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ORIGINAL ARTICLE

Evaluation of fragmented embryos implantation potential using time-lapse technology

Shirly Lahav-Baratz[1] | **Idit Blais**[1] | **Mara Koifman**[1] | **Martha Dirnfeld**[1,2] | **Galia Oron**[1,2]

[1]Division of Reproductive Endocrinology and In Vitro Fertilization (IVF), Department of Obstetrics and Gynecology Lady Davis Carmel Medical Center, Haifa, Israel

[2]Ruth and Bruch Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel

Correspondence
Shirly Lahav-Baratz, Division of Reproductive Endocrinology and In Vitro Fertilization (IVF), Department of Obstetrics and Gynecology Lady Davis Carmel Medical Center, 7 Michal St., Haifa 3436212, Israel.
Email: lahav_shirly@clalit.org.il

Abstract

Aim: To examine the implantation potential of fragmented embryos that underwent morphokinetic evaluation in a time-lapse incubator.

Methods: A retrospective study analyzing 4210 Day 5 embryos which were incubated in a time-lapse incubator, between 2013 and 2019. Embryos with more than 5% fragmentation (379 embryos) were included in the study. Embryos selected using the general model and re-examined by our in-house model. Embryo fragmentation percentage was documented from the first cell-division (start fragmentation) to its maximal percentage (final fragmentation), and the ratio between them (fragmentation worsening). Data were analyzed with relation to embryo development, embryos transfer or freezing, clinical pregnancy, and live birth rates.

Results: Embryo fragmentation and morphokinetics were found to be independent variables for clinical pregnancy achievements. A higher fragmentation worsening was noted among discarded embryos compared to transferred or frozen embryos ($p < 0.0001$). Advanced maternal age had a significant negative effect on fragmentation ($p < 0.001$). Missed abortion rates were similar in fragmented embryos that implanted compared with the overall population. Live birth rates were comparable among embryos which were selected for transfer or freezing by their morphokinetics and had different severity of fragmentation.

Conclusion: Our study shows that fragmented embryos have a potential to implant and therefore should be selected for transfer. Laboratories which do not use time-lapse incubators for embryo selection, should consider transferring fragmented embryos, since they have an acceptable chance for live birth. Calculation of fragmentation worsening may enhance our ability to predict embryo development. Further research with analysis of more fragmented embryo maybe beneficial. This study was approved by the local ethics committee No. 0010-19 CMC on April 18th, 2019.

KEYWORDS
embryo selection, fragmentation, in vitro fertilization (IVF), morphokinetics, time-lapse

INTRODUCTION

Morphological evaluation of embryos in in vitro fertilization (IVF) treatment is the most common method for embryo selection. Embryo quality assessment is based on cell number, cell symmetry, and fragmentation.[1] The presence of fragments in the embryos, has been considered as a negative marker for their potential to develop and implant.[2,3] In a prospective study fragmentation was considered as one of the variables most negatively associated with the chance for live birth.[4] Moreover, it was suggested that fragmentation on day 3 should be taken into account in the selection of the best blastocyst for transfer.[2] Fragments have been linked to abnormalities in cell metabolism and may reflect an apoptotic process.[5] Apoptotic markers such as Bax, Fas, and caspase-3 were found in

Martha Dirnfeld and Galia Oron authors contributed equally to this study.

fragmented embryos.[6] Fragmentation formation was also correlated with abnormalities in the oocyte membrane[7] and anomalies in chromosomal segregation.[8] Recent data suggests that fragments can be seen in embryos from the first cell division and are in association with the progression through meiotic and mitotic cell cycles.[9,10] It was shown that fragmentation can occur in embryos of various species in vivo[7] and no difference in fragmentation was found between stimulated and unstimulated cycles.[11] The correlation of fragmentation with maternal age remains in debate. Some researchers found that age does not influence the extent of fragmentation[12,13] while others suggested a direct relationship between maternal age and embryo fragmentation.[14,15]

In most of the studies, the degree of fragmentation was determined by morphological estimation. Fragmentation severity is usually categorized as up to 10%–50% and above 50% fragments,[2,13,16] or as up to 25% and above 25%.[17] Alkiani et al. used microscopic magnification of ×600 to estimate fragmentation degree and divided fragmentation to 0%–5%, 6%–15%, 16%–25%, 26%–35% and more than 35%.[14] Time-lapse systems (TLS) developed for assisted reproductive technology, can substantially increase accuracy in fragmentation measurement by using its measurements tools. Measurement tools provided by TLS which were developed for assisted reproductive technology, and enable area calculation can substantially increase accuracy in fragmentation measurement.

