Clinical outcomes of uninterrupted embryo culture with or without time-lapse-based embryo selection versus interrupted standard culture (SelecTIMO): a three-armed, multicentre, double-blind, randomised controlled trial

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Summary

Background Time-lapse monitoring is increasingly used in fertility laboratories to culture and select embryos for transfer. This method is offered to couples with the promise of improving pregnancy chances, even though there is currently insufficient evidence for superior clinical results. We aimed to evaluate whether a potential improvement by time-lapse monitoring is caused by the time-lapse-based embryo selection method itself or the uninterrupted culture environment that is part of the system.

Methods In this three-armed, multicentre, double-blind, randomised controlled trial, couples undergoing in-vitro fertilisation or intracytoplasmic sperm injection were recruited from 15 fertility clinics in the Netherlands and randomly assigned using a web-based, computerised randomisation service to one of three groups. Couples and physicians were masked to treatment group, but embryologists and laboratory technicians could not be. The time-lapse early embryo viability assessment (EEVA; TLE) group received embryo selection based on the EEVA time-lapse selection method and uninterrupted culture. The time-lapse routine (TLR) group received routine embryo selection and uninterrupted culture. The control group received routine embryo selection and interrupted culture. The co-primary endpoints were the cumulative ongoing pregnancy rate within 12 months in all women and the ongoing pregnancy rate after fresh single embryo transfer in a good prognosis population. Analysis was by intention to treat. This trial is registered on the ICTRP Search Portal, NTR5423, and is closed to new participants.

Findings 1731 couples were randomly assigned between June 15, 2017, and March 31, 2020 (577 to the TLE group, 579 to the TLR group, and 575 to the control group). The 12-month cumulative ongoing pregnancy rate did not differ significantly between the three groups: 50·8% (293 of 577) in the TLE group, 50·9% (295 of 579) in the TLR group, and 49·4% (284 of 575) in the control group (p=0·85). The ongoing pregnancy rates after fresh single embryo transfer in a good prognosis population were 38·2% (125 of 327) in the TLE group, 36·8% (119 of 323) in the TLR group, and 37·8% (123 of 325) in the control group (p=0·90). Ten serious adverse events were reported (five TLE, four TLR, and one in the control group), which were not related to study procedures.

Interpretation Neither time-lapse-based embryo selection using the EEVA test nor uninterrupted culture conditions in a time-lapse incubator improved clinical outcomes compared with routine methods. Widespread application of time-lapse monitoring for fertility treatments with the promise of improved results should be questioned.

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Introduction

The culture of embryos for medically assisted reproduction in an in-vitro fertilisation (IVF) laboratory usually involves regular morphological evaluations of the developing embryos outside the incubator. Nowadays, more and more IVF laboratories use time-lapse monitoring for the assessment of embryos. In time-lapse incubators, embryos are closely monitored by built-in cameras that take images at fixed time intervals resulting in videos of embryo development that can be analysed by embryologists, computer software, or artificial intelligence. Because embryo development is recorded continuously, and therefore in more detail than with standard assessments, important developmental events will not be missed.[1,2] With this method, embryo selection could be improved. In addition, as there is no need to remove embryos from the incubator for morphological assessments, stable culture conditions are provided. Potentially detrimental effects due to changes in temperature, gas concentration, or culture-medium pH are reduced while beneficial autocrine

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Research in context

Evidence before this study

Time-lapse monitoring is routinely offered to patients in in-vitro fertilisation (IVF) centres worldwide even though its clinical benefits are still controversial. Two mechanisms are thought to have a role in improving clinical results: the time-lapse-based embryo selection procedures and the uninterrupted culture conditions that are part of the system. Systematic reviews of the available data from earlier studies show that there is insufficient good quality evidence for improved pregnancy results and well designed randomised controlled trials are needed to assess the clinical value of time-lapse monitoring for IVF and intracytoplasmic sperm injection (ICSI) treatments.

Added value of this study

The SelecTIMO study is the largest, sufficiently powered, multicentre, randomised controlled trial on time-lapse monitoring to date providing cumulative ongoing pregnancy and livebirth results for a follow-up period of 1 year. The comparison of three treatment groups allows, for the first time in one study, the distinction of the two mechanisms suspected to improve clinical results.

