Influence of different oocyte insemination techniques on early and late morphokinetic parameters: retrospective analysis of 500 time-lapse monitored blastocysts

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Objective: To determine how standard IVF vs. intracytoplasmic sperm injection (ICSI) fertilization influences early and late morphokinetic parameters during prolonged embryo culture.
Design: Five-hundred expanded blastocysts that were monitored in a time-lapse monitoring incubator were analysed retrospectively. Early (pronuclear fading [PNf], t2–t9) and late (start of blastulation, expanded blastocyst) morphokinetic variables were scored according to published consensus criteria.
Setting: Private infertility clinic.
Patient(s): A total of 209 consecutive infertile patients (mean ± SD age, 38.4 ± 4 years; range, 28–47 years) undergoing 238 natural IVF/minimal ovarian stimulation cycles during 2012–2014.
Intervention(s): Minimal ovarian stimulation, oocyte retrieval, fertilization with standard IVF or ICSI, prolonged embryo culture in a time-lapse monitoring incubator.
Main Outcome Measure(s): Differences in morphokinetic parameters according to insemination techniques.
Result(s): In total, 29% and 71% of the whole cohort was fertilized with standard IVF and ICSI, respectively. During early cleavage stages (PNf to t4) there was a statistically significant delay (+1.5 to +1.1 hours) among IVF-fertilized embryos. By contrast, at the expanded blastocyst stage IVF-fertilized embryos showed faster development (−3.3 to −4.1 hours). After normalizing to the time point of PNf, differences in cleavage-stage parameters disappeared, but those at all blastocyst stages increased even further in favor of IVF-fertilized embryos (−3.2 to −5.7 hours).
Conclusion(s): The observed 1.5-hour time difference between standard IVF- and ICSI-fertilized embryos is an artificial phenomenon. At the blastocyst stages, however, genuine timing differences arise between IVF- and ICSI-fertilized embryos, possibly related to their different quality. Normalization to a common time point permits the joint analysis of IVF- and ICSI-fertilized embryos, thus increasing the size of studied cohorts. (Fertil Steril® 2015;104: 1175–81. ©2015 by American Society for Reproductive Medicine.)
Key Words: Time-lapse monitoring, blastocyst culture, ICSI, single-embryo transfer, in vitro fertilization

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In recent years time-lapse monitoring (TLM) of human embryos during IVF treatment has emerged as a promising, noninvasive tool to enhance classic morphology-based embryo selection and possibly even improve overall clinical outcome (1–3). Sophisticated incubators with built-in cameras and dedicated software were developed, allowing the

accurate measurement of numerous morphokinetic variables, proposed to be used in models that aimed at predicting blastocyst formation, aneuploidy status, and the chance of implantation (4–6). Time-lapse variables, however, are also affected by a number of external factors, including laboratory and culture conditions (fertilization method, oxygen concentration, culture media) as well as patient- (obesity, smoking) and treatment-related characteristics (gonadotropin dose, stimulation protocol) (7–14).

Among these factors the influence of different oocyte insemination techniques (standard IVF vs. intracytoplasmic sperm injection [ICSI]) has only been investigated by a handful of studies to date, yielding somewhat contradictory results (8, 9). Although it was already known that by circumventing early fertilization events ICSI zygotes developed faster, from existing time-lapse studies it was unclear whether this difference in timing persists beyond the early cleavage stages. This has considerable implications if mixed cohorts of IVF-/ICSI-fertilized embryos were to be analyzed together. On the other hand most TLM predictive models published to date, by design, involved ICSI-fertilized embryos exclusively, and no models have been developed to date for IVF-fertilized or mixed cohorts (4–6).

Recently it was demonstrated that available published TLM models are largely center-specific and could not be easily transferred to different settings (15, 16). As a consequence, each IVF unit is encouraged to develop its own predictive model based on its own data, which reflect its local laboratory practices, including the proportion of ICSI- and IVF-fertilized treatment cycles. According to the most recently published registry data from the United States and Europe, the ICSI procedure was performed in approximately two-thirds of all treatment cycles (almost identical in both regions at 67%–68%) (17, 18). In Europe, however, where country-specific data were also available, there was a large variation among countries in the proportion of ICSI cycles, ranging from 21% to 96% (17).

