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Non-invasive embryo evaluation and selection using time-lapse monitoring: Results of a randomized controlled study

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ABSTRACT

Objective: Continuous monitoring of embryos via time-lapse (TL) provides more information on embryo kinetics and morphology compared to standard daily evaluation. Embryo selection by TL could support single embryo transfer (SET). With SET multiple gestations are avoided and perinatal outcome is improved. Our primary goal was to determine whether selection of a single blastocyst based on an algorithm comprising kinetic and morphologic scores assessed through continuous TL monitoring results in superior clinical outcome compared to embryo selection based on morphology alone. A secondary goal was to assess whether a time-lapse score based on kinetic and morphologic parameters was predictive of implantation.

Study design: Randomized controlled trial performed in two private IVF centers in Hungary. Infertile couples scheduled to undergo 1st or 2nd IVF cycles were enrolled. Female age had to be under 36 years. The intervention was embryo evaluation/selection based on TL algorithm. Patients were randomized to SET with TL monitoring (TL-eSET) vs. SET with standard evaluation (control-eSET). Assuming an increase in pregnancy from 44% to 58%, a sample size of 202 per group was calculated based on the interim analysis at 10% information fraction. The primary outcome of the study was pregnancy rate. Secondary outcomes were miscarriage rates, live birth, perinatal outcome and the ability of a time-lapse score constructed based on kinetic and morphologic parameters to predict implantation.

Chi-square tests, likelihood-ratio tests and exact tests were used for the analysis of categorical variables. Continuous variables were compared using independent group *t*-test and analysis of variance.

Results: The study was closed after three years. Eventually 161 patients were randomized and analyzed (N = 80 TL-eSET and N = 81 control-eSET). Pregnancy rate did not significantly differ between the groups though there was a trend favoring TL selection (TL-eSET: 46.3% vs control-eSET: 34.6%, p = 0.150; OR: 1.628 (95% CI: 0.857–3.092)). The time-lapse score based on morphologic and kinetic parameters was significantly higher for blastocysts that implanted vs. those that did not (14.5 ± 1.8 vs. 12.1 ± 2.9, p = 0.0001). There were no adverse effects of the intervention.

Conclusions: Selection of a single blastocyst based on information derived from time-lapse monitoring can aid embryo selection for SET.

Introduction

In vitro fertilization (IVF) is considered to be successful when it leads to the birth of a healthy full-term singleton [1]. In order to maximize the chance of optimal IVF outcome, a single embryo should be transferred (SET) at a time: the embryo that has the highest chance of implantation. However, in the majority of the IVF cycles two or even more embryos are transferred worldwide [2,3]. In most embryology labs single daily evaluation of morphology is used for embryo selection [4]. Success rates are disappointingly low since only about 1/4th-1/3rd of the IVF cycles result in live birth [2,3]. Over the past 1–1.5 decades several methods have been evaluated as tools to improve embryo selection [5]. It is time-lapse (TL) technique and preimplantation genetic screening (PGS) that grow today as techniques with the potential to aid embryo evaluation [6–8].

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TL technology allows practitioners to continuously observe the embryos without removing them from the culture environment. Significantly more information can thus be collected on morphological changes and kinetics compared to single daily assessments. This additional information could help embryo selection [9–11]. To date, only few randomized controlled trials (RCT) have been published that evaluated the clinical benefits of TL systems [7,12–17]. There is considerable heterogeneity in the patient populations and methods that make it challenging to compare their findings. The latest Cochrane report, in 2015, reported that there was insufficient data to prove the clinical benefit of TL [17].

Our primary objective was to determine whether selection of a single blastocyst based on an algorithm comprising kinetic and morphologic scores assessed through TL monitoring improves clinical outcome compared to selection based on single morphology assessment score alone. A secondary objective was to assess whether a time-lapse score based on kinetic and morphologic parameters was predictive of implantation.

