Blastocyst collapse is not an independent predictor of reduced live birth: a time-lapse study

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Objective: To ascertain the rate of blastocyst collapse observed by time-lapse monitoring in a retrospective cohort of unselected infertile patients undergoing single blastocyst transfer and to determine its association with live birth.
Design: Blastocyst collapse and morphokinetic variables were scored according to previously published criteria. The association between blastocyst collapse and live birth was evaluated by a multivariate logistic regression analysis including morphokinetic variables and other confounders.
Setting: Private infertility clinic.
Patient(s): Patients who underwent 277 consecutive single blastocyst transfers (mean age, 38.4 ± 3.9 years; range, 28–47 years) after minimal ovarian stimulation.
Intervention(s): Minimal ovarian stimulation, prolonged embryo culture in time-lapse monitoring incubator, elective vitrification with subsequent vitrified-warmed single blastocyst transfer.
Main Outcome Measure(s): Live birth rate per single blastocyst transfer in different blastocyst collapse groups (no, single, multiple collapses).
Result(s): No, single, or multiple blastocyst collapses occurred in 54% (150/277), 22% (61/277), and 24% (66/277) of the cohort, respectively. In the multiple collapse group on average 2.9 contractions were seen (range, 2–9 contractions). Live birth rate decreased progressively between blastocyst collapse groups (36%, 31%, 14%); significantly lower if multiple collapses occurred. In a multivariate analysis, however, blastocyst collapse was not found to be a significant predictor and was confounded by stronger predictors such as morphokinetic variables t2, texpB_2, and female age.
Conclusion(s): Blastocyst collapse pattern should not be evaluated alone without taking into account morphokinetic variables that are stronger predictors of reproductive outcome. (Fertil Steril® 2016;105:1476–83. ©2016 by American Society for Reproductive Medicine.)
Key Words: Time-lapse monitoring, blastocyst collapse, single embryo transfer, in vitro fertilization

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In recent years time-lapse monitoring (TLM) technology has emerged as a promising new way to augment the classic morphological selection of embryos leading to a potential improvement in clinical outcome (1, 2). A number of studies (3–7) have shown that optimal values of certain morphokinetic variables were closely correlated with blastocyst formation, implantation potential, and even aneuploidy status. Using these findings several groups have created sophisticated, hierarchical predictive models that were shown to improve clinical outcome in the setting of randomized clinical trials (8, 9). These usually included the same static and interval morphokinetic variables from the early divisions of a cleavage-stage embryo (i.e., t3, t5, cc2a, s2). At present only one published model (that incorporated tSB and tB) has focused on TLM blastocysts and could successfully predict aneuploidy status and implantation potential (6, 10).

In addition to these morphokinetic variables other studies (11–16) focused on qualitative markers of cleavage-stage embryos that were associated with a reduced implantation potential and could be used as deselection criteria such as direct cleavage,

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multinucleation, and abnormal cleavage patterns. In a recently published study (17) another negative prognostic marker emerged, but in a blastocyst-stage embryo. In this retrospective review blastocyst collapse, precisely observed by TLM, was associated with a reduced rate of implantation (35% vs. 48.5%). Marcos et al. (17) strongly recommended against transferring such blastocysts (if any alternative is available) and encouraged to include this marker as an additional criterion in existing embryo selection models. Although this previous study was the first to investigate the influence of blastocyst collapse on reproductive outcome, its predictive value of a negative test (PVN) still has to be confirmed on embryos that originated from different patient populations and clinical settings. Therefore the aim of the present retrospective analysis was to determine the rate of blastocyst collapse observed by TLM in a cohort of blastocysts from unselected infertile patients undergoing single blastocyst transfer after mild ovarian stimulation and to determine its exact influence on live birth outcome.

MATERIALS AND METHODS

Study Patients and Follow-up

All consecutive infertile patients whose embryos were submitted to prolonged embryo culture and reached the expanded blastocyst stage (n = 501) in a TLM incubator between October 2012 (the acquisition of a single EmbryoScope incubator) and December 2014 at our center (Kobe Motomachi Yume Clinic, Kobe, Japan) and subsequently underwent vitrified-warmed ETs until August 2015 (n = 291) were included in this retrospective analysis. Fourteen cases involving zona pellucida (ZP)-free embryos were excluded because blastocyst collapse could not be precisely evaluated in these embryos (18). The outcome of ETs was followed-up until a live birth occurred (including 4 still ongoing pregnancies beyond 20 gestational weeks at the time of writing) (Supplemental Fig. 1, available online). Institutional Review Board approval was not required for the present study because in our center all patients undergoing IVF treatment gave informed consent to the anonymous use of their data for retrospective reviews.