TLS technologies have been shown in multiple clinical trials to improve embryo selection and clinical outcomes versus traditional morphological evaluation of embryos.[18–20] Generally applicable morphokinetic algorithms were developed for embryo selection, such as "KIDScore™ D3" for Day 3 transferred embryos,[21] and "KIDScore™ D5" for Day 5 transferred embryos.[22] These algorithms for selection and frequently deselection of embryos is routinely applied in IVF labs that use the EmbryoScope TLS incubator (Vitrolife, Goteborg, Sweden). Mizobe et al. used TLS for embryos incubation, but embryo selection for transfer was performed primarily by morphological estimation. They graded fragmentation up to 10% and 10%–50% and concluded that embryos which formed two cells during the first division and four cells during the second division, regardless of the presence of fragments, were most likely to achieve a pregnancy.[16] Stensen et al. showed that embryos with high degree of fragmentation (>50%) are characterized by a delayed first cell division, an early start of the second division and a longer duration of the third cell cycle.[9] Kong et al. found in their study using TLS that embryos with an increased tendency for fragmentation formation had a decreased cell number on Day 3.[23]

Several studies showed that there is a need for an individual laboratory-adapted model for embryo selection in addition to the general models.[24] We developed a laboratory-adapted model based on our known implantation data (KID) embryos. This in-house model was successfully calibrated and validated, and it has recently been implemented at our IVF laboratory for embryo selection including the fragmented embryos. It seems now in hindsight that our in-house model is more accurate and has additive information that helps us in embryos selection.

The aim of the present study was to re-examine the impact of embryo fragmentation on implantation and live-birth rates with the perspective of TLS embryo evaluation.

We investigated whether general models and in-house models could predict the implantation rate and live birth rate of these fragmented embryos including the severely fragmented ones.

TABMATERIALS AND METHODS

Stimulation protocol

All women were treated with either a gonadotropin releasing hormone (GnRH) agonist or antagonist protocol. In long agonist cycles, downregulation was achieved using GnRH agonist (0.2 mg of decapeptyl, Ferring Pharmaceuticals, Switzerland) from mid-luteal phase, and ovarian stimulation was achieved using recombinant FSH (Gonal-f; Merck Serono, Geneva Switzerland, or Puregon; Schering-Plough; Kenilworth, NJ), human menopausal gonadotropin (hMG) (Menopour, Ferring, Langley, United Kingdom), or a combination of recombinant FSH and luteinizing hormone LH (Pregoveris; Merck Serono). In antagonist cycles, stimulation by gonadotrophins was performed as described above and a GnRH antagonist (Orgalutran 0.25 mg, Schering-Plough; Kenilworth, NJ or Cetrotide 0.25 mg, Merck Serono, Geneva Switzerland) was administered daily from the sixth day of the cycle. Final follicular maturation was triggered with recombinant hCG (Ovitrelle; Merck Serono) when at least two leading follicles of 18 mm in mean diameter were measured. Triggering with GnRH agonist (0.2 mg Decapeptyl, Ferring) was used to avoid ovarian hyper stimulation syndrome. Oocyte retrieval was performed 36–37 h later by transvaginal ultrasound-guided needle aspiration of follicles (Day 0). Informed consent and animal studies: animals were not used in this study. Since it is a retrospective study, no informed consent was requested by the local ethics committee (0010-19 CMC, April 18th, 2019).

Fertilization and embryo culture

After retrieval, collected cumulus-oocyte complexes were placed in HTF medium (Qulnn's Advantage Fertillization [HTF] Medium, Sage, Trumbull, USA) supplemented with 10% Serum Protein Substitute under oil (Sage