Materials and methods

This was a prospective, randomized, controlled study in which two embryo selection methods were compared: Group 1: TL algorithm and eSET (TL-eSET), Group 2.: standard morphology based selection for eSET (control-eSET). (IRB approval 11061-7/2011/HER; Clinical Trial Registry [NCT:01694641]).

The study was carried out in two private clinics between 10/2012-04/2015. Good prognosis infertile couples requiring IVF or intracytoplasmic sperm injection (ICSI) treatment were enrolled. Full inclusion-exclusion criteria are shown in Table 1. Cycles were considered drop-outs if there were <3 good quality embryos on day 3 or if there was only a single good quality blastocyst on day 5 as in these cases embryo selection per se was not applicable.

Written informed consent was obtained following verbal explanation of study aims and details. Randomization to Groups 1 or 2 was performed prior to stimulation, in 1:1 allocation ratio. The randomization list was generated by randomly selecting TL or control assignments from closed, opaque envelopes in blocks of two by the principle investigator. Embryologists who performed embryo evaluation, scoring and selection were not blinded.

None of the studies published before the start of our trial provided grounds for proper power calculations [9,10]. However we assumed a 30% relative increase in pregnancy rate (PR) from 42% (PR in the clinics the year before the study) to 55%. We planned to run the study with 1:1 ratio between the groups and to do one interim monitoring after 10% enrollment. Using two-sided test and Pocock boundaries for estimation, 282 patients in each group was calculated to achieve a power of 80%, at a significance level of 5%. Assuming a 10% dropout rate we needed to enroll 620 patients into the RCT.

Table 1
Study inclusion-exclusion criteria.

Inclusion criteria	Exclusion criteria
age <36 years	advanced stage endometriosis
1st/2nd IVF/ICSI treatment	polycystic ovary syndrome
autologous oocyte use	surgical sperm extraction
normal ovarian reserve (day-3 follicle stimulating hormone [FSH] level <10 IU/l)	PGD involved
regular cycles (21–35 days)	
acceptance of eSET	

Interim analysis

The PR in Group 1 was relatively 30% higher than in Group 2 (58.3% vs. 44.0%) at interim analysis (61 patients randomized, 49 completed). Assuming a 58% PR in Group 1 and 44% in Group 2, a sample size of 202 per group was calculated with two-sided test to achieve a power of 80%, at a significance level of 5%. Assuming 10% dropout rate a total sample of size of 446 (223 in each arm) was defined for completing the RCT.

Interventions

The embryos in the TL-eSET group were evaluated using the images from the time-lapse observation. A score was composed for this group comprising kinetic and morphological parameters as seen in time-lapse. The embryo with the highest score was selected for transfer. In the control group (control-eSET) embryos were selected based on morphology. All other interventions for the groups were identical and standard.

Embryos were cultured in groups in sequential culture medium (Origio Sequential Series, Origio AS, Denmark) using standard dishes (Primo Vision Dish, Vitrolife AB, Sweden). Same type standard incubators (Binder CB 60, Binder GmbH, Germany) were used for all cases (37 °C; 6% C02, 5% O2, 89% N2).

Embryo handling and manipulations outside of the incubator were identical: day 1: fertilization check; day 3: culture medium change; day 5: transfer. Embryos were morphologically assessed under an inverted microscope in all cycles on days 3 and 5. The morphological scores of the embryos in Group 1 were determined based on the TL images only, while in Group 2 the evaluation and selection was according to the Gardner score given based on the evaluation under an inverted microscope before transfer [18].

TL images were taken every 10 min; image stacks of 11 images were taken at different focal planes every 60 min. The Primo Vision software (Vitrolife AB, Sweden) was used for the analysis of images. The first appearance of the 2–5 blastomeres (t2–t5, respectively) were annotated. Timing of the kinetic events was calculated from the time of the fertilization (t0). T0 was defined for the ICSI as the midpoint of the time interval of the injection of the oocyte cohort while for IVF it was the time when the semen droplet was added to the oocytes. There were no adjustments of the kinetic events depending on the method of fertilization. Duration from fertilization until the end of the 1st cell cycle, the length of the 1st cytokinesis, and the length of the 2nd cell cycle were evaluated. A score was given for kinetic events and morphologic features for all embryos as shown in Table 2. The reference ranges were defined and fixed before the start of the recruitment, by September 2012, based on existing published literature and our own database [9,10,19].