The aim of the present retrospective analysis was to determine how standard IVF vs. ICSI fertilization affected early and late morphokinetic parameters during each step of the prolonged embryo culture in a large sample of time-lapse monitored blastocysts. Furthermore, we have also tested whether standardizing to a common time point after fertilization could diminish the observed differences and thus permit the joint analysis of mixed embryo cohorts.

MATERIALS AND METHODS

Study Patients

All consecutive infertile patients who underwent treatment between October 2012 (the acquisition of a single TLM incubator) and December 2014 at our center (Kobe Motomachi Yume Clinic, Kobe, Japan) were eligible for inclusion in this retrospective analysis. Inclusion criteria were as follows: [1] fertilized oocytes that underwent prolonged embryo culture in a time-lapse incubator; [2] embryos that had developed into expanded blastocysts and were electively vitrified (or transferred in fresh state). Institutional review board approval was not required for the present study because in our center all patients undergoing IVF treatment were informed about and gave consent to the anonymous use of their data for retrospective analyses. A flowchart depicts the number of gametes/embryos in the IVF- and ICSI-fertilized groups from oocyte fertilization up until the expanded blastocyst stage (Fig. 1).

Natural Cycle IVF and Minimal Ovarian Stimulation Protocols

Clomiphene citrate (Serofene, Merck Serono) or letrozole (Femara, Novartis) based minimal stimulation was used in the majority of cycles, whereas unstimulated natural cycle IVF or other mild stimulation protocols using low-dose hMG (Ferring) represented only a smaller proportion of cases. Details of the clomiphene citrate–based minimal stimulation and natural cycle IVF protocols were described previously (19, 20). Patients were not selected according to their age, and this treatment option was offered over a wide age range.

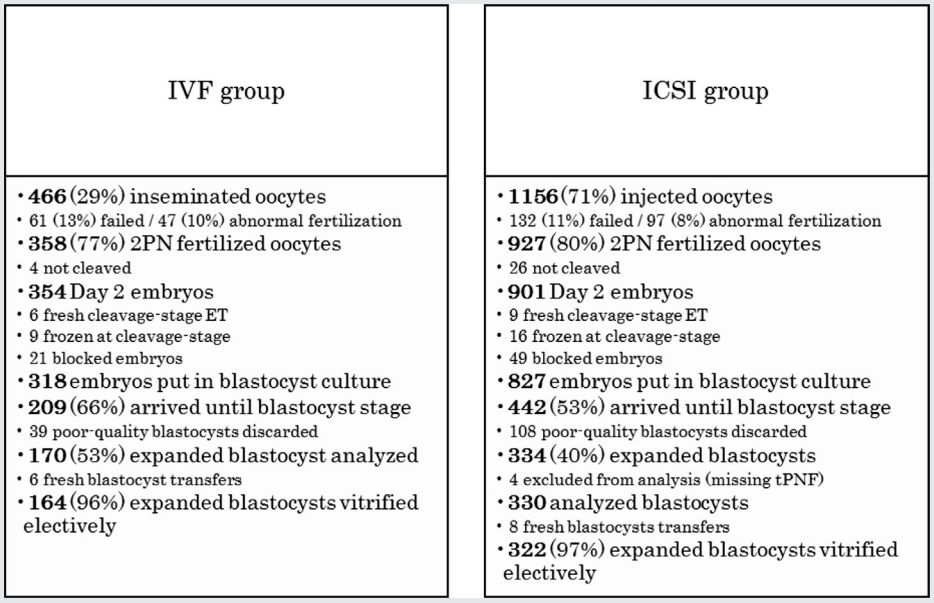
Oocyte Retrieval and Fertilization with Conventional IVF or ICSI