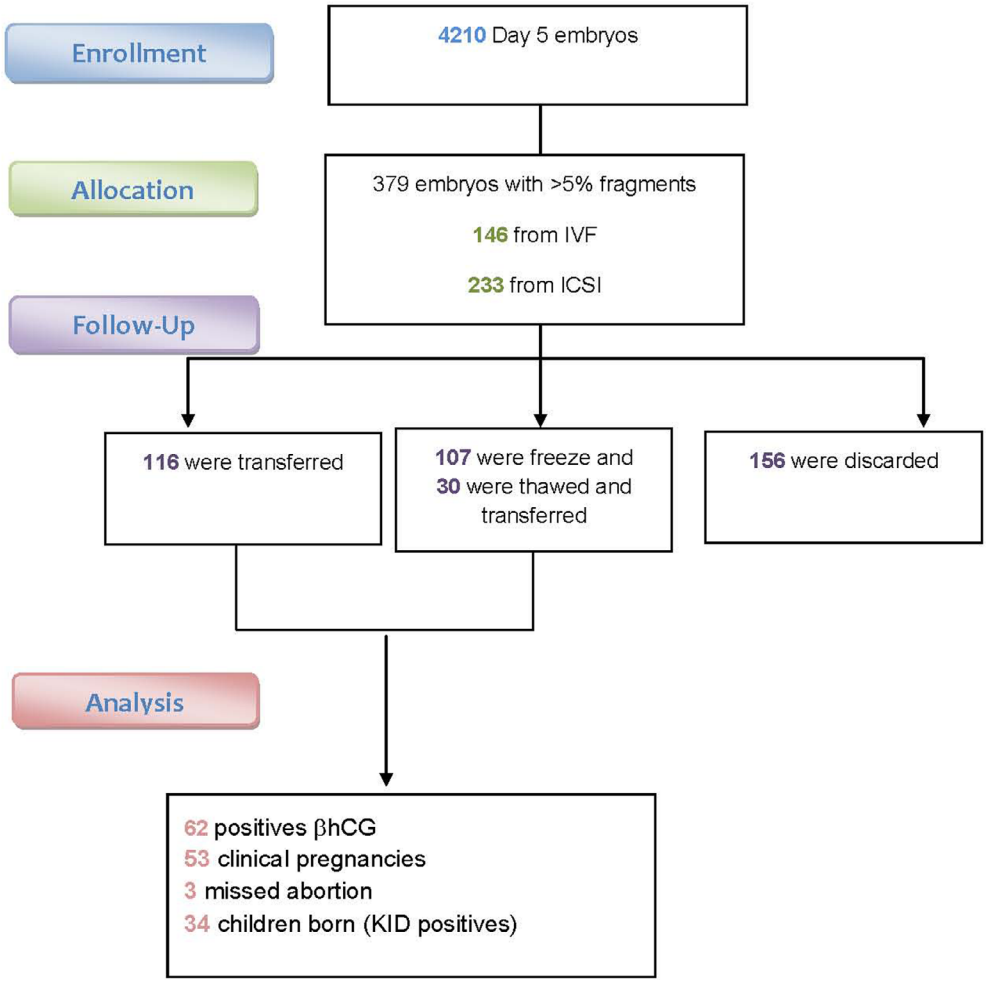

FIGURE 1 Patient's characteristics.

USA) overlay and incubated at 37°C in 6% CO_2 and 5% O_2.

Fertilization was performed using IVF or intracytoplasmic sperm injection (ICSI). Fertilized oocytes were placed in individual micro wells within a pre-equilibrated specific culture slide (EmbryoSlide; Vitrolife) in "one-step" culture medium (Global total, LifeGlobal group) overlaid with oil (Sage, USA). The slides were loaded into the EmbryoScope (Vitrolife), a tri-gas incubator. Embryos were cultured at 37°C with 6% CO_2 and 5% O_2. Images of each embryo were acquired automatically every 15 min, on seven focal planes.

All embryos in our IVF unit are incubated in the EmbryoScope to day 2, 3 or 5–6. About 60% of the embryos are incubated to day 5. In this study we included only embryos which were incubated to day 5.

Embryo transfer (ET) was performed under transabdominal ultrasound guidance using soft catheters (Wallace; Cooper Surgical, Trumbull, USA).

Embryo cryopreservation and embryo warming was done using vitrification and vitrification warming kits (Sage USA). Embryos were freeze on cryotops (Kitazato Shizuoka Japan) or cryolocks (Biotech INC, USA).

Patient's characteristics

Retrospective data analysis included data on all embryos which were incubated in a time-lapse incubator (EmbryoScope; Vitrolife) to Day 5 (4210 embryos) (Figure 1). Three hundred seventy-nine embryos had more than 5% fragmentation on the first cell division and were included in the study. Of the 379 embryos (derived from 141 cycles), 116 embryos were transferred, 107 were frozen, of which 30 were thawed, and 156 embryos were discarded (Figure 1).

Embryo estimation and selection

Embryos were selected for transfer or freezing if they reached the morula stage on Day 5 or the blastocyst stage on Day 6. Embryo selection was based on morphokinetic general model (KIDScore Day 5 model). Morphological parameters such as degree of blastocyst expansion, quality of inner cell mass, and quality of trophectoderm are included in the general model.

Embryo annotations were performed to all embryos in accordance with published consensus criteria "Proposed

guidelines on the nomenclature and annotation"[25] and the Vitrolife Technotes for KIDScore D5 v.3.[26] Morula were annotated at the end of compaction process (M); start of blastulation was set as the first frame when initiation of a cavity formation was observed (SB); annotation of a full blastocyst was done at the last image before the embryo starts to compress the zona pellucida (B); expanded blastocyst was annotated when the zona pellucida was about 50% thinner in thickness (EB). The EmbryoViewer software (version 7.3.200.16739, Vitrolife) was used for annotation of embryo blastomeric division timings. The annotations were performed by the same three senior embryologists. Comparative annotation is performed periodically. Images of each embryo were acquired automatically every 15 min on seven focal planes which were also used for fragmentation measurements. Continuous surveillance of the embryo, measurement, and annotation of cell diameter absence of nucleus and lack of cell division all enabled us to accurately detect fragments.[27]

Following embryo scoring by KIDScore D5 v.3 model, the embryos were examined also by our in-house model.[24] For some severely fragmented embryos it was not possible to recognize all their division times and therefore to perform a full annotation. To that end we used the timing of first cell division (from one cell to two cells) t2 and the timing of start blastulation (when the cavity appear) tSB, which can usually be identified, for embryo estimation. For all 379 fragmented embryos, t2 and tSB timings were tested using the group quartile table developed in our lab for the in-house model, in order to assess the potential of these fragmented embryos to implant (Table S1, Supporting Information). The division timings were converted from continuous variables into categorical variables by dividing them into groups based on their quartiles (as described earlier by Meseguer et al.[4]). Each embryo was evaluated according to its fertilization method (IVF or ICSI).

Fragmentation evaluation was performed using the EmbryoViewer software (Vitrolife) using its measuring tools as demonstrated in Figure S1. The cells and the fragments were marked by the embryologist, their area was noted by area calculating tool and according to these values and percentage of fragmentation was calculated. Fragmentation was documented from the first cell division to Day 5. Fragmentation measured following first cell division was defined as start fragmentation. The maximal fragmentation measured was defined as final fragmentation, usually following the third or fourth division. During blastocyst expansion the fragments are marginalized to the edge of the perivitelline space so we could not measure them at this stage. Fragmentation worsening was calculated as the ratio between final and start fragmentation.