Blastocyst morphology was assessed using the Gardner score, at the time-point when the embryo reached the most expanded stage in the TL sequence (Table 2b). Degree of fragmentation and cytoplasmic abnormalities were also recorded.

To adjust for the weight of abnormal early embryo development the final score was halved in case of direct cleavage from 1 to 3 cells, multinucleation at 2 or 4 cells or uneven blastomere size at 2 cells [9].

Embryo transfer was performed using soft catheters (Wallace, Smith Medical, UK) while surplus embryos were vitrified (Vitrifreeze, Fertipro, Belgium) in all groups.

Outcomes, data collection

The primary end point was PR per patient based on intention-to-treat. Further endpoints were ongoing PR (OPR), pregnancy loss, live birth rate (LBR), gestational age (GA) at delivery and birth weight. Furthermore, the ability of the time-lapse score to predict

Table 2
Time-lapse parameters and scores assigned to them; D5: day 5; MK: compact morula stage embryo; cf: cleavage furrow.

(a) Scoring system for evaluating embryo kinetics and morphology

	0 point	1 point	2 point
Duration from fertilization until the end of the 1st cell cycle: t2 – t0	<16 or >35 hours	16-20 or 32–35 hours	20–32 h
Length of the 1st cytokinesis: t2 – tcf	>45 min	35–45 min	0–35 min
Length of the 2nd cell cycle in the embryo: t3 – t2 (cc2a)	<6 or >14 h	6–8 or 12–14 h	8–12 h
The synchronicity of the two blastomere divisions within the second embryonic cleavage cycle: t4 – t3 (S2)	>90 min	70–90 min	0–70 min
Duration from fertilization until the 5-cell stage (t5 – t0)	<37 or >72 h	37–47 or 63–72 h	47–63 h
Fragmentation	>20%	transient/reabsorbing fragments or <20%	no fragmentation
Vacuolization	if vacuoles are present	if no vacuoles during the 5 days	–

(b) Scoring system for evaluating blastocyst morphology

	0 point	1 point	2 point	3 points
Blastocyst morphology on D5	Any other morphology	MK, 4BB, 3BB, 2BB	3AB, 2AB, 3BA, 2BA	3AA, 2AA, 4AB, 4BA, 4AA

implantation was evaluated within Group 1 by comparing the scores in cycles with or without implantation.

Data for baseline demographic characteristics, stimulation/laboratory parameters and treatment outcome were collected.

A βhCG level >10 IU/l was consistent with a biochemical pregnancy. The definitions of outcome parameters are shown in Table 3.

Statistical analysis

Chi-square tests, likelihood-ratio tests and exact tests were used for the analysis of categorical variables. Continuous variables were compared using independent group *t*-test and ANOVA. Normality and homogeneity of variances were examined. At the interim analysis of the trial the primary endpoint (PR) between Groups 1 and 2 was compared with Fisher's exact test. After closing the trial the primary endpoint in Groups 1 and 2 was compared with Fisher's exact test. Bonferroni correction was used for the p-value at the end of the trial.

All other p-values were considered exploratory in nature. During the analyses the extent and distribution of missing data were also examined. No imputations were made for missing data.

Analyses were performed using Stata 14 software (StataCorp. [2015]).

Results

Patient recruitment started in 10/2012, and was stopped in 04/2015. Follow-up of patients and collecting the data of all deliveries lasted until 10/2016.