Transvaginal ultrasound–guided oocyte retrieval was performed without anesthesia using a very thin 21–22 G needle (Kitazato Medical). Mature (metaphase II) oocytes were inseminated by conventional IVF or injected by ICSI. Subsequently injected oocytes were immediately placed in pre-equilibrated slides (EmbryoSlide, Unisense Fertilitech), whereas inseminated oocytes were first cultured at 7.3 pH, and 5% O_2, 5.5 %CO_2 atmosphere in a conventional, tri-gas, water-jacket incubator (Astec) in Quinn's Advantage Fertilization Medium (Sage) and transferred to a TLM incubator next morning if fertilization was confirmed.

Prolonged Embryo Culture in a TLM Incubator, Elective Vitrification, and ET

In our center elective blastocyst culture, vitrification, and subsequent vitrified–warmed single blastocyst transfer are routinely practiced in cases with previous failed cleavage-stage embryo transfer cycles (19). Prolonged embryo culture was performed in a time-lapse incubator (EmbryoScope, Unisense Fertilitech) according to previously described methodology (4). Briefly, normally fertilized two pronuclei zygotes were incubated at 37°C and 7.2 pH in a 5% O_2, 5.5% CO_2 atmosphere and cultured individually until day 2–3 in Quinn's Advantage Cleavage Medium and subsequently until blastocyst stage to day 5–7 in Quinn's Advantage Blastocyst Medium. During cleavage stage, morphologic selection was performed by classic criteria according to consensus criteria (21). Embryos that reached the blastocyst stage by day 5 or 6 were eligible for elective vitrification as soon as they expanded to a size of at least 160 μm. Expanded blastocysts were graded into five categories (A–E), depending on female age and time to reach blastocyst expansion as per previously published criteria of our group (22). Almost all expanded blastocysts were electively vitrified using the Cryotop vitrification method (Kitazato Medical) as described previously (23, 24).

FIGURE 1



Flow chart of study events.

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Subsequently, vitrified–warmed blastocyst transfers were performed 1–3 months later in a spontaneous natural or hormonal replacement cycle. In natural cycles, blastocysts were transferred on day 5 after ovulation was confirmed. In hormonal replacement cycles, transdermal E_2 patches were started from cycle day 2, and duphaston was added from cycle day 11, after which blastocysts were transferred 7 days later (19). All ET procedures were performed under vaginal ultrasound guidance using a specially designed soft catheter (Kitazato) by placing a single embryo in minimal volume to the mid-uterine cavity (25).

Time-lapse Annotations

Seven plane focal images were taken and recorded every 20 minutes shortly after insemination up until a period of approximately 160 hours. Early (pronuclear fading [PNf], t2–t9) and late (start of blastulation and full blastocyst) morphokinetic time points were scored in accordance with recently published consensus criteria (26). Time to morula formation was not included in the analysis owing to the considerable subjectivity of evaluating full compaction. To determine the degree of blastocyst expansion more objectively, two additional TLM variables (texpB_1 and $_2$) were also introduced. These were the time points when the horizontal inner diameter of the expanded blastocyst reached 130 and 160 μm, respectively. Annotations were performed using EmbryoViewer by two experienced embryologists (J.Y.S. and T.S.) and rechecked by a third person (D.B.). Those blastocysts in which timing of PNf was missing were excluded from the analysis (n = 4).

Outcome Measures and Statistical Analysis

Main outcome measures were differences in standardized and nonstandardized morphokinetic parameters at cleavage and blastocyst stage according to the oocyte insemination technique used. Metric variables were compared by the Mann–Whitney *U* test owing to a nonnormal distribution. Nominal variables were analyzed by the χ^2 test. $P<.05$ was considered statistically significant.