Natural Cycle IVF and Minimal Ovarian Stimulation Protocols

Clomiphene citrate (CC) or letrozole-based minimal stimulation was used in most cycles whereas unstimulated natural cycle IVF or other mild stimulation protocols represented only a smaller proportion of cases. Details of the CC-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

Transvaginal ultrasound-guided oocyte retrieval was performed without anesthesia using a very thin 21–22 G needle (Kitazato Medical Co., Ltd.). Mature (metaphase II) oocytes were inseminated by conventional IVF or intracytoplasmic sperm injection (ICSI). Subsequently ICSI injected oocytes were placed in pre-equilibrated slides (EmbryoSlide, Vitrolife), whereas IVF inseminated oocytes were first cultured in a conventional, trigas, water jacket incubator (Astec), and transferred to a TLM incubator the next morning if fertilization was confirmed.

Prolonged Embryo Culture in a TLM Incubator and Elective Vitrification

In our center elective blastocyst culture, vitrification, and subsequent vitrified-warmed single blastocyst transfer are routinely practiced in most patients. Prolonged embryo culture was performed in a time-lapse incubator (EmbryoScope, Vitrolife) according to previously described methodology (1). Briefly, normally fertilized 2-pronuclei (PN) zygotes were incubated at 37°C in a 5% O_2 atmosphere, cultured individually until days 2–3 in a Quinn's Advantage Cleavage Medium (SAGE), and subsequently until days 5–7 in a Quinn's Advantage Blastocyst Medium (SAGE). Cleavage-stage selection was performed by classic morphology criteria according to published consensus criteria (21). According to the protocol of our group embryos that reached the blastocyst stage by day 5 or 6 were eligible for elective vitrification (Cryotop, Kitazato Medical Co., Ltd.) as soon as they expanded to a size ≥ 160 μm. Expanding blastocyst were observed two to four times a day (22). Expanded blastocysts were graded into high-quality (inner cell mass [ICM] and trophoectoderm score: AA, AB, or BA) or low-quality (ICM and trophoectoderm score: BB, BC, CB, or CC) categories (7, 23). Electively vitrified blastocysts were transferred in the next 1–3 months after the oocyte retrieval in a spontaneous natural or hormonal replacement (HR) cycle (24).

