birth outcome paired with morphokinetic data and were used to assess the association of fertilization and cleavage morphokinetic parameters with live birth achievement and maternal age. Of these 1,390 embryos with a known live birth outcome, 168 achieved a live birth (LB) and 1,222 did not (NLB). The 168 LB embryos derived from 134 patients, and the 1,222 NLB embryos derived from 669 patients. Double and triple patient representation in the NLB group was due to participation with consecutive cycles (detailed above in "Patients and Experimental Design") as well as to DETs and TETs not resulting in any live birth. On the other hand, double patient representation in the LB group was exclusively due to DETs resulting in twins; 168 LB embryos derived from 134 patients (100 SET and 34 DET). Seven hundred three embryos transferred in DET or TET resulting in a number of babies lower than the number of embryos transferred were excluded from the analysis for not having a known live birth outcome.

Baseline clinical characteristics of all patients included in the study are presented in Table 1. Patients reaching a live birth were younger and yielded a larger number of oocytes, but presented similar BMI compared with those providing only NLB embryos.

Effects of Embryo Competence and Maternal Age on Early Morphokinetics

When all patients were analyzed together regardless of their age, all morphokinetic end points (tPNf, t2, t3, t4, and t8) were reached earlier in LB than in NLB embryos (Table 2). On the other hand, different morphokinetic patterns were observed in embryos from patients grouped according to

TABLE 2

Fertilization and cleavage morphokinetic parameters of all embryos produced, embryos that were transferred and had a birth outcome paired with morphokinetic information, those embryos that achieved a live birth, and those that did not achieve a live birth.

Parameter	Total	Transferred	NLB	LB
n	4,915	1,390	1,222	168
tPNf	24.1 ± 3.6 (n = 4,915)	23.7 ± 3.3 (n = 1,390)	23.9 ± 3.4[a] (n = 1,222)	22.5 ± 2.7[a] (n = 168)
t2	27.0 ± 4.0 (n = 4,915)	26.6 ± 3.7 (n = 1,390)	26.8 ± 3.8[a] (n = 1,222)	25.0 ± 2.7[a] (n = 168)
t3	38.0 ± 5.2 (n = 4,758)	37.4 ± 4.4 (n = 1,350)	37.6 ± 4.5[a] (n = 1,183)	36.1 ± 3.3[a] (n = 167)
t4	39.8 ± 6.1 (n = 4,646)	38.7 ± 4.9 (n = 1,321)	39.0 ± 5.1[a] (n = 1,155)	36.8 ± 3.6[a] (n = 166)
t5	50.6 ± 7.5 (n = 4,224)	49.8 ± 6.2 (n = 1,119)	50.0 ± 6.4[b] (n = 974)	48.8 ± 4.9[b] (n = 145)
t8	57.8 ± 9.6 (n = 3,565)	54.5 ± 6.6 n = (904)	55.0 ± 6.6[c] (n = 769)	52.7 ± 6.1[c] (n = 145)

Note: Values are presented as mean ± standard deviation, unless stated otherwise. Annotation of t3, t4, t5, and t8 was not possible when their occurrence preceded embryo transfer, which explains the decrease in the number of observations from t2 to t8. LB = live birth; NLB = did not result in live birth; tPNf = time of pronuclear fading; t2 = time at which two separate and distinct cells were identified; t3 = time at which a 3-blastomere embryo was identified; t4 = time at which a 4-blastomere embryo was identified; t5 = time at which a 5-blastomere embryo was identified; t8 = time at which an 8-blastomere embryo was identified.
[a,b,c] Differences between LB and NLB embryos: [a] $P<.0001$; [b] $P=.009$; [c] $P=.0002$.

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maternal age; all morphokinetic parameters were achieved earlier in LB than in NLB embryos from patients aged <37 years, although no differences were observed between LB and NLB embryos from patients aged ≥37 years (Table 3). Curves illustrating morphokinetic patterns of LB and NLB embryos from patients aged <37 and ≥37 years are shown in Figure 1. Clearly separated curves were observed for LB and NLB embryos from patients aged <37 years, while nearly coincident curves were observed for patients aged ≥37 years. In addition, except for t8 ($P=.07$), all morphokinetic parameters were reached earlier in LB embryos obtained from patients aged <37 years than in LB embryos obtained from patients aged ≥37 years (Table 3).