RESULTS

Baseline Characteristics of the IVF- and ICSI-fertilized Groups

Altogether 1,622 oocytes were inseminated or injected; 1,285 (79%) of them fertilized normally, and 1,145 underwent prolonged embryo culture (Fig. 1). There were no differences in fertilization rates between the IVF and ICSI groups (77% vs. 80%, $P=.14$). After prolonged embryo culture in a time-lapse incubator a total of 500 expanded blastocysts were analyzed. Only 14 of them were transferred in a fresh state, whereas the majority was vitrified electively (97%). During the study period 231 vitrified blastocysts were thawed (3% did not survive thawing), resulting 224 vitrified–thawed blastocyst transfers (Fig. 1).

The cohort of analyzed blastocysts originated from 209 consecutive infertile patients (female age 38.4 ± 4 years [mean ± SD]; range, 28–47 years) who underwent 238 natural IVF or minimal ovarian stimulation cycles. Baseline cycle characteristics according to the method of fertilization used are summarized in Table 1. Female patients undergoing IVF-fertilized cycles were slightly younger (37.1 ± 3.7 vs.

TABLE 1

Baseline characteristics in IVF- and ICSI-fertilized groups.

Treatment cycle	IVF (n = 80)	ICSI (n = 158)	*P* value
Female age (y)	37.1 ± 3.7	39 ± 4	.0008[a,c]
BMI, (kg/m^2)	20.2 ± 2.1	20.6 ± 2	.38[a]
Basal FSH (IU/mL)	8.9 ± 4.1	9.8 ± 5.7	.26[a]
Primary infertility	53 (66)	99 (63)	.58[b]
Nulliparous	77 (96)	139 (88)	.04[b]
Duration of marriage (y)	5.9 ± 2.8	7 ± 4	.11[a]
No IVF treatment (at other centers)	41 (51)	46 (29)	.0008[b,c]
Current cycle rank			.43[b]
1–2 cycle	19 (24)	43 (27)	
3–4 cycle	31 (39)	48 (30)	
5 or higher	30 (37)	67 (42)	
Stimulation type			.53[b]
Natural cycle	11 (14)	21 (13)	
Clomiphene	56 (70)	102 (65)	
Letrozole	13 (16)	32 (20)	
Other	0 (0)	3 (2)	
Retrieved eggs (n)	5.4 ± 3.3	4.8 ± 3.4	.12[a]
Male partner age (y)	38 ± 4.8	41 ± 5.1	<.0001[a,c]
OAT	6 (8)	68 (43)	<.0001[b,c]
Total mobile sperm count (×10^6/mL)	156 ± 126	67 ± 86	<.0001[a,c]

Note: Values are mean ± SD or number (percentage). ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; OAT = oligoasthenoteratozoospermia (according to World Health Organization 2010 criteria).
[a] Mann–Whitney *U* test.
[b] χ^2 test.
[c] Statistically significant.

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39 ± 4 years, *P*=.0008), and more of them were nulliparous (96% and 88%, *P*=.04) and without any previous IVF treatments in other centers (51% and 29%, *P*=.0008). Their male partners were approximately 3 years younger (38 ± 4.8 vs. 41 ± 5.1 years, *P*<.0001), with a lower proportion of oligoasthenoteratozoospermia (8% vs. 43%, *P*<.0001) and higher total motile sperm counts (156×10^6/mL ± 126×10^6/mL vs. 67×10^6/mL ± 86×10^6/mL, *P*<.0001).

Blastocyst Development Rates and Embryo Quality among IVF- and ICSI-fertilized Groups

The proportion of cultured embryos that reached blastocyst stage was significantly higher in the IVF-fertilized group (66% vs. 53%, *P*=.0002). Similarly, there were more expanded blastocysts among IVF-fertilized embryos (53% vs. 40%, *P*<.0001). Furthermore, according to the blastocyst grading system used by our group (22), there was a higher proportion of good-quality blastocysts in the IVF-fertilized group compared with their ICSI-fertilized counterparts (*P*=.0007) (Supplemental Fig. 1, available online).