The trial was stopped before reaching the target numbers for reasons apparently independent of trial findings. The accumulation of TL data during the years of our RCT led to the generation of algorithms with higher predicting power (KIDScore, described in Petersen et al., 2016). Patients would benefit more from the use of these new universally applicable algorithms [20]. Moreover, speed of recruitment was slower than originally calculated; only about 40% of the target sample size was reached in three years. In addition, the participating clinics were about to introduce laboratory changes that would have resulted in deviations from the study protocol. For this reason we have stopped using our composed score for embryo evaluation and consequently stopped the recruitment and proceeded with data collection and analysis.

In total, 161 patients were randomized to Groups 1 (n = 80) and 2 (n = 81). Twenty-two patients (Group 1: 12; Group 2: 10) dropped out for various reasons and 139 completed the trial (Patient flow chart see Fig. 1).

Demographic parameters were well balanced except for a 0.9-year age difference favoring the TL-eSET group. Response to stimulation and laboratory parameters were similar in Groups 1 and 2 (Table 4).

The pregnancy rate in TL-eSET was relatively 34% higher than in control-eSET though this difference did not reach statistical significance (46.3% vs. 34.6%, p = 0.150; OR: 1.628 (95% CI: 0.857–3.092)). Pregnancy loss rate was low and not different between Groups 1 and 2 (3.8% and 2.5%).

Within Group 1 the TL score was significantly higher in cycles that resulted in pregnancy when compared to those that failed (14.5 ± 1.8 vs. 12.1 ± 2.9, p = 0.0001).

Table 3
Definitions and endpoints used in during the trial.

Parameter	Definition
pregnancy rate (PR)	rise in β human chorionic gonadotropin [hCG] per patient based on intention-to-treat
ongoing pregnancy rate (OPR)	pregnancy that progresses beyond 12 weeks gestation per patient based on intention-to-treat
pregnancy loss	loss of pregnancy after an initial rise in βhCG up to 12 weeks gestation based on cycles started
live birth rate (LBR)	delivery of a live infant beyond 24 weeks per patient based on intention-to-treat
gestational age (GA) at delivery	weeks of gestation at delivery
good quality day 3 embryo	≥6 cells and <20% fragmentation on day 3
clinical pregnancy	intrauterine gestational sac 4 weeks after transfer
full term delivery	Delivery between 37th–42nd week of gestation
preterm delivery	delivery before the 37th week of gestation
very preterm delivery	delivery before the 32nd week of gestation
normal weight at birth	birth weight over 2500 g
low birth weight (LBW)	birth weight <2500 g
very low birth-weight (VLBW)	birth weight <1500 g