Implications of all the available evidence

We found no evidence that time-lapse monitoring improves the cumulative ongoing pregnancy or livebirth rate within 1 year, time to pregnancy, or pregnancy results after fresh embryo transfer only. Neither embryo selection based on a time-lapse-based selection algorithm in combination with morphology nor the uninterrupted culture conditions in a time-lapse incubator improved clinical results after IVF or ICSI treatments. Our findings, together with the available evidence from earlier studies, suggest that the widespread application of time-lapse monitoring for IVF and ICSI treatments with the promise of improved outcomes should be questioned. In the absence of adequately designed and executed trials proving efficacy, the practice to financially charge patients for the use of time-lapse monitoring as an add-on cannot be justified.

and paracrine factors could be accumulated. Furthermore, embryo handling and the risk of cell damage or loss are minimised.[3,4]

Time-lapse-based embryo culture and selection is offered increasingly to couples undergoing IVF with the promise of improved clinical results.[5] However, systematic reviews have concluded that there is currently insufficient high-quality evidence supporting the routine use of time-lapse monitoring and that well designed randomised controlled trials (RCTs) are needed to assess its clinical value.[6–8]

We did a multicentre, double-blind, RCT to evaluate whether time-lapse monitoring can increase clinical results and if so, whether an improvement is caused by the time-lapse-based embryo selection method itself or the uninterrupted culture environment that is part of the system. A three-armed design along with culturing all embryos in the same incubator allows for a distinction between both suspected mechanisms for the first time in one RCT.[8]

Methods

Study design

The Embryo Selection Using Time-Lapse Monitoring (SelecTIMO) study was designed as a three-armed, multicentre, double-blind, RCT. The IVF laboratory procedures were done in five IVF laboratories in the Netherlands. Couples were recruited from 15 affiliated fertility clinics in the Netherlands. The trial protocol of this phase 4, interventional study was first approved on Dec 22, 2016, by the Central Committee on Research involving Human Subjects (The Hague, Netherlands) and by the board of directors of each participating clinic. The Netherlands Society of Obstetrics and Gynaecology consortium supported the trial and did independent audits. Good Clinical Practice guidelines and the principles of the Declaration of Helsinki were followed. Accuracy and completeness of the data and fidelity of the trial to the protocol are assured by the authors.

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Participants

Couples scheduled for their first, second, or third IVF or intracytoplasmic sperm injection (ICSI) oocyte retrieval cycle, who were planning to have a fresh single embryo transfer, were invited to participate. Couples could only participate in one IVF or ICSI oocyte retrieval cycle. The exclusion criteria were: (1) planned double embryo transfer; (2) planned freeze all cycle without a fresh embryo transfer; (3) participation in another scientific study; (4) use of donor gametes; (5) preimplantation genetic diagnosis; and (6) the use of thawed oocytes. Eligible couples were counselled about the study by their fertility doctors and received a patient information letter during their scheduled visits. Female age was not considered for inclusion, but all women were 42 years or younger since IVF treatment is only covered by Dutch health insurances until the age of 42. There was no upper or lower limit on the number of follicles present before and during stimulation. Participating couples provided written informed consent. Controlled ovarian hyperstimulation was done according to the local protocols of each fertility clinic.

Randomisation and masking

At least one follicle had to be present to be scheduled for oocyte retrieval. Randomisation was done centrally using Castor, a web-based, online, computerised randomisation service 1 day before or on the day of oocyte retrieval by a

laboratory technician or embryologist. Randomisation was stratified by laboratory site and oocyte retrieval cycle number. A random permuted block design with block size four, six, or eight was used to ensure a balanced allocation of couples to the three groups. Couples and physicians were unaware of treatment allocation but embryologists and laboratory technicians could not be masked.

Participating couples were randomly allocated to one of three groups. The first group, named time-lapse early embryo viability assessment (EEVA; TLE), received embryo selection based on the EEVA test and uninterrupted culture. The second group, named time-lapse routine (TLR), received routine morphological embryo selection and uninterrupted culture. The third group was the control group, which received routine morphological embryo selection and interrupted culture.

Procedures

All embryos in all three groups were cultured in a Geri+ time-lapse incubator (Genea Biomedx, Sydney, NSW, Australia) under 5% O_2. Geri+ incubator software (version 5.3–6.0), Geri connect software (version 1.0–2.0), and EEVA system (version 3.0–3.1) were used in this study. Images in 11 focal planes were captured for each embryo in the TLE and TLR group every 5 min with a total light exposure time of around 125 s per day per embryo. The EEVA[9] test is a day 3 time-lapse algorithm used adjunctively with morphology to predict blastocyst formation on day 3 without the need for extended culture until day 5. The goal was to investigate whether the application of the EEVA test on day 3 can be used as a substitute for extended blastocyst culture and lead to improved clinical results.