Comparison of Morphokinetic Variables among IVF- and ICSI-fertilized Groups

When examining early cleavage-stages parameters (PNf to t4) there was a statistically significant delay for IVF-fertilized embryos (on average between +1.5 to +1.1 hours). In contrast, the average differences (between −1.2 to +0.9 hours) were not significantly different for late cleavage stage (t5 to t9) and interval parameters (cc2a/b and s2/3). By contrast, when comparing blastocyst TLM parameters, it was IVF-fertilized embryos that showed faster development, which gradually became more pronounced and statistically significant at the expanded blastocyst stage (on average between −3.3 to −4.1 hours for texpB$_1$ and $_2$) (Table 2). After normalizing all morphokinetic variables to the time point of PNf, the previously observed differences in cleavage-stage

TABLE 2

Morphokinetic variables among IVF- and ICSI-fertilized groups.

Nonstandardized TLM variables[a]	IVF fertilization (n = 170)	ICSI fertilization (n = 330)	Δ mean IVF-ICSI	*P* value[a]
tPNf	24.1 ± 3.4	22.6 ± 2.9	+1.5	<.0001[b]
t2	26.7 ± 3.4	25.3 ± 3.1	+1.4	<.0001[b]
t3	37.7 ± 4.5	36.4 ± 4.1	+1.3	.005[b]
t4	38.9 ± 4.4	37.8 ± 4.6	+1.1	.006[b]
t5	51.7 ± 6.9	50.7 ± 7.0	+1	.17
t6	54.0 ± 6.4	53.4 ± 7.2	+0.6	.27
t7	56.6 ± 7.9	55.4 ± 7.9	−1.2	.10
t8	59.1 ± 9.1	58.8 ± 9.4	+0.6	.66
t9	73.5 ± 9.5	72.6 ± 10.0	+0.9	.50
cc2a (t3-t2)	11.3 ± 2.1	11.1 ± 2.1	+0.2	.87
cc2b (t4-t2)	12.3 ± 2.3	12.3 ± 2.4	0	.60
s2 (t4-t3)	1.12 ± 2.2	1.41 ± 3.0	−0.3	.16
s3 (t8-t5)	7.7 ± 8.3	9.4 ± 10.1	−1.7	.15
tSB	102.2 ± 9.4	104 ± 10.5	−1.8	.10
tfB	112.7 ± 10.5	114.5 ± 13.0	−1.8	.21
texpB$_1$	112.7 ± 9.5	116 ± 12.6	−3.3	.013[b]
texpB$_2$	121.9 ± 11.6	126 ± 13.1	−4.1	.0009[b]

Note: Values are expressed as mean ± SD, in hours. ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; TLM = time-lapse monitoring; tPNf = time to pronuclear fading; t2–t9 = time to two to nine discrete cells; cc2a/cc2b/s2/s3 = duration of different embryo cell cycle events; tSB = time to initiation of blastulation; tfB = time to full blastocyst; texpB$_1$ = time to reach expanded blastocyst size of 130 μm; texpB$_2$ = time to reach expanded blastocyst size of 160 μm.
[a] Mann–Whitney *U* test.
[b] Statistically significant.

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parameters practically disappeared, but those at all blastocyst stages increased even further in favor of IVF-fertilized embryos (between −3.2 and −5.7 hours), reaching high statistical significance (Table 3).

Supplemental Figure 2 depicts the average values of TLM parameters before and after standardization in the IVF- and ICSI-fertilized groups. It can be readily appreciated that during cleavage stages in the nonstandardized, original dataset the IVF curve is slightly above the ICSI curve (+1.5 hours), whereas after standardization both curves become completely superimposed. During blastocyst stages, however, the IVF and ICSI curves are diverging increasingly, which was most pronounced in the standardized dataset.

DISCUSSION

Our study involving a large of number time-lapse monitored blastocysts has confirmed that during early cleavage stages an approximately 1.5-hour lag could be observed in zygotes fertilized with standard IVF. If all morphokinetic parameters were adjusted to the time of PNf these differences vanished, aligning all embryos to a common starting point irrespective of their fertilization method. By contrast, at the blastocyst stage there was an increasing developmental advance favoring standard IVF-fertilized embryos, more pronounced when standardized parameters were used.