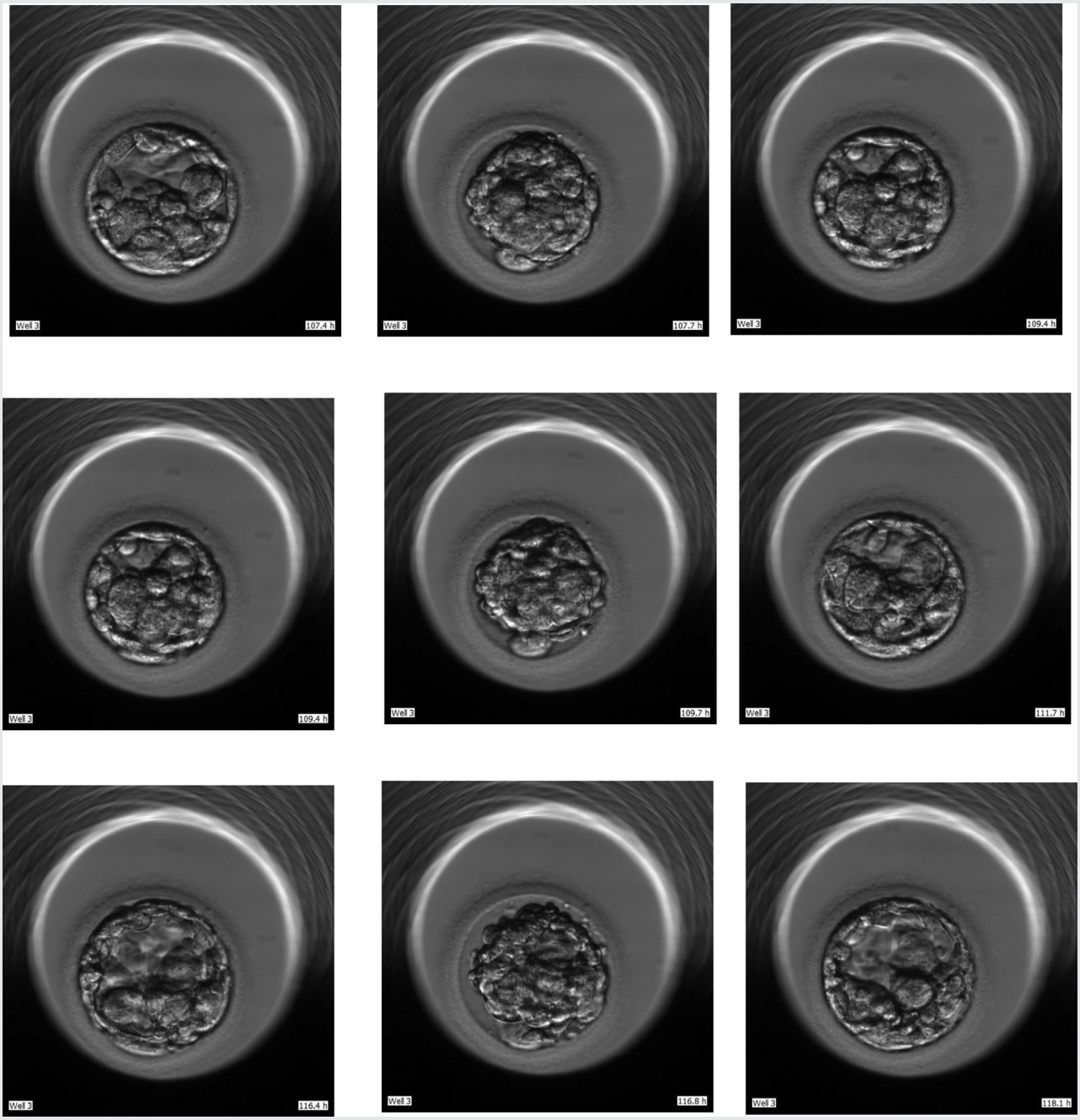
Time-lapse Annotations

Seven plane focal images were taken and recorded every 20 minutes shortly after insemination for approximately 160 hours. The duration of the TLM observation from the time point when the blastocyst reached full expansion and started pushing on the ZP (tfullB) until it reached a sufficiently expanded size for elective vitrification, and was removed from the TLM incubator, was calculated for each blastocyst. Early (PNf, t2–t9) and late (start of blastulation and full blastocyst) morphokinetic time points were scored in accordance with recently published consensus criteria (25). To determine the degree of blastocyst expansion more objectively, two additional TLM variables ($texpB_1$, $texpB_2$) were also introduced (the time point when the horizontal diameter of the expanded blastocyst reached 130 and 160 μm) (26). Annotations were performed retrospectively using EmbryoViewer by two experienced embryologists (J.Y.S. and T.S.) and rechecked by a third person (D.B.). To control for different insemination techniques used (34% and 66% of the cohort was fertilized with conventional IVF or ICSI, respectively) static morphokinetic variables were standardized to the common postfertilization time point of pronuclear fading (26–28).

Definition of Blastocyst Collapse(s)

Blastocyst collapse was defined according to the methodology described in Marcos et al. (17). Briefly, each video sequence produced by EmbryoViewer was carefully analyzed—from full blastocyst expansion (the frame before the ZP starts to thin) until approximately 160 hours after insemination—to detect instances when the surface of the trophoectoderm cells separated >50% from the inner side of the ZP (collapse or strong contraction). A separation of <50% (weak contraction) was not considered as collapse. When necessary drawing tools were used to ascertain the degree of separation (Fig. 1 and Videos 1 and 2, available

FIGURE 1



Multiple blastocyst collapses observed with Embryo Viewer (in a 39-year-old patient with a negative pregnancy test). *Upper row*: first collapse at 107.7 hours and re-expansion by 109.4 hours; *middle row*: second collapse at 109.7 hours and re-expansion by 111.7 hours; *lower row*: third collapse at 116.8 hours and re-expansion by 118.1 hours.

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online, are showing a blastocyst with a single and multiple collapses). The number of observed blastocyst collapses were counted and the entire cohort was divided into three groups (no collapse, a single collapse, and multiple collapses).

Outcome Measures and Statistical Analysis

The main outcome measure was live birth rate per single ET in blastocyst collapse groups. Baseline cycle characteristics were also compared between groups. Metric variables were compared by the one-way analysis of variance (ANOVA) test. Nominal variables were analyzed by the χ^2 test. $P<.01$ was considered statistically significant. The association between live birth and blastocyst collapse was examined by a multivariate logistic regression analysis. In addition to the blastocyst collapse variable (no, single, multiple collapses) the model included eight categorical morphokinetic variables (categorized into inside or outside of the optimal live birth ranges) that were shown to be associated with live birth outcome in our previous work. In addition eight patient-related and cycle-related confounding factors, such as female age (28–34 years [reference], 35–40 years, 41–47 years), body mass index (BMI; in kg/m^2), parity (yes/no), history of previous IVF treatment at other center (yes/no), current cycle rank (cycles 1–2 [reference], 3–4, $\geq$5), stimulation type (unstimulated [reference]/CC/letrozole/other), blastocyst quality (low/high), and duration of TLM observations (hours), were included. These were chosen because in our previously published dataset they were significantly associated with live birth (29). Statistical calculations were performed with the "R" software package.