In the current study, the 379 fragmented embryos were examined for their cell division timing in relation to their fragmentation percentage according to both models (the KIDScore Day 5 model and our in-house model).

KID embryos are defined as embryos with known implantation data; KID positive embryos are embryos that implanted and resulted in a clinical pregnancy which is defined as visualization of the gestational sac with heartbeat by ultrasound examination.

Statistical analysis

Differences in clinical characteristics were compared using independent *t* test or the Mann–Whitney for the continuous variables, and chi square test for the categorical variables. The continuous variables are presented as mean ± standard deviation; median and IQR.

Differences in fragmentation and fragmentation ratio among different clinical characteristics with more than two categories were compared using the Kruskal–Wallis test followed by Mann–Whitney for pairwise comparison with Bonferroni correction, $p < 0.005$ was considered statistically significant.

Variables with two categories were examined using the Mann–Whitney test.

Association between categorical variables were examines using the chi square test.

ROC (receiver operating curve) analysis test followed by Youden Index was applied for fragmentation cut off.

Statistical analysis was performed by using IBM statistics software (SPSS) vs. 24.

Ethics statement

This study was approved by the local ethics committee No. 0010-19 CMC on April 18th, 2019.

RESULTS

Embryo fragmentation and morphokinetics

By using the cell division timing grouped quartiles (previously published by our group and presented in Table S1),[24] we found that fragmentation and morphokinetics were independent variables for clinical pregnancy in embryos which had up to 32% final fragmentation. We were able to show that there was no correlation between morphokinetics and degree of fragmentation up to 32% fragmentation. Therefore, while in the general population of embryos the t2 and tSB of embryos that implanted were mainly in quartile 1 (Q1), the t2 and tSB of fragmented embryos were equally distributed in the first three quartiles (Table 1) and were not concentrated in quartile 3 or 4 as one may assume. Only embryos with very high fragmentation (32.5% as average and 25% median) were concentrated in quartile 4 and this was significant (Table 1).

The percentage of start and final fragmentation was similar in quartiles 1–3 for t2 (Table 1). Embryos with a

TABLE 1 Comparison between first cell division timing (t2) quartiles (Q1, Q2, Q3, and Q4) and embryo fragmentation percentage.

t2 quartile	% of start fragmentation	% of final fragmentation	Fragmentation ratio (worsening)
Q1 ($n = 64$)			
Mean ± SD	15.9 ± 13.3	23.6 ± 16.4	1.8 ± 1.5
Median (IQR)	12.5 (8; 17)	20 (14; 25.8)	1.25 (1.05; 1.78)
Q2 ($n = 97$)			
Mean ± SD	13.7 ± 8.7	20.5 ± 13.9	1.6 ± 0.8
Median (IQR)	12 (8; 17)	15 (10; 30)	1.35 (1.12; 1.94)
Q3 ($n = 79$)			
Mean ± SD	13.6 ± 9.4	20.0 ± 12.9	1.6 ± 0.9
Median (IQR)	10 (8; 16.5)	15 (10.5; 25)	1.40 (1.12; 1.94)
Q4 ($n = 139$)			
Mean ± SD	23.7 ± 18.8[a]	32.5 ± 21.2[a]	1.6 ± 1.0
Median (IQR)	17 (10; 30)	25 (15; 50)	1.33 (1.11; 1.75)
p-Value	$p < 0.0001$	$p < 0.0001$	NS

Note: Kruskal–Wallis followed by Mann–Whitney for pairwise comparison with Bonferroni correction.
Abbreviation: *n*, number of embryos.
[a]The differences between Q4 and Q1Q3 quartiles.

TABLE 2 Comparison between start blastocyst timing quartiles (tSB) and embryo fragmentation percentage.

tSB quartile	% of start fragmentation	% of final fragmentation	Fragmentation ratio (worsening)
Stage 0 ($n = 165$)			
Mean ± SD	25.1 ± 18.6[a]	36.6 ± 20.1[a]	1.8 ± 1.2
Median (IQR)	20 (10; 30.3)	33.5 (20; 50)	1.4 (1.1; 2.0)
Q1 ($n = 28$)			
Mean ± SD	11.0 ± 6.6	16.1 ± 9.5	1.6 ± 0.6
Median (IQR)	10 (7; 14)	14 (10; 22)	1.3 (1.1; 1.75)
Q2 ($n = 43$)			
Mean ± SD	11.7 ± 8.3	15.4 ± 9.8	1.4 ± 0.5
Median (IQR)	10 (6; 15)	15 (8; 20)	1.3 (1.0; 1.7)
Q3 ($n = 69$)			
Mean ± SD	12.9 ± 6.5	18.4 ± 11.5	1.5 ± 1.2
Median (IQR)	12 (8; 16)	15 (10.5; 20.5)	1.3 (1.1; 1.6)
Q4 ($n = 74$)			
Mean ± SD	12.1 ± 7.0	16.3 ± 8.0	1.6 ± 0.9
Median (IQR)	10 (7; 16)	15 (10; 20)	1.3 (1.1; 1.7)
p-Value	$p < 0.0001$[b]	$p < 0.0001$[b]	NS