Before the advent of the modern time-lapse era it was already known that cycle dynamics of human embryos vary according to the time of oocyte fertilization. Although it was based only on observations performed every 2 hours, it was recognized that injected oocytes usually undergo pronucleus development and first cleavage division approximately 2–4 hours earlier than zygotes derived from standard IVF (27, 28). This time difference comes from the fact that initial fertilization events, such as sperm penetration of the cumulus cells, interaction with the zona pellucida, and sperm–oocyte fusion, are simply bypassed by the ICSI procedure.

To date only a few time-lapse studies have examined the relationship between morphokinetic parameters and different oocyte insemination techniques. An early time-lapse study by Lemmen et al. (29), using in-house-developed equipment, examined the timing and coordination of early development, by monitoring 102 fertilized oocytes during 20–24 hours, and found that ICSI-fertilized four-cell embryos spent a significantly shorter period as two-cell compared with IVF-fertilized ones. A more comprehensive investigation by Dal Canto et al. (9) involved a total sample of 151 standard IVF- or ICSI-fertilized zygotes that successfully developed until the blastocyst stage. Although the study's main objective was to predict blastocyst development and implantation, as a collateral finding in their mixed cohort the authors also noticed a significant variation in TLM variables according to the insemination technique used. A statistically significant delay was seen in early cleavage times among standard IVF-fertilized zygotes (+1.4 and +1.1 for t2 and t3, respectively), which disappeared by the six-to eight-cell stage. This prompted the authors to analyze the entire, mixed cohort together without any adjustment. The authors hypothesized that the observed gradual alignment suggested that development to the blastocyst stage is guided by precise temporal checkpoints. Unfortunately, no data were presented on any timing differences in blastocyst-stage parameters, and the study's findings could also have been affected by its relatively small sample size.

A subsequent, larger, single-center, retrospective study by a Spanish group—which has pioneered TLM research—involved 1,203 cleavage-stage embryos and a subgroup of 650 blastocysts in patients undergoing oocyte donation exclusively (8). In line with our study, Cruz et al. also found that embryo development after ICSI was slightly faster than after conventional IVF, observing significant differences both for early and late cleavage-stage parameters (for t2 and t3 but also for t5, t7, and t9), ranging between +1.2 and +1.5 hours. When PNf rather than the time of insemination was established as t0, the differences in means were reduced for all parameters (ranging between 0 to +1.6 hours

TABLE 3

Morphokinetic variables among IVF- and ICSI-fertilized groups (standardized to PNf).

Standardized TLM variables	IVF fertilization (n = 170)	ICSI fertilization (n = 330)	Δ mean IVF-ICSI	*P* value[a]
t2	2.7 ± 0.6	2.8 ± 0.9	−0.1	.08
t3	13.8 ± 2.1	13.8 ± 2.2	0	.48
t4	14.9 ± 2.3	15.2 ± 2.9	−0.3	.57
t5	27.7 ± 5.2	28.1 ± 5.8	−0.4	.31
t6	30.0 ± 4.2	30.8 ± 5.9	−0.8	.20
t7	32.5 ± 6.2	32.7 ± 6.6	−0.2	.83
t8	35.1 ± 7.5	36.2 ± 8.5	−0.8	.31
t9	49.7 ± 8.0	50.1 ± 8.8	−0.4	.51
tSB	78.2 ± 8.5	81.4 ± 10.1	−3.2	.0007[b]
tfB	88.7 ± 9.8	92.0 ± 12.5	−3.3	.007[b]
texpB_1	88.7 ± 8.6	93.4 ± 11.9	−4.7	<.0001[b]
texpB_2	97.8 ± 10.9	103.5 ± 12.5	−5.7	<.0001[b]

Note: Values are expressed as mean ± SD, in hours. Abbreviations and variables defined in Table 2 footnote.
[a] Mann–Whitney *U* test.
[b] Statistically significant.