Univariate and multivariate logistic analyses were performed to better characterize the impact of age on the association of early morphokinetic parameters with live birth (Supplemental Table 1, available online at www.fertstert.org). All morphokinetic parameters were associated with live birth on an individual basis, with odds ratios (OR) indicating earlier occurrence associated with live birth. On the other hand, in the multivariate analysis, only t2 ($P=.007$) and t5 ($P=.006$) were significantly associated with live birth independently from the influence of maternal age and BMI. Yet, although the odds ratio of t2 (OR 0.6) was consistent with its earlier occurrence in LB compared with NLB embryos (Tables 2 and 3), the same was not observed for t5 (OR 1.1). The same trend for t5 was observed for tPNf, although the P value did not reach the stipulated significance level ($P=.055$; OR 1.4).

DISCUSSION

Time-lapse microscopy has rapidly spread despite lacking evidence that the adoption of morphokinetic markers in embryo selection can indeed improve live birth rates (15, 19). In parallel, the efficacy of TLM-based strategies has been suggested to vary with maternal age (20, 21), although its impact on embryonic morphokinetics has not been sufficiently addressed. Here, we present solid novel findings indicating that faster fertilization and cleavage kinetics are indeed associated with competence to provide a live birth, and that early kinetics of competent embryos slows as maternal age advances. Despite their retrospective nature, the present results derive from a large number of patients and embryos, allowing appropriate and straightforward assessment of our hypotheses, and provide valuable parameters for the improvement and development of embryo selection strategies.

Our data indicate that embryos competent to provide a live birth achieve tPNf, t2, t3, t4, and t8 faster than embryos that do not reach a live birth, which is in agreement with previous studies assessing the relationship between early developmental kinetics and embryo quality estimated by different outcomes. Faster tPNf and t2 were associated with better embryo morphology on day 3 (3), and faster t2 was also reported to reflect blastocyst formation and implantation competence (8, 30). Similarly, earlier t3, t4, and t5 have been associated with higher implantation rates, t4 also reflecting blastocyst quality (8, 30). Moreover, shorter t8

TABLE 3

Morphokinetic parameters of all embryos produced, embryos that were transferred and had a birth outcome paired with morphokinetic information, those embryos that achieved a live birth, and those that did not achieve a live birth in different maternal age groups.

	<37 y				≥37 y			
Parameter	Total (n = 2,378)	Transferred (n = 573)	NLB (n = 448)	LB (n = 125)	Total (n = 2,537)	Transferred (n = 817)	NLB (n = 774)	LB (n = 43)
tPNf	23.9 ± 3.5 (n = 2,378)	23.3 ± 3.2 (n = 573)	23.6 ± 3.3^{a,c} (n = 448)	22.3 ± 2.7^{a,c} (n = 125)	24.2 ± 3.6 (n = 2,537)	24.0 ± 3.3 (n = 817)	24.1 ± 3.4^{c} (n = 774)	23.2 ± 2.4^{c} (n = 43)
t2	26.8 ± 4.0 (n = 2,378)	26.1 ± 3.7 (n = 573)	26.5 ± 3.8^{a,e} (n = 448)	24.8 ± 2.8^{a,c} (n = 125)	27.1 ± 4.0 (n = 2,537)	26.8 ± 3.6 (n = 817)	26.9 ± 3.7^{e} (n = 774)	25.7 ± 2.5^{c} (n = 43)
t3	37.9 ± 5.3 (n = 2,322)	37.1 ± 4.4 (n = 564)	37.4 ± 4.6^{b} (n = 440)	35.7 ± 3.1^{a,d} (n = 124)	38.2 ± 5.2 (n = 2,436)	37.6 ± 4.4 (n = 786)	37.7 ± 4.4 (n = 743)	37.4 ± 3.4^{d} (n = 43)
t4	39.6 ± 6.2 (n = 2,270)	38.2 ± 4.9 (n = 554)	38.8 ± 5.2^{a} (n = 431)	36.4 ± 3.5^{a,d} (n = 123)	39.9 ± 6.1 (n = 2,376)	39.1 ± 4.9 (n = 767)	39.2 ± 5.0 (n = 724)	37.9 ± 3.6^{d} (n = 43)
t5	50.3 ± 7.5 (n = 2,126)	49.2 ± 6.0 (n = 495)	49.6 ± 6.3^{b,c} (n = 386)	48.1 ± 4.7^{b,d} (n = 109)	50.8 ± 7.4 (n = 2,098)	50.3 ± 6.3 (n = 624)	50.3 ± 6.4^{e} (n = 588)	50.8 ± 5.0^{d} (n = 36)
t8	57.6 ± 9.7 (n = 1,846)	54.0 ± 6.6 (n = 423)	54.6 ± 6.8^{b} (n = 321)	52.2 ± 6.0^{b,f} (n = 102)	57.9 ± 9.6 (n = 1,719)	55.2 ± 6.5 (n = 481)	55.3 ± 6.5 (n = 448)	54.2 ± 6.2^{f} (n = 33)