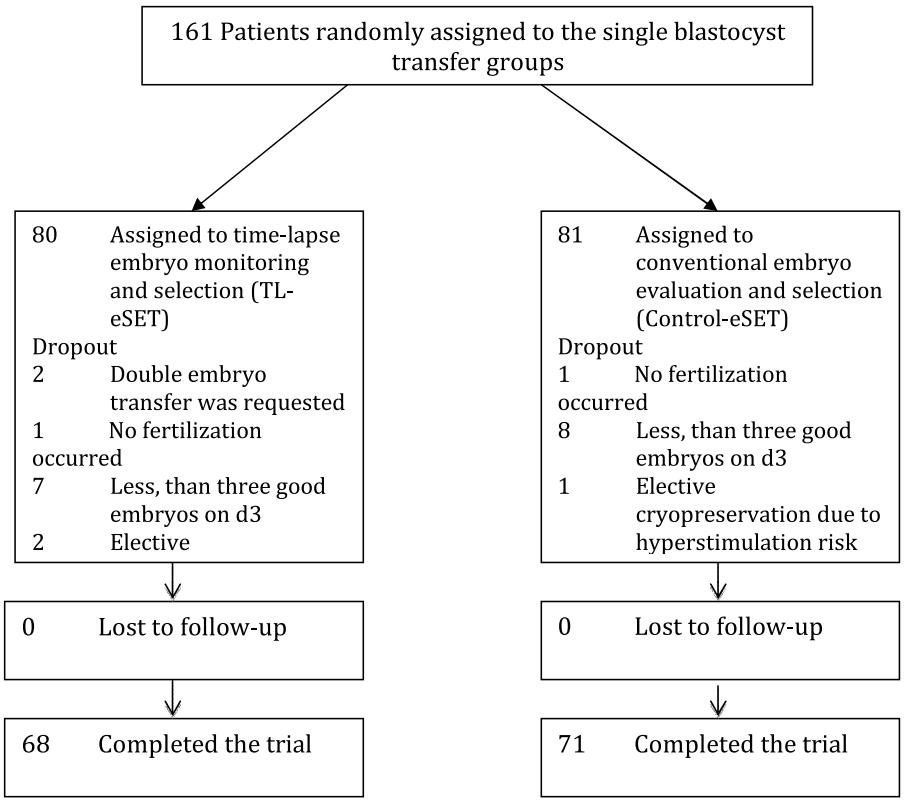

Fig. 1. Description of study groups and flow of patients.

Perinatal outcome was similar in the two groups; 91.2% (TL-eSET) and 88.4% (control-SET) of the deliveries were at full term. The percent of full term/ preterm deliveries as well the proportion of normal weight vs. low birth weight newborns were comparable in the two eSET groups (Table 5).

Comment

Our objective was to assess whether TL-algorithm based selection of a single blastocyst for transfer in good prognosis patients improved clinical outcome during IVF. We did find a trend favoring TL-algorithm based selection but the 34% relative increase in PR did not reach statistical significance. Our TL score is constructed based on early kinetic markers and parameters of blastocyst morphology. The score was significantly higher for embryos that implanted compared to those that failed to implant. While there was an overlap in the score when successful and unsuccessful cycles were considered this finding suggests that beyond the undisturbed culture conditions, algorithms based on parameters obtained via TL monitoring could aid embryo selection.

Identification of the embryo with the highest implantation potential would increase the motivation for single embryo transfer. The current gold standard involving a single daily assessment of embryos is not sufficiently effective [4].

Time-lapse monitoring is a non-invasive tool for embryo evaluation; it offers an order of a magnitude more data to assess embryo morphology compared to the routine daily observations, potentially leading to more reliable assessment of embryo quality and improved IVF outcome [21,22].

Table 4
Baseline demographic and stimulation parameters and clinical outcome in the study groups. N/A: not available.

	Group 1: TL-eSET (N = 80)	Group 2: Control-eSET (N = 81)	P value
Age (yrs)	31.2 ± 2.7	32.1 ± 2.5	0.03
Baseline FSH (IU/l)	6.9 ± 1.9	6.8 ± 1.4	0.84
BMI (kg/m2)	22.3 ± 3.3	22.2 ± 3.0	0.85
Gonadotropin dose (IU)	1484.0 ± 400.5	1482.2 ± 451.5	0.80
Peak E2 (pmol/l)	7481.0 ± 4175.7	7222.5 ± 3421.2	0.66
Follicles > 14 mm	10.0 ± 4.0	9.3 ± 3.5	0.29
Days of stimulation	11.0 ± 1.6	10.7 ± 1.9	0.32
Endometrial thickness (mm)	11.2 ± 1.9	11.0 ± 1.7	0.65
No. of oocytes	11.2 ± 4.4	10.8 ± 4.6	0.56
No. of MII oocytes	9.2 ± 4.0	9.0 ± 3.9	0.74
No. of fertilized oocytes	6.9 ± 3.4	7.2 ± 3.6	0.62
No. of good quality day 3 embryos	5.5 ± 6.0	5.2 ± 3.1	0.62
No. of good quality day 5 embryos	4.6 ± 5.0	4.3 ± 2.9	0.56
Pregnancy rate (%)	46.3%	34.6%	0.14
Ongoing pregnancy rate (%)	42.5%	32.1%	0.19
Live birth rate (%)	42.5%	32.1%	0.19

Table 5
Perinatal outcome in the study groups. None of the differences are significantly different.