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and becoming nonsignificant). In contradiction to our investigation this Spanish study did not observe any significant difference in (both standardized and nonstandardized) blastocyst-stage parameters of ICSI- vs. IVF-fertilized embryos, even if this might be simply related to notable differences in the two studies' patient populations, such as female/male age, oocyte source, stimulation protocols used, and the presence of male factor. Interestingly, however, after adjustment it was similarly IVF-fertilized blastocysts that consistently displayed faster development in the Spanish study (between −1 to −1.1 hours, although not significant), and they have also shown a higher trend toward optimal morphokinetic categories compared with their ICSI counterparts.

All time-lapse monitored blastocysts in our study originated from mildly stimulated or natural-cycle IVF treatments. It is unknown whether this fact could have influenced our findings in comparison with the existing studies in which all embryos came from conventional ovarian stimulation cycles. Although a few studies have already compared the effect of different stimulation protocols on time-lapse kinetics, to our knowledge none of them compared the potential differences between cohorts derived from mild or conventional ovarian stimulation (10, 11).

Our findings support the general recommendation that if mixed, IVF/ICSI-fertilized cohorts were to be analyzed together it is necessary to adjust all static (referring to a single time point) morphokinetic variables to the time point of PNf, otherwise timings for IVF-fertilized zygotes would be imprecise, affecting the validity of any derived model. By contrast, it is not necessary to adjust any interval parameters (such as cc2 a/b, cc3, and s2/3) because they are already calculated ones, expressing the duration of different cell cycle events. Adjusting allows the joint analysis of mixed embryo cohorts, increasing the available sample size, which is crucial if predictive models are to be developed for less frequently used but clinically more relevant outcome variables, such as live birth. Already-published models that were developed for ICSI-fertilized cohorts could not be directly compared with models that use PNf-adjusted morphokinetic variables, but such a comparison probably would be of limited importance because it has already been demonstrated that existing models cannot be easily transferred between different centers (15, 16).

Normalization could be done to any morphokinetic parameter of the first cell cycle, but the time point of pronuclear breakdown is the most suitable one because it happens "abruptly" (usually can be observed no longer than for a single frame only) and is also easily discernable even by observers with different levels of annotation experience. According to the only study that investigated the inter- and intraobserver consistency of time-lapse annotations, PNf showed the highest degree of almost perfect correlation, with an inter-intraclass correlation coefficient: 0.999 and intra-intraclass correlation coefficient: 1.0 (30).

Interestingly, our study also found that at the blastocyst stage there were gradually increasing timing differences in favor of IVF-fertilized embryos. These were already present in the nonadjusted variables but became even more pronounced after adjusting, reaching a considerable −5.7-hour difference at the expanded blastocyst stage. We also observed a higher proportion of optimal-quality embryos among IVF-fertilized ones, suggesting that differences in morphokinetics might be related to differences in blastocyst quality. We did not evaluate differences in reproductive outcome between the IVF- and ICSI-fertilized groups because only a part of the analyzed blastocysts was transferred.

The study's main strength is the large sample of 500 embryos that were time-lapse monitored throughout and fully annotated from fertilization until the expanded blastocyst stage. On the other hand the main limitation of our study is its retrospective nature, which could have led to some inherent biases between the standard IVF- and ICSI-fertilized groups. There were significant differences between the groups for some confounders, such as female/male age, parity, or previous infertility treatment, and by definition there was a higher proportion of male factor infertility among ICSI-fertilized cases. The only way to avoid this would have been to randomize prospective patients to different insemination techniques, which is contrary to usual clinical practice by exposing some patients' gametes to an unnecessary procedure. As a slight difference compared with their ICSI-fertilized counterparts, during a short period of time IVF-inseminated oocytes were incubated in a conventional incubator (although in the same culture medium and O_2 tension as in the Embryoscope). This was in line with all previous TLM studies involving IVF-fertilized embryos.