RESULTS

Blastocyst Collapse Groups and Their Reproductive Outcome

During the study a total of 277 single blastocyst transfers were performed from all consecutive infertile patients (female age, 38.4 ± 3.9 years; range, 28–47 years) whose embryos underwent prolonged embryo culture in a time-lapse incubator. The overall live birth rate per (single) ET in the examined cohort was 30% (82/277). One or more blastocyst collapse occurred in 46% (127/277) of the examined embryos. In 22% (61/277) of the cases only a single collapse was observed, whereas in 24% (66/277) multiple collapses were seen. In the multiple collapse group an average number of 2.9 collapses were registered (range, 2–9 collapses).

Baseline characteristics of the ET cycles according to blastocyst collapse groups are summarized in Table 1. There were no significant differences among baseline variables such as female age, BMI, basal FSH, type of infertility, parity, the history of previous IVF treatment at other centers, current cycle rank, stimulation type, the number of retrieved eggs, and the male partner age. Female age did not influence the occurrence of blastocyst collapse(s) (Supplemental Table 1). However, the proportion of high-quality blastocysts decreased gradually in each blastocyst collapse group (38%, 21%, and 8%, respectively; $P<.0001$). The duration of the time lapse observations was increasingly longer among groups (no collapse, 13.1 ± 5.7 hours; single collapse, 16.7 ± 6.1 hours; multiple collapses, 24.6 ± 8.3 hours).

Live birth rates per single blastocyst transfer decreased progressively in blastocyst collapse groups (36%, 31%, and 14%, respectively; $P=.004$), but statistically significant

TABLE 1

Baseline cycle characteristics in groups with no, 1, or >1 blastocyst collapse(s).

ET cycles	No collapse (n = 150)	1 collapse (n = 61)	>1 collapse (n = 66)	*P* Value
Female age (y)	38.4 ± 4	38.6 ± 3.9	39.1 ± 3.6	.16[a]
BMI, (kg/m^2)	20.4 ± 2.3	20.3 ± 2.5	20.8 ± 2.4	.49[a]
Basal FSH (IU/mL)	9 ± 4.9	9.5 ± 5.4	9.3 ± 5.2	.80[a]
Primary infertility, n (%)	102 (68)	42 (69)	39 (59)	.39[b]
Nulliparous, n (%)	136 (91)	56 (92)	60 (91)	.97[b]
Duration of marriage (y)	6.7 ± 3.9	6.8 ± 3.5	7.1 ± 3.9	.77[a]
No IVF treatment (at other centers), n (%)	62 (41)	17 (28)	27 (41)	.17[b]
Current cycle rank				.49[b]
1–2 cycles, n (%)	47 (31)	17 (28)	16 (24)	
3–4 cycles, n (%)	57 (38)	20 (33)	22 (33)	
5 or higher, n (%)	46 (31)	24 (39)	28 (42)	
Stimulation type				.91[b]
Natural cycle, n (%)	15 (10)	6 (4)	4 (3)	
Clomiphene citrate, n (%)	110 (73)	44 (29)	47 (31)	
Letrozole, n (%)	22 (15)	10 (7)	14 (9)	
Other, n (%)	3 (2)	1 (0.7)	1 (0.7)	
Retrieved mature (MII) eggs, n	5.8 ± 3.4	5.7 ± 3.3	5.3 ± 3.2	.68[a]
High-quality blastocysts, n (%)	57 (38)	13 (21)	5 (8)	<.0001[b]
Male partner age (y)	39.7 ± 5.1	40.9 ± 5.6	40.8 ± 5.7	.23[a]

Note: MII = metaphase II.
[a] One-way analysis of variance test.
[b] χ^2 test.

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differences were only observed between the multiple collapse and the other two groups ($P=.0009$ and $P=.02$).

Comparison of Morphokinetic Variables among Blastocyst Collapse Groups

There were no significant differences observed between blastocyst collapse groups in cleavage stage, static (t2–t9), and interval parameters (cc2a, cc2b, s2, s3). In contrast when comparing blastocyst-stage TLM parameters (from tSB to $texpB_2$), there was a statistically significant, gradually increasing delay among blastocyst collapse groups (Table 2 and Supplemental Fig. 2, available online).