	GA at delivery	PTD	VPTD	Mean birth weight (g ± SD)	LBW	VLBW
Group 1: TL-eSET (N = 34)	39.0 ± 2.3	2 (5.9%)	1 (2.9%)	3325 ± 486	0	1 (2.9%)
Group 2: Control-eSET (N = 26)	38.8 ± 2.5	2 (7.7%)	1 (3.9%)	3238 ± 603	1 (4 %)	1 (3.9%, min. 1240 g)

Although recent clinical studies reported higher implantation rates using TL technique, it has never been made clear whether improvements are due to the undisturbed culture conditions or to better embryo selection [7,9,23–25].

Our study differs from the previously published RCTs [7,12–16]. We used a well-defined study population and applied predefined cancellation criteria. The culture conditions and patient characteristics were similar for TL and control groups, thus the observed differences in PR can primarily be attributed to differences in the embryo selection strategies. When the groups were compared, we found a 34% relative increase in the clinical pregnancy rate in the TL group (OR: TL-eSET vs. control-eSET: 1.628; (95% CI: 0.857–3.092) though this finding was not significant.

Our TL algorithm combined scores for classic morphologic parameters as seen on TL and for kinetics. The score successfully identified the embryo with the highest implantation potential. In 50% of the cycles a different embryo was selected for transfer compared to what would have been selected based on morphology alone. The individual parameters/scores and their weights represent our knowledge as of 2012.

Our study also has important limitations. We restricted the study population to good prognosis cases therefore the conclusions can be applied to similar patients and cannot be generalized. Although the study was designed and conducted to avoid the risk of biases, the characteristics of the design and the study flow still raises the possibility of minor biases. The principle investigator randomized patients by the means of selecting TL or control assignments from closed, opaque envelopes. Randomization was in blocks of two that raises possibility for selection bias. Patients were blinded to their assignment but embryologists and gynecologist performing the transfer were not. Despite incomplete blinding, we feel that the outcomes (objective TL and outcome parameters) are unlikely to be influenced by lack of blinding.

Interim monitoring performed at 10% information fraction may also pose some risks, however it was compensated for in the analysis. There was a baseline imbalance (the average age of patients in the groups differed: 31.2 ± 2.7 vs. 32.1 ± 2.5), although the authors don't regard the age difference of 0.9 years in an overall young patient population clinically significant.

Patient recruitment to the randomized groups was stopped after three years for multiple reasons explained in the Results section. With the included sample size our study is underpowered to show a clear benefit of TL culture and algorithm based embryo selection. This however is true for most RCTs in the field [12,13,15]. The only properly powered study however suffers from other weaknesses [7]. Rubio et al., allowed cross-over from one arm of the study to the other in their RCT. They included a rather heterogeneous patient population (own vs. donated oocytes, fresh retrievals vs. frozen oocytes, day 3 vs. day 5 ET, SET vs. DET) and the incubation conditions were not defined for the control group. Yet the smaller RCTs can contribute useful information for a meta-analysis. Recently, Pribenszky et al., have published their meta-analysis based on the 5 RCTs that used time-lapse algorithm for embryo selection and compared it to standard, actual morphology based selection. The meta-analysis is based on the results of 1637 IVF cycles. Ongoing pregnancy rates were significantly higher (OR: 1.54; 95% CI: 1.21–1.96), pregnancy loss rates were lower (OR: 0.66; 95% CI:0.46–0.93) and live birth rates were also significantly higher (OR: 1.66; 95% CI: 1.13–2.45) in the TL group [26].

Conclusions

The use of TL systems is safe and offers significant advantages. We have shown that an algorithm based on time-lapse kinetic and morphologic markers can improve the embryologist's ability to select a single embryo for transfer. We found a trend for improved IVF outcome in eSET cycles that utilized a TL algorithm for embryo selection. The full benefit of TL systems (undisturbed culture conditions and algorithm-based embryo selection) will need to be evaluated using the latest algorithms with improved predictive ability.

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None.

Conflict of interest

CP is also active as senior scientist at Vitrolife AB Research Department.

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