Note: The table shows tSB Quartile stage 0 for embryos that did not reach SB developmental stage and tSB timing quartiles; Q1, Q2, Q3, and Q4 for embryos that did.
Kruskal–Wallis followed by Mann–Whitney for pairwise comparisons with Bonferroni correction.
Abbreviation: *n*, number of embryos.
[a]The difference between start and final fragmentation, between stage 0 to all tSB timing quartiles is significant ($p < 0.0001$).
[b]Sig after Bonferroni correction between embryo stage 0 and Q1, Q2, Q3, and Q4.

start fragmentation high as 23.7 ± 18.8 and final fragmentation high as 32.5 ± 21.2 were significantly more prevalent in quartile 4 (Table 1). About 65% of embryos from the first three quartiles were transferred or frozen, while from the fourth quartile, only 47% were transferred or frozen ($p < 0.003$). These embryos cleaved slower into two cells as analyzed by the EmbryoScope and therefore gained a low morphokinetic score. This followed by the appearance of more than 50% fragmentation.

For the tSB timing, no difference was found between start fragmentation, final fragmentation and fragmentation worsening in all four quartiles of timing. One hundred and sixty-five embryos were defined as Stage 0—embryos that did not reach the start blastulation (SB) developmental stage and therefore had no tSB timing. These embryos had a significantly higher start and final fragmentation compared with embryos that reached the SB stage (at Q1–4) ($p < 0.0001$) (Table 2).

TABLE 3 Comparison between embryo stage of development to Day 5 and embryo fragmentation percentage.

Embryo stage at day 5	% of start fragmentation	% of final fragmentation	Fragmentation ratio (worsening)
Group 0 ($n = 129$)			
Mean ± SD	28.9 ± 19.5*	42.7 ± 19.2*	1.9 ± 1.2***
Median (IQR)	25 (14; 40)	45 (28; 60)	1.4 (1.1; 2.1)
Group 1, Morula ($n = 31$)			
Mean ± SD	12.4 ± 7.0	19.4 ± 10.3	1.8 ± 1.2
Median (IQR)	12 (8; 17)	15 (11; 27)	1.4 (1.1; 2.0)
Group 2, Start Blast ($n = 49$)			
Mean ± SD	14.4 ± 8.8	20.1 ± 9.8**	1.6 ± 1.0
Median (IQR)	13 (8; 17)	20 (12; 25)	1.3 (1.1; 1.9)
Group 3, Blast ($n = 100$)			
Mean ± SD	11.9 ± 7.1	16.4 ± 10.4	1.5 ± 1.1
Median (IQR)	10 (7; 15)	15 (10; 20)	1.3 (1.1; 1.7)
Group 4, Expanded Blast ($n = 70$)			
Mean ± SD	11.6 ± 5.7	14.2 ± 5.8	1.3 ± 0.4
Median (IQR)	11 (7; 15)	15 (10; 18)	1.2 (1.0; 1.5)
p-Value	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

Note: Kruskal–Wallis followed by Mann–Whitney for pairwise comparisons.
*The difference between start and final fragmentation of group 0 and all other stages were statistically significant ($p < 0.0001$).
**The differences between group 2 to groups and 4 in the final fragmentation was significant ($p < 0.0001$ respectively).
***The difference in fragmentation worsening between group 0 to group 4 was significant ($p < 0.0001$).

Fragmentation and fragmentation worsening

No correlation was found between fragmentation worsening and clinical pregnancy and live birth. However, we found that when final fermentation per start fragmentation (fragmentation worsening) is above 1.76; most of these embryos were not transferred or frozen.

The average fragmentation following first cell division (the start fragmentation) in all 379 embryos was 17.9% ± 14.8% and the final (approximately 110 h post insemination) was 25.5% ± 18.0%. Those embryos were divided into three groups according to the start fragmentation. The three groups were set according to similar groupings previously reported.[14,17] The first group ($n = 227$ embryos) consisted of embryos with up to 15% at the start of fragmentation, the second group ($n = 111$ embryos) with 15%–35% fragmentation and the third group ($n = 41$ embryos) with more than 35% fragmentation from the first cell division. Fragmentation worsening was calculated for each group. A significant difference was found in fragmentation worsening between embryos with start fragmentation of up to 15% and embryos with start fragmentation of 15%–35% (worsening of ×1.9 ± 1.3 and ×1.4 ± 0.4, respectively; $p < 0.001$). We found that above 35% fragmentation at first cell division it was impossible to accurately calculate the fragmentation worsening.

Embryo stage on Day 5 and fragmentation

Embryos were divided according to their developmental stage at Day 5; group 0 for embryos that did not reach the morula stage, group 1 for embryos at the morula stage, group 2 for start blastocyst, group 3 for blastocyst and group 4 for expanded blastocyst stage (Table 3). Significant difference in percentage of fragmentation was found between embryos which did not develop to the morula stage on Day 5 (group 0) compared with embryos that continued to develop to morula, start blastocyst, blastocyst, or expanded blastocyst stages (groups 1–4) ($p < 0.0001$). This group of embryos (group 0) had high fragmentation (28.9% ± 19.5% at start fragmentation and 42.7% ± 19.2% at their final fragmentation) compared with other embryos which developed at least to morula stage (groups 1–4) (Table 3).