In conclusion, the observed 1.5-hour time difference between IVF- and ICSI-fertilized embryos during early cleavage stages is an artificial phenomenon. However, at the blastocyst stage genuine differences arise between IVF- and ICSI-fertilized embryos, possibly related to their different intrinsic quality. Normalization to a common time point of PNf permits the joint analysis of IVF- and ICSI-fertilized embryos, thus increasing the size of studied cohorts.

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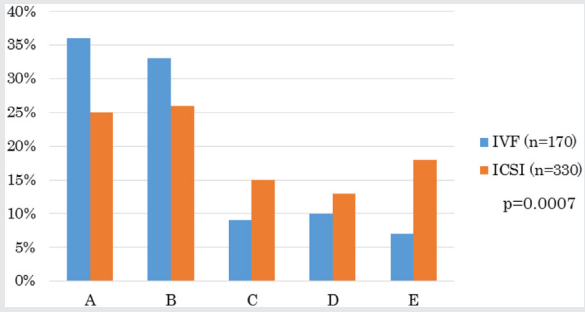
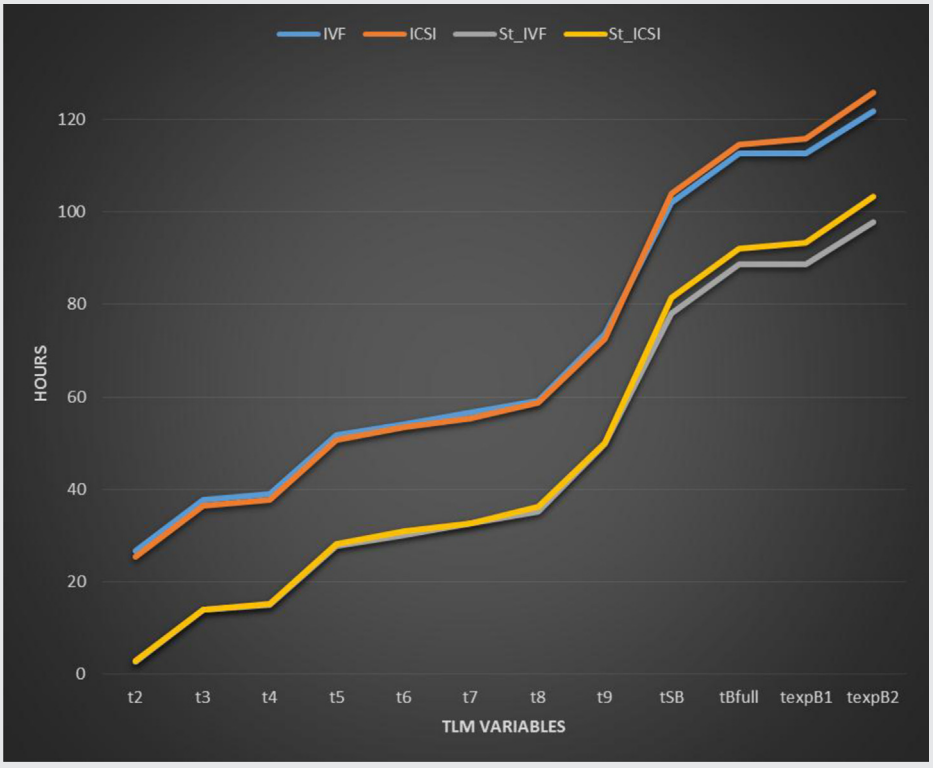
SUPPLEMENTAL FIGURE 1



Blastocyst quality categories among IVF- and ICSI-fertilized groups (A to E blastocyst categories are based on a combination of female age and time to reach the expanded blastocyst stage, as described by Kato et al. (22)).

Bodri. TLM variables and insemination techniques. Fertil Steril 2015.

SUPPLEMENTAL FIGURE 2



Average values of morphokinetic variables for standard IVF- and ICSI-fertilized groups, before and after standardizing (in hours).

Bodri. TLM variables and insemination techniques. Fertil Steril 2015.