Association between Live Birth and Blastocyst Collapse in a Univariate/Multivariate Analysis

Unadjusted odds ratios (ORs) showed that only "multiple blastocyst collapse" was associated with a decreased probability of live birth (OR, 0.28; 95% confidence interval [CI] 0.12–0.59; $P=.001$). In contrast "single blastocyst collapse" did not decrease significantly the chance of a live birth (OR, 0.80; 95% CI 0.42–1.51; $P=.502$). Among patient-related confounders female age (≥41 years), higher blastocyst quality, and longer duration of TLM observations were inversely associated with live birth (Table 3).

After adjusting for eight TLM variables and eight patient-related and cycle-related confounders the previously shown association for the multiple blastocyst collapse group disappeared (OR, 0.86; 95% CI 0.26–2.74; $P=.803$). In the resulting model among TLM variables only $texpB_2$ (adjusted OR, 4.76; 95% CI 1.61–15.51; $P=.006$), t3 (albeit at a less significant level, $P=.038$), and t2 (adjusted OR, 2.50; 95% CI 1.29–4.95; $P=.007$) remained significantly associated with live birth. Among patient-related confounders the only variable, that still had a significant impact was female age >41 years (adjusted OR, 0.21; 95% CI 0.07–0.61; $P=.005$), whereas the previously observed association for low-quality blastocysts and duration of TLM observations disappeared (Table 3).

DISCUSSION

Our study involving a cohort of unselected advanced aged infertile patients undergoing minimal ovarian stimulation has found that blastocyst collapses (including multiple ones), observed by TLM, occurred frequently (in almost 50% of entire cohort). Although live birth rates decreased when blastocyst collapse(s) were observed (especially if they were multiple) blastocyst contraction was not an independent predictor of reduced live birth and was confounded by cleavage stage and blastocyst stage morphokinetic variables and female age.

This study to our knowledge is the second one that has investigated the influence of time-lapse observed blastocyst collapse(s) on reproductive outcome after IVF treatment. A recently published article (17) by a Spanish group that pioneered TLM research presented the results of a two-center, retrospective review involving 715 transferred blastocysts during a 1-year period. They found that 19.4% of the analyzed blastocysts presented collapse including 1.4% with multiple (2 or 3) collapses. Baseline characteristics were not significantly different between the two groups with the exception of a higher proportion of donor cycles in the group of collapsed blastocysts. Interestingly, embryo/blastocyst quality assessed by classic morphology criteria was not significantly different, but timing for several morphokinetic parameters (i.e., t2, t3, t4, tB) were shorter for collapsed embryos (implying faster development). Reproductive outcome was evaluated in a smaller subset of 502 embryos due to the necessary reliance on known-implantation or known-implantation data. The investigators found that implantation rates (albeit only in a univariate analysis) were significantly lower (35% vs. 48.5%) in collapsed blastocysts. They concluded that these blastocysts should not be replaced

TABLE 2

TLM timings in groups with no, 1, or >1 blastocyst collapse(s).

TLM variables (mean ± SD)	No collapse (n = 150)	1 collapse (n = 61)	>1 collapses (n = 66)	*P* value[a]
t2	2.7 ± 0.7	2.7 ± 0.9	2.8 ± 0.9	.96
t3	14.0 ± 1.7	14.0 ± 1.8	14.1 ± 1.9	.86
t4	14.7 ± 2.2	14.8 ± 2.1	15.1 ± 2.4	.5
t5	28.2 ± 4.9	27.5 ± 5.5	28.2 ± 4.8	.58
t6	29.8 ± 4.2	29.2 ± 5.0	30.1 ± 4.1	.56
t7	31.4 ± 4.9	31.6 ± 5.3	31.9 ± 4.8	.82
t8	33.7 ± 6.5	35.1 ± 7.8	35.8 ± 7.7	.15
t9	49.9 ± 8.7	51.6 ± 7.8	49.8 ± 8.7	.42
cc2a (t3-t2)	11.2 ± 1.7	11.2 ± 1.7	11.4 ± 2.0	.84
cc2b (t4-t2)	12.1 ± 2.1	12.2 ± 1.7	12.4 ± 2.6	.53
s2 (t4-t3)	0.9 ± 1.6	1.0 ± 1.5	1.1 ± 2.3	.71
s3 (t8-t5)	5.6 ± 5.3	7.7 ± 8.6	7.8 ± 7.5	.054
tSB	77.7 ± 8.5	78.6 ± 11.5	81.4 ± 8.7	.027
tfB	88.2 ± 10.5	89.7 ± 13.0	92.5 ± 9.6	.030
$texpB_1$	88.4 ± 9.7	90.5 ± 12.0	94.4 ± 9.2	.0004
$texpB_2$	96.6 ± 10.8	99.0 ± 12.1	108.9 ± 10.2	<.0001

Note: cc2a/cc2b/s2/s3 = duration of different embryo cell cycle events; PNf = pronuclear fading; t2–t9 = time to 2 to 9 discrete cells; tSB = time to initiation of blastulation; $texpB_1$ = time to reach expanded blastocyst size of 130 μm; $texpB_2$ = time to reach expanded blastocyst size of 160 μm; tfB = time to full blastocyst. All time-lapse monitoring (TLM) variables were standardized to PNf.
[a] One-way analysis of variance test.