The five IVF laboratories adhered to the study protocol, but otherwise applied their own laboratory procedures (appendix p 1). Geri+ culture dishes for the TLE and TLR group, along with conventional dishes for the control group, were pre-equilibrated with culture medium overnight. After oocyte retrieval, oocytes of all three groups were cultured in the Geri+ incubator in standard dishes. Fertilisation by IVF or ICSI was done according to standard protocol of each participating IVF laboratory. Cumulus cells were removed from the oocytes and zygotes as much as possible during denudation on day 0 for ICSI and during the fertilisation check on day 1 for IVF. Fertilisation was checked in the morning of day 1 outside of the Geri+ incubator in all groups using conventional microscopes in laminar-flow cabinets with a temperature-controlled surface.

In the TLE and TLR group, zygotes were transferred to Geri dishes after the fertilisation check on day 1 of embryo development and were not removed from the Geri+ incubator until day 3, thereby providing uninterrupted culture conditions. In the control group, routine laboratory procedures were applied: embryo culture continued in standard dishes after fertilisation check and embryo morphology was assessed outside the Geri+ incubator once or twice a day, thereby providing interrupted culture conditions. Between day 1 and day 3, embryos in the control group were removed from the incubator on two additional occasions compared with those in the TLE and TLR groups.

Embryo morphology was assessed in all three groups by recording the number of blastomeres, degree of fragmentation, and blastomere symmetry between day 1 and day 3. The Geri+ images were used for morphological evaluations in the TLE and TLR group on a computer screen. A conventional microscope outside the Geri+ incubator was used for morphological evaluations in the control group.

In the TLE group, embryo selection was based on the EEVA test in combination with morphology: the results of the EEVA prediction report (1=highest EEVA result to 5=lowest EEVA result) were used together with morphological assessments to select a single embryo for transfer on day 3 based on a decision tree (appendix p 4). The embryologist or embryological technician, who did the embryo assessments on day 3, was also asked to identify the embryo that would be selected for embryo transfer based on morphology only, independent of the outcome of the EEVA test prediction report. In the TLR and control group, embryo selection on day 3 was based on routine morphological embryo selection procedures.

Embryo culture continued in the Geri+ incubator in all groups until the moment of cryopreservation. In the TLE group, embryos were selected for cryopreservation if they fulfilled the local freezing criteria and were ranked according to their EEVA test result in combination with morphology. This process meant that the embryos with a higher EEVA score were frozen and thawed first, followed by the embryos with a lower EEVA test result. Embryos exhibiting abnormal cleavage (ie, direct division of one cell into three cells or two cells into five cells) who fulfilled cryopreservation criteria were marked and cryopreserved to be thawed last.

See Online for appendix

In the TLR and control group, embryos that fulfilled local freezing criteria were cryopreserved and ranked based on their morphology. This way, the embryos with the best morphology were frozen and thawed first, followed by the embryos with inferior morphology.

The follow-up included the result of the fresh embryo transfer, all frozen–thawed embryo transfers from the initial oocyte retrieval cycle, and natural conceptions within 12 months of randomisation.

Outcomes

The co-primary endpoints were: (1) the cumulative ongoing pregnancy rate after fresh single embryo transfer and all transfers of cryopreserved embryos from the study cycle or natural conceptions occurring within 12 months in all women; and (2) the ongoing pregnancy rate after fresh single embryo transfer in a good prognosis