In embryos which developed to the morula, start blastocyst, blastocyst, and expanded blastocyst stage at Day 5 (groups 1–4), a similar start fragmentation was measured (Table 3). The average final fragmentation rates in embryos which reached the morula or start blastocyst stage were similar (groups 1–2) (19.4% ± 10.3% and 20.1% ± 9.8%, respectively) but was significantly different from embryos at the blastocyst and expanded blastocyst stage (groups 3–4) (16.4 ± 10.4% and 14.2% ± 5.8%, respectively; $p < 0.005$) (Table 3).

Fragmentation and embryo fate

Embryos which were transferred, or frozen had a significantly lower start fragmentation (12.0% ± 7.1%) and final fragmentation (15.7% ± 8.6%) compared with discarded embryos. The discarded embryos had 25.8%

TABLE 4 The association between embryo selection fate and embryo fragmentation percentage.

Fate	% of start fragmentation	% of final fragmentation	Fragmentation ratio (worsening)
Transfer/Frozen ($n = 223$)			
Mean ± SD	11.8 ± 6.8	16.1 ± 9.0	1.5 ± 0.9
Median (IQR)	10 (7; 15)	15 (10; 20)	1.3 (1.0; 1.6)
Discarded ($n = 156$)			
Mean ± SD	26.1 ± 18.6	39.3 ± 18.9	1.9 ± 1.2
Median (IQR)	20 (12; 35)	40 (23; 55)	1.4 (1.2; 2.2)
p-Value	$p < 0.001$	$p < 0.001$	$p < 0.001$

Note: Mann–Whitney test.
Abbreviation: *n*, number of embryos.

TABLE 5 Start fragmentation cut off for embryo transfer/freezing or deselection.

	Transfer/frozen (percent of total)	Discard (percent of total)	Positive LB-KID (total LB-KID)
≤19.5% fragmentation (*n*)	197 (88.3%)	69 (44.2%)	29 (127)
>19.5% fragmentation (*n*)	26 (11.7%)	87 (55.8%)	1 (18)
Total embryo number (*n*)	223 (100%)	156 (100%)	30 (145)

Note: Start fragmentation of 19.5% is the cut off for embryo transfer/freezing or deselection (AUC: 0.77 95%CI [0.72–0.82]).
Abbreviation: *n*, number of embryos, LB, live birth, KID, known implantation data.

± 18.5% start and 38.8% ± 18.9% final fragmentation ($p < 0.0001$) (Table 4).

The fragmentation worsening was also found to be significantly higher among discarded embryos (×1.5) than among transferred or frozen embryos (×1.3) ($p < 0.0001$) (Table 4).

In a retrospective examination, using ROC analysis we found that 19.5% fragmentation on first cell division was the cut-off for embryo selection (AUC: 0.77 95%CI [0.72–0.82]; $p < 0.001$). More than 88% of the embryos which were transferred, or frozen had up to 19.5% start fragmentation. For final fragmentation, the cut-off was 27.5% fragmentation (AUC: 0.87 95%CI [0.84–0.91]; $p < 0.001$). More than 92% of the embryos which were transferred, or frozen had up to 27.5% final fragmentation (Tables 5, 6). ROC analysis (Figure S2A,B) shows that the chance for embryo selection for transfer or freezing decrease beyond 19.5% for start fragmentation and 27.5% for final fragmentation.

Patient's age and embryo fragmentation

The mean age of patients with Day 5 embryos with more than 5% fragmentation ($n = 379$ embryos) was 32.7 ± 5.4 years; similar to the entire study population of patients with Day 5 embryos during the study period (33.0 ± 5.0 years old [$n = 4210$ embryos]). Among these fragmented embryos, a significant difference in fragmentation was found between patients aged <35 compared with ≥35 years; start fragmentation was 16.5% ± 13.4% and 20.8% ± 17.1%, respectively ($p < 0.001$) and final fragmentation 23.3% ± 17% and 30.2% ± 19.3%, respectively ($p < 0.0001$) (Table 7). A correlation test between patient age and fragmentation showed a low but statistically significant correlation ($r = 0.23$; $p < 0.0001$).

ICSI and IVF

One hundred and forty-six embryos (52 patients) were IVF embryos, and 233 (89 patients) were ICSI embryos. No difference was found in fragmentation between embryos from IVF or ICSI. The start fragmentation in IVF embryos was 19.6% ± 17.7% compared with 18.4% ± 13.8% in ICSI embryos. The final fragmentation was 27.1% ± 20.4% in IVF embryos compared to 26.8% ± 16.8% in ICSI embryos.

Pregnancy and live birth rates

One hundred forty-one cycles included fragmented embryos (379 embryos). From those cycles, as high as 129 cycles included fragmented embryos that was selected for transfer of for freezing (Figure 1). Live birth (44%) and miscarriage (5%) rates were similar to the entire D5 embryo population (4210 embryos).

Among the 379 fragmented embryos, 145 are KID embryos from which 34 embryos were KID positive resulted in a live birth (Figure 1).