Note: Values are presented as mean ± standard deviation, unless stated otherwise. Annotations of t3, t4, t5, and t8 were not possible when their occurrence preceded embryo transfer, which explains the decrease in the number of observations from t2 to t8. LB = live birth; NLB = did not result in live birth; tPNf = time of pronuclear fading; t2 = time at which two separate and distinct cells were identified; t3 = time at which a 3-blastomere embryo was identified; t4 = time at which a 4-blastomere embryo was identified; t5 = time at which a 5-blastomere embryo was identified; t8 = time at which an 8-blastomere embryo was identified.

a,b"Differences between NLB and LB embryos from patients aged <37 years ($^{a}P<.0001$; $^{b}P<.015$). No significant difference was observed between NBL and LB embryos from patients aged ≥37 years ($P>.1$).

c,e Differences between embryos in equivalent outcome groups from different maternal ages; $^{c}P<.03$; $^{e}P\leq.01$; $^{d}P>.05$; $^{f}P\leq.07$.

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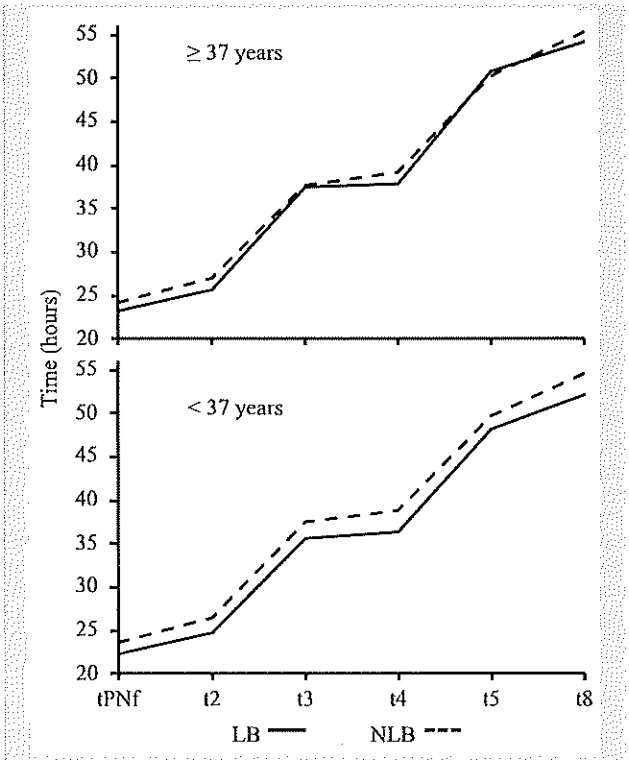
FIGURE 1



Graphical representation of the effect of maternal age on early developmental kinetics of embryos providing a live birth (LB) and embryos that did not reach a live birth (NLB).

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intervals have been associated with improved blastocyst formation and ability to implant (4, 31). To our knowledge, the present study is the first to report an association between fertilization and cleavage kinetics with competence to provide a live birth. The only previous study linking embryonic developmental kinetics and live birth potential focused on blastulation dynamics, suggesting that it constitutes a valuable parameter for later embryo selection and transfer (16).

Maternal age has long been known to inversely reflect IVF success (32, 33), which has mostly been attributed to increasing rates of aneuploidy, but also linked to compromised early embryonic development (26, 34, 35). Variations in maternal age have been suggested as a potential cause of inconsistent results achieved with TLM-based strategies in different clinical settings (21, 22). However, the clarification of the influence of maternal age on embryo morphokinetics has been troubled by experimental designs using different age cutoffs and, most importantly, grouping embryos in accordance with maternal age but regardless of their developmental competence. Discrepant data have been reported with this type of design; two studies using age cutoffs ~38–42 years suggested that maternal age does not affect cleavage kinetics (23, 24), whereas another study, comparing patients aged 20–30 years with patients aged 30–40 years, suggested that maternal age delays tPNf and cleavage kinetics until the 4-blastomere stage (25). In the present study, by assessing the impact of maternal age on developmental kinetics of embryos with the same competence status, we could demonstrate that this maternal variable is indeed associated with slower fertilization and cleavage kinetics of embryos competent to provide a live birth. In addition, morphokinetic markers encompassing fertilization and cleavage were only significantly different between LB and NLB embryos in patients younger than 37 years. This is reflected by nearly coincident curves of early developmental kinetics for LB and NLB embryos from patients ≥37 years, whereas well separated curves were observed for LB and NLB embryos from patients <37 years.