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TABLE 3

Results of univariate and multivariate analysis on the association between live birth and categorical TLM variables and other confounders.

TLM variables	Unadjusted ORs	*P* value	Adjusted ORs	Adjusted *P* value[a]
Blastocyst collapse				
No collapse (reference)	—	—	—	—
1 collapse	0.80 (0.42–1.51)	.502	1.87 (0.80–4.40)	.148
>1 collapse	0.28 (0.12–0.59)	.001	0.86 (0.26–2.74)	.803
$texpB_2$	4.09 (2.31–7.52)	<.0001	4.76 (1.61–15.51)	.006
$texpB_1$	2.13 (1.26–3.67)	.005	1.06 (0.32–3.43)	.917
tfullB	2.20 (1.27–3.9)	.005	0.89 (0.22–3.42)	.867
t4	1.84 (1.08–3.17)	.026	1.24 (0.56–2.78)	.593
t3	1.72 (1.02–2.94)	.044	2.23 (1.05–4.86)	.038
t2	2.29 (1.35–3.9)	.002	2.50 (1.29–4.95)	.007
cc2a	1.94 (1.14–3.31)	.014	1.54 (0.73–3.27)	.254
cc2b	2.06 (1.20–3.54)	.009	1.79 (0.75–4.36)	.190
Female age				
28–34 y (reference)	—	—	—	—
35–40 y	0.61 (0.31–1.21)	.152	0.70 (0.29–1.66)	.413
41–47 y	0.21 (0.09–0.48)	.0002	0.21 (0.07–0.61)	.005
BMI	0.97 (0.86–1.08)	.593	1.02 (0.88–1.19)	.770
Previous live birth	0.92 (0.34–2.20)	.854	1.04 (0.30–3.34)	.950
Previous IVF treatment	0.84 (0.49–1.43)	.508	0.86 (0.42–1.79)	.691
Stimulation type				
Natural cycle (reference)	—	—	—	—
Clomiphene citrate	0.55 (0.24–1.32)	.171	1.04 (0.32–3.61)	.946
Letrozole	0.35 (0.12–1.01)	.053	1.19 (0.27–5.35)	.813
Current cycle rank				
1–2 cycles (reference)	—	—	—	—
3–4 cycles	1.20 (0.64–2.27)	.563	1.64 (0.73–3.81)	.237
5 or higher	0.64 (0.32–1.24)	.186	0.79 (0.32–1.95)	.611
Low-quality blastocyst	0.48 (0.27–0.84)	.009	0.73 (0.34–1.58)	.428
Duration of TLM observations	0.94 (0.90–0.97)	.001	1.00 (0.93–1.07)	.966

Note: cc2a/cc2b/s2/s3 = duration of different embryo cell cycle events; OR = odds ratio; PNf = pronuclear fading; t2–t9 = time to 2 to 9 discrete cells; 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; tSB = time to initiation of blastulation. All time-lapse monitoring (TLM) variables were standardized to PNf.

[a] Adjusted for eight TLM variables and nine confounders (blastocyst collapse, female age, body mass index (BMI), parity, history of previous IVF, cycle rank, stimulation type, blastocyst quality, duration of time-lapse observation).

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(except if no better alternatives are available) and that the collapse pattern should be considered as an additional criterion that could improve embryo selection.

Our study partly confirms, but also partly contradicts, the findings observed in the previously mentioned study. First, the overall rate of blastocyst collapse(s) was much higher in our cohort (46% vs. 19.4%) and also more multiple (<9) collapses were seen (22% vs. 1.4%). Second, findings were different in relation to blastocyst quality (decreasing quality for collapsed blastocysts in ours vs. similar quality in the Spanish study) and timing of morphokinetic parameters. The collapsed group blastocyst-stage TLM parameters were on average longer in our study but shorter in the Marcos et al. study (17). All of these discrepancies could be explained by a radically different patient profile (beside ethnicity) between the two studies. Our study involved advanced aged, nondonor, infertile patients exclusively, whereas more than half of the Spanish cohort (17) were young oocyte donors. In addition there were some differences in culture conditions (e.g., different brands of sequential media, low vs. atmospheric O_2 concentration).