Interestingly, we found that 83% of the patients who had fragmented embryos, had additional fragmented embryos in that cycle and 57% of those patients, had fragmented embryos in a previous cycle (for 31% of these patients, this was their first and only cycle).

TABLE 6 Final fragmentation cut off for embryo transfer/freezing or deselection.

	Transfer/frozen (percent of total)	Discard (percent of total)	Positive LB-KID (total LB-KID)
≤27.5% fragmentation (*n*)	207 (92.8%)	47 (30.1%)	29 (132)
>27.5% fragmentation (*n*)	16 (7.2%)	109 (69.9%)	1 (13)
Total embryo number	223 (100%)	156 (100%)	30 (145)

Note: Final fragmentation of 27.5% is the cut off for embryo transfer/freezing or deselection (AUC: 0.87 95%CI [0.84–0.91]).
Abbreviations: *n*, number of embryos, LB, live birth, KID, known implantation data.

TABLE 7 Fragmentation percentage according to patients' age.

Age	% of start fragmentation	% of final fragmentation	Fragmentation ratio (worsening)
≤35 ($n = 257$)			
Mean ± SD	16.5 ± 13.4	23.3 ± 17.0	1.6 ± 1.0
Median (IQR)	13 (7; 20)	17 (11; 30)	1.4 (1.1; 1.8)
>35 ($n = 122$)			
Mean ± SD	20.8 ± 17.1	30.2 ± 19.3	1.7 ± 1.1
Median (IQR)	14.5 (10; 26.3)	25 (15; 45)	1.3 (1.1; 1.9)
p-Value	$p = 0.005$	$p < 0.001$	NS

Note: Mann–Whitney test was used.
Abbreviation: *n*, number of embryos.

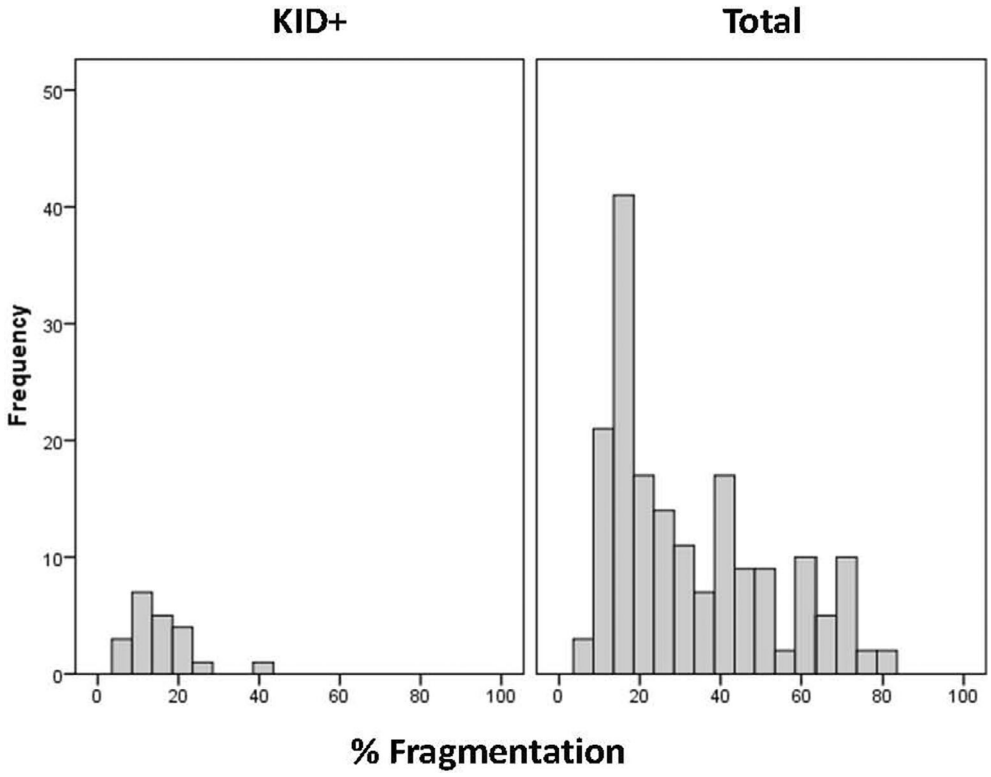

FIGURE 2 The frequency of embryo numbers according to fragmentation percentage in KID positive embryos (left side) and fragmentation division in total fragmented embryo populations.

KID positive embryos and fragmentation

No difference was found between start fragmentation, final fragmentation, and the fragmentation worsening, between the KID positive and KID negative embryos. All KID positive embryos which yield a live birth had up to approximately 30% fragmentation except for one case with 43% final fragmentation (Figure 2).

DISCUSSION

In our study we found that morphokinetics and embryo fragmentation were two independent variables. For embryos with a fragmentation of up to 32%, the main factor affecting the implantation potential was the morphokinetics and not the presence of fragments. Ultimately 58% of the fragmented embryos were transferred or frozen.

Evaluating fragmented embryos using time-lapse technology gives the possibility to compare morphologic parameters and morphokinetic parameters. Therefore, the aim of our study was to learn about the implantation potential of fragmented embryos that were transferred according to their morphokinetic time-lapse parameters and to evaluate what degree of fragmentation would still result in a clinical pregnancy and live birth.