The impact of age on early embryonic morphokinetics was also reinforced by a multivariate analysis, in which t2 was the only parameter significantly associated with live birth independently from age, while providing an OR compatible with its earlier occurrence in LB embryos as indicated by the rest of our data. Interestingly, although the multivariate analysis also indicated t5 to be associated with live birth, the OR indicated slightly later occurrence associated with live birth, in disagreement with the rest of our data and previous literature (8). This discrepancy likely reflects the strong influence of maternal age on live birth, as well as distinct kinetic patterns of LB and NLB embryos from patients at different age groups in the multivariate analysis. Therefore, this finding reinforces the rest of our data to indicate that maternal age does affect developmental kinetics, altering its relationship with competence to reach a live birth.

We acknowledge that our study is limited by its retrospective nature and by the use of data generated in a single IVF center. We also recognize that variation in the number of embryos transferred, unequal patient representation in the database, and potential intra- and interoperator variation in annotation may have influenced our results. On the other hand, the assessment of a robust number of embryos with a known live birth outcome is an important strength of our study, having allowed us to properly evaluate the impact of maternal age on embryo morphokinetics for the first time.

In conclusion, this study presents novel evidence that competence to provide a live birth is associated with faster fertilization and cleavage kinetics in a maternal age-dependent manner. More specifically, the present data indicate that the relationship of early developmental kinetics with embryo competence fades with maternal age. Therefore, early morphokinetic markers may represent a valuable reference for embryo selection in younger IVF/ICSI patients, whereas their clinical relevance appears to be very limited in advanced maternal age. While contributing for a better characterization of developmental kinetic patterns linked with embryo competence, this study provides important references for the improvement and adaptation of embryo selection strategies taking into account maternal age.

Acknowledgments: The authors thank Dr. Rita Vassena for critical reading and suggestions; and Dr. Thaisy T. Dellaqua for assistance during manuscript preparation.

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La fecundación y cinética de división más rápidas reflejan la capacidad para lograr un recién nacido vivo tras la inyección intracitoplasmática de espermatozoides, pero esta asociación se desvanece con la edad materna.

Objetivo: Evaluar la relación de la cinética del desarrollo temprano con la capacidad para generar un recién nacido vivo y el impacto de la edad materna en este contexto.

Diseño: Estudio de cohorte retrospectivo que incluyó 4.915 embriones, de los cuales 1.390 fueron transferidos y generaron un resultado clínico, emparejado con los datos morfocinéticos; 168 de ellos resultaron en un recién nacido vivo (LB) y 1,222 no (NLB). Se compararon los parámetros morfocinéticos tempranos entre embriones LB y NLB de pacientes estratificados en dos grupos de edad (<37 y ≥37 años), y entre embriones en el mismo grupo de competencia de pacientes de <37 y ≥37 años. Se probó la asociación de los parámetros morfocinéticos con los recién nacidos vivos mediante análisis univariado y multivariado.

Lugar: Clínica de fertilidad.

Paciente(s): La población de estudio incluyó a 1.066 pacientes que realizaron un ciclo de inyección intracitoplasmática de espermatozoides autólogos con transferencia en fresco de un embrión (SET), dos (DET) o tres (TET) en el día 2 o 3 de desarrollo. De ellos, 669 pacientes produjeron embriones NLB y 134 produjeron embriones LB.

Intervención(es): Ninguna.

Medida(s) del resultado principal: Parámetros morfocinéticos de fecundación y división y nacidos vivos.

Resultado(s): En la población total de pacientes, todos los parámetros morfocinéticos se alcanzaron antes en los embriones LB en comparación con los NLB. Se observó lo mismo en pacientes <37 años ($p < 0.015$), pero no en las ≥37 años. A excepción del t8 (momento en el que se identificó un embrión de 8 blastómeros), todos los parámetros morfocinéticos se alcanzaron antes en los embriones LB de pacientes <37 años en comparación con los embriones LB de pacientes ≥37 años. El análisis univariante reveló que la aparición más temprana de todos los parámetros morfocinéticos se asoció con el recién nacido vivo, aunque solo el t2 temprano (momento en el que se identificaron dos células separadas y distintas) se asoció con el recién nacido vivo independientemente de la edad materna en el análisis multivariado.

Conclusión(es): A pesar de su naturaleza retrospectiva y de su realización en un solo centro de FIV, este estudio presenta datos novedosos que indican que los embriones capaces de proporcionar un nacimiento vivo muestran una cinética de desarrollo temprana, en general más rápida, en comparación con los embriones que no logran un nacimiento vivo tras la transferencia. Una diferencia que, sin embargo, se estrecha a medida que avanza la edad materna. Los hallazgos sugieren que los parámetros morfocinéticos de fecundación y división pueden constituir referencias valiosas para las estrategias de selección de embriones con el objetivo de mejorar las tasas de nacidos vivos, específicamente antes de la edad materna avanzada, mientras que tienen una utilidad limitada en la edad materna avanzada.