However, both studies were concordant in that, at least in a univariate analysis, they have found decreased implantation rates in the group of collapsed blastocysts. Regarding the group of multiple collapses this finding was statistically significant in our study but could not be determined exactly in the Spanish study (17) due to the small number of observed multiple collapse cases (n = 10). Even more important, however, in our study decreased implantation rate in the collapse groups was not present anymore after adjusting for confounding variables in a robust multivariate analysis. This suggests that the influence of blastocyst collapse is strongly confounded by a handful of important morphokinetic parameters that influence reproductive outcome (in our study, t2, t3, $texpB_2$). Although a less robust multivariate analysis was also part of the Spanish study (17), the six chosen covariates were patient-related, or cycle-related only and did not include the morphokinetic parameters (t2, t3, t4, tB). Therefore it is impossible to determine whether the original Spanish findings would have been concordant with ours had a more extensive multivariate analysis been performed. This strongly implies that although the blastocyst collapse pattern might become a relatively important predictive variable it should not be evaluated without taking into account other (cleavage stage or blastocyst stage) morphokinetic variables that already have been shown to be strong predictors of implantation and have been included in various hierarchical predictive models (9, 10, 30).

At present many animal studies (31–33) investigated the phenomenon of blastocyst contraction, its molecular mechanisms, and the relation to embryo hatching. Niimura (34) suggested that in mouse embryos weak contractions play a positive role in hatching, whereas strong ones are inhibitory. It is unclear, however, how the previously mentioned findings could be extrapolated to in-vitro cultured human blastocysts. Marcos et al. (17) hypothesized that blastocyst collapse may be linked to the implantation process, either through embryo quality (caused by decreased oocyte quality or suboptimal culture conditions) or by directly affecting the blastocyst's implantation ability (through abnormal hatching or other mechanisms). Our findings suggest that collapsing blastocysts also display suboptimal timing of morphokinetic variables, implying that the underlying common cause is the defective embryo quality, which ultimately manifests itself in a reduced implantation potential. For example, a link between suboptimal morphokinetical timing and aneuploidy rates (and decreased implantation) was already established in previous studies (6, 35).

The main limitation of our study was the relatively low number of single blastocyst transfers (277). This sample size, however, which was achieved in a single center during a 2-year study period, is comparable with those published in other relevant TLM articles and is certainly sufficient to develop an initial, center-specific predictive model that later could be improved on. Second, the duration of the TLM observations was increasingly longer for the collapse groups that, in our opinion, is not an inherent bias, but that (especially repeated) collapse/re-expansion cycles increased the time needed to reach the necessary criteria for elective vitrification. Third, elective vitrification itself, which is a prominent feature of our clinic's treatment protocol, was not practiced in the Spanish study (17), where only fresh ET cycles were included. As this has equally affected all blastocyst transfer cycles in our cohort it is unlikely that it could have introduced any bias affecting live birth rates. As a considerable strength, by our center's routine policy, our dataset included only single ETs, thus we did not have to rely on known-implantation data embryos, only avoiding any potential bias that might arise from analyzing embryos with a "double or nothing" implantation potential.

In conclusion in a group of unselected infertile patients undergoing minimal ovarian stimulation and vitrified-thawed single blastocyst transfer, single and multiple blastocyst collapses occurred frequently, but were not independently associated with a decreased chance of a live birth.