Many efforts are invested in selecting the best embryo for single embryo transfer that will result in a live birth.[19] Fragmentation of human embryos is a common observation[14,28] and it is considered to be in correlation with a decreased chance for pregnancy.[14,29,30] Lately, morphokinetic time-lapse evaluation of embryos is a widespread tool used for embryo selection. We previously reported that the different morphokinetic quartiles are in correlation with clinical pregnancy and that shorter division timings yielded a significantly higher pregnancy rate.[24] Rhenman et al. found that the degree of fragmentation is an independent significant predictor for success rate. However, in their study, fragmentation was compared with other morphological parameters without morphokinetics.[4]

Chavez et al., suggested that the addition of fragmentation criteria such as the degree of cellular fragmentation in combination with cell cycle parameter might aid in embryo assessment.[8] We calculated fragmentation worsening as the ratio between final and start fragmentation. Fragmentation worsening was significantly higher in discarded embryos. We found that embryos with a low start fragmentation and high final fragmentation (meaning a high fragmentation worsening) have a higher chance to be discarded compared with embryos with a steady fragmentation rate throughout cell divisions. These results demonstrate that fragmentation worsening was an important factor in embryo selection and that once a fragmented embryo is selected, its chance to achieve a pregnancy is based mainly on its morphokinetics. Hardarson et al.[31] showed a case report where the blastomers of an embryo reabsorb two of its fragments. We did not find fragmentation reduction over time. Possibly, some embryos did reabsorb a few fragments, however, when measuring, the overall amount of fragments – we did not see a reduction over time.

Fragmentation in a way affects the chance of an embryo to reach blastulation, however, a fragmented embryo developing into an utilizable blastocyst has a high chance to implant. The correlation between embryo fragmentation and the developmental stage of the embryos was previously described and fragmented embryos were categorized according to their ability to develop into low- or high-quality blastocyst. Yu et al., found that fragmented embryos that developed into low quality blastocysts had significantly less blastomeres on Day 3.[32] Additionally, Yang et al. found that fragmentation and abnormal division significantly influenced blastocyst formation and despite the correlation between fragmentation and embryo development, 45% of the fragmented embryos still developed to a blastocysts.[33] It is known that some of the severely fragmented embryos with a poor implantation potential (according to their morphology on Day 2 and 3), may ultimately blastulate and "rescue themselves."[34] We previously described a case report, where an embryo with 43% fragmentation developed into a blastocyst, was transferred and resulted in a live birth of a healthy child.[35]

The correlation between fragmentation and maternal age is still controversial. In some studies increased embryo fragmentation was found to be correlated with advanced maternal age[15,28] whereas in others no correlation was found.[12,36,37] In this study the maternal age of patients who had fragmented embryos (above 5% fragmentation) was similar to the entire study population of patients with embryos that were cultured to the blastocyst stage. However, when examining the fragmented embryos ($n = 379$), start fragmentation and final fragmentation were significantly higher in older patients (>35 years) compared with younger patients.

It is suggested that oocyte quality is a key factor of embryo quality and that embryo fragmentation is correlated to oocyte cytoplasmic and nuclear competence.[7] A recent study using morphokinetics showed a correlation between embryo cell division timing and embryo quality including fragmentation with sperm quality.[38] We found no difference in fragmentation of embryos originating from ICSI or IVF, designating the possible role of the oocyte in embryo fragmentation as suggested by Ebner et al.[30,39]

Our results suggest that a fragmented embryo once implanted, has a high chance to yield a live birth. We noticed that the percentage of miscarriage and late abortion in fragmented embryos was similar to the rates following the transfer of non-fragmented embryos at our IVF unit. Kirillova et al. reached a similar conclusion, that poor quality embryos, once successfully implanted had the same potential to result in a live birth as embryos of fair and good quality.[40] Ebner et al. found that although fragmentation showed a significant influence on clinical pregnancy and implantation rate, no such relation was reported regarding late pregnancy concerning later complications.[30] Oron et al. also reported that poor quality embryos had a lower chance to implant, however, once implanted there was no difference in the obstetric or perinatal outcome and no increased risk of maternal or neonatal complications.[41]

Our study shows that fragmented embryos with up to 32% fragmentation should not be discarded and should be considered for transfer since they have an acceptable chance for live birth. Our findings may be applicable to laboratories with no use of time-lapse incubators basing their decision of embryo selection only on morphology. Embryo evaluation for fragmentation and the calculation of fragmentation worsening may enhance the ability to predict embryo development and lead to decreased embryo wastage.

AUTHOR CONTRIBUTIONS

All authors significantly contributed to the study and manuscript. Shirly Lahav-Baratz: contribution to the conception and design of the work, analysis, and interpretation of data for the work. Ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Writing the Manuscript. Idit Balis: contribution to the conception and design of the work. Revising the manuscript critically for important intellectual content and final approval of the version to be published. Mara Koifman: contributions to the conception and design of the work and analysis. Martha Dirnfeld: Drafting the work and revising it critically. Final approval of the version to be published. Galia Oron: Drafting the work and revising it critically. Final approval of the version to be published.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and in its online supplementary material. The data will be also shared on reasonable request to the corresponding author.

ORCID

Shirly Lahav-Baratz https://orcid.org/0000-0002-3870-3735

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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