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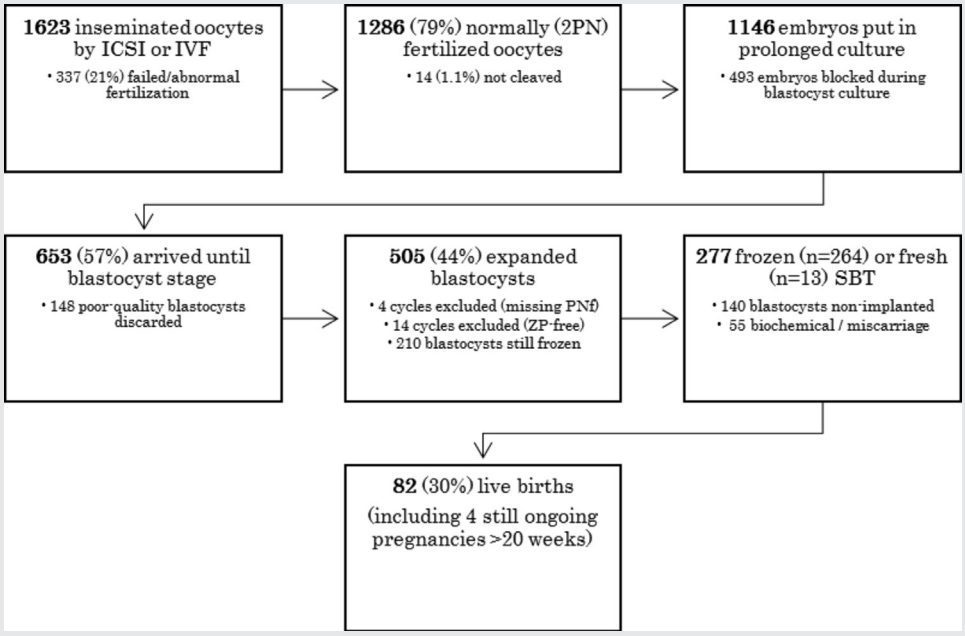
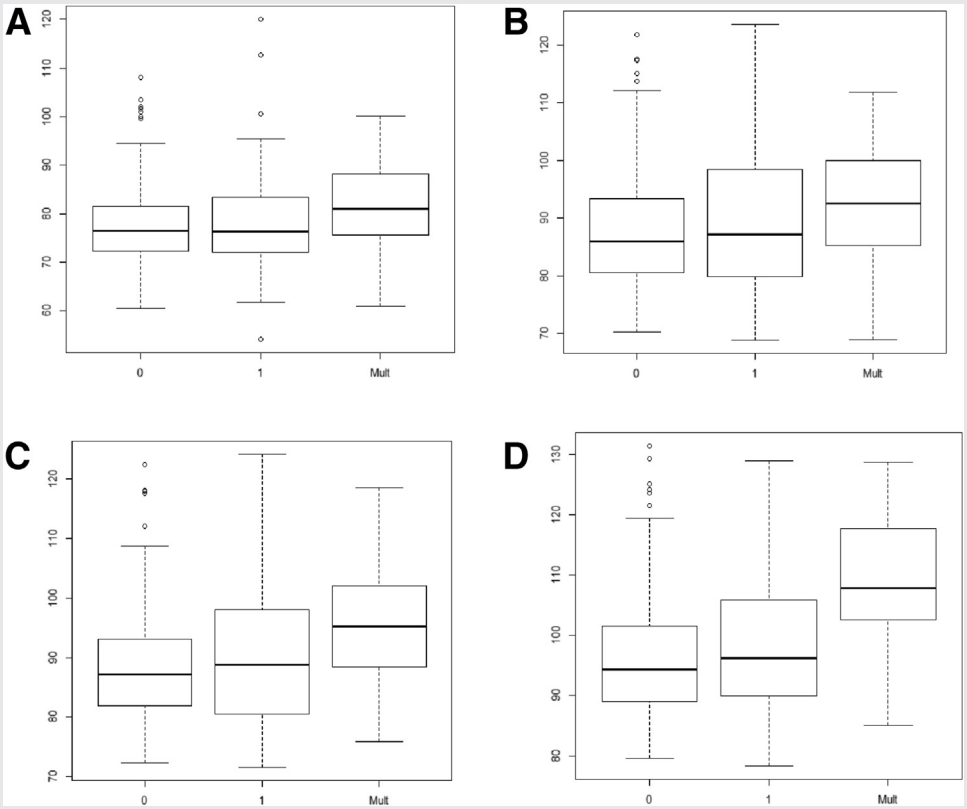
SUPPLEMENTAL FIGURE 1



Flow chart of study events. ICSI = intracytoplasmic sperm injection; PN = pronuclei; PNf = pronuclear fading; SBT = single blastocyst transfer; ZP = zona pellucida.

Bodri. Blastocyst collapse and live birth. Fertil Steril 2016.

SUPPLEMENTAL FIGURE 2



Time-lapse monitoring variables according to blastocyst collapse groups (0/1/multiple collapse(s). (**A**) tSB P=.019 between "0" and "Mult"; (**B**) tfullB P=.023 between "0" and "Mult"; (**C**) texpB$_1$ P=.0002 between "0" and "Mult"; (**D**) texpB$_2$ P<.0001 between "0" and "Mult" and between "1" and "Mult." PNf = pronuclear fading; texpB$_1$ = time to reach expanded blastocyst size of 130 μm; texpB$_2$ = time to reach expanded blastocyst size of 160 μm; tSB = time to initiation of blastulation; tfB = time to full blastocyst. All time-lapse monitoring variables standardized to PNf.

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SUPPLEMENTAL TABLE 1

Relationship between blastocyst collapse patterns and female age groups.

Female age versus blastocyst collapse patterns[a]	28–34 y (n = 44)	35–40 y (n = 146)	41–47 y (n = 87)
No collapse n, (%)	28 (64)	79 (54)	43 (49)
1 collapse n, (%)	7 (16)	34 (23)	20 (23)
>1 collapse n, (%)	9 (20)	33 (23)	24 (28)

[a] χ^2 test, $P = .59$.

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