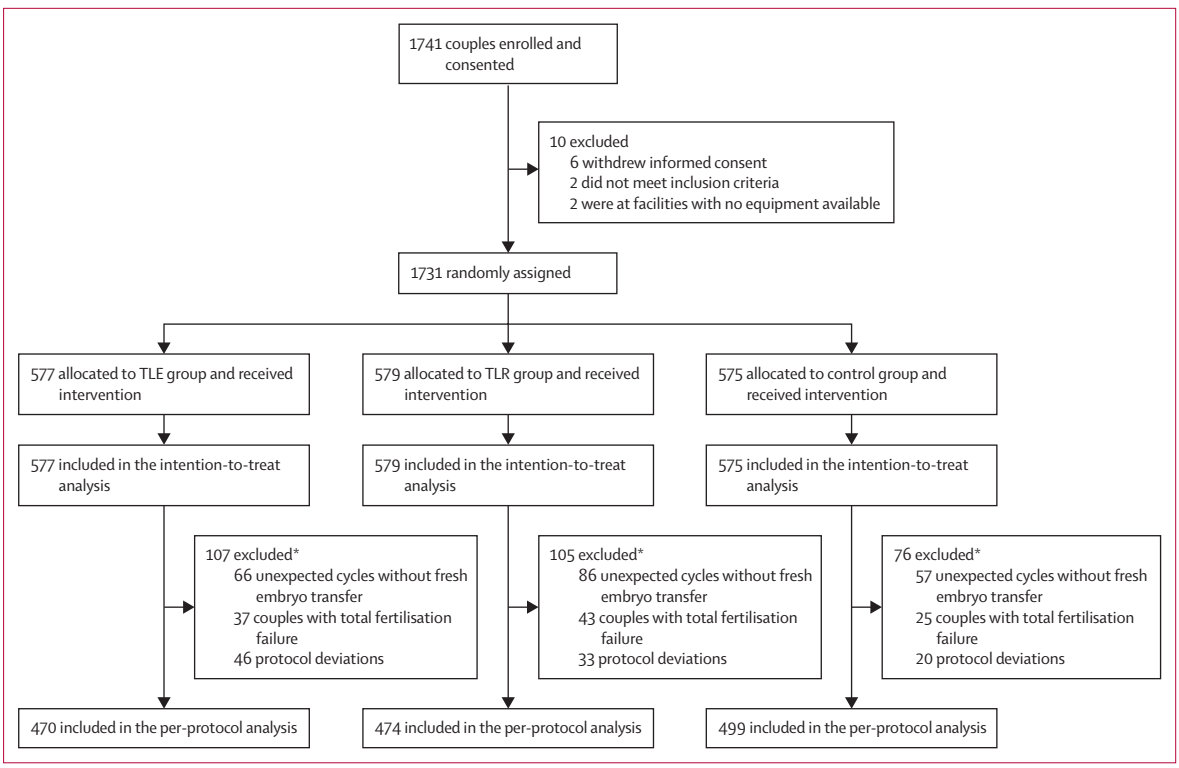

Figure 1: **Trial profile**
TLE=time-lapse early embryo viability assessment. TLR=time-lapse routine. *A couple could be excluded for more than one reason.

population defined as women younger than 41 years who had five or more oocytes and four or more fertilised oocytes. Secondary outcomes were positive hCG rate after fresh single embryo transfer, livebirth rate after fresh single embryo transfer, cumulative livebirth rate, miscarriage rate, time to pregnancy within 1 year, embryo morphology, embryo utilisation rate, and ongoing pregnancy rate in three female age subgroups.

Serious adverse events were reported to the coordinating investigator as well as ethical commitee and were analysed immediately.

Statistical analysis

The overall null hypothesis specified that all three embryo selection strategies would result in the same cumulative ongoing pregnancy rate. We assumed the 1-year cumulative ongoing pregnancy rate to be 27% with conventional procedures based on cumulative results of a Dutch IVF trial.[10] An absolute increase from 27% to 34·5% was expected for the cumulative ongoing pregnancy rate from time-lapse-based embryo selection based on the results of an earlier RCT.[11] Recruiting a study group of 1740 patients and allocating them on a 1:1:1 basis to the three groups would give us a power of 89% in rejecting the null hypothesis of no differences between the three groups for the cumulative ongoing pregnancy rate.

We tested the null hypothesis using a two-sided χ^2 test statistic at a 0·05 significance level, which allowed us to compare all three treatment groups. Subsequently, we did pairwise comparisons between the groups using logistic regression analysis. Odds ratios (ORs) and absolute differences were calculated with 95% CIs, adjusted for the stratification variables laboratory site and oocyte retrieval number. Kaplan-Meier survival curves were made for each treatment group to summarise time to ongoing pregnancy. Statistical analysis was based on the intention-to-treat principle.

We did a planned and prespecified subgroup analysis for three female age groups (<35·0, 35·0–38·9, ≥39·0 years) and a post-hoc subgroup analysis based on IVF laboratory site. A test for interaction was done for the two subgroup analyses. The effects of these subgroups were examined by adding the subgroup-by-treatment-group interaction parameters to the logistic regression model. We also did a per-protocol analysis using the same statistical methods. The per-protocol analysis excluded unexpected cycles without a fresh embryo transfer, couples with total fertilisation failures, and any instances of protocol deviations. A planned interim analysis was done by an independent statistician after 50% of the couples were randomly assigned. Because this analysis was done with the use of the Haybittle–Peto boundary principle, no adjustment was made in the final p values. All analyses were done with IBM SPSS statistics (version 28).

This trial is registered with WHO's ICTRP Search Portal under NTR5423.

For **trial registration** see https://trialsearch.who.int/Trial2.aspx?TrialID=NTR5423

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between June 15, 2017, and March 31, 2020, a total of 1741 couples consented and were enrolled in the trial (figure 1). Ten couples could not participate because informed consent was withdrawn in the laboratory phase (n=6), inclusion criteria were not fulfilled (n=2), or laboratory equipment was unavailable (n=2). The remaining 1731 couples were randomly assigned to one of the three study groups.

Baseline characteristics are shown in table 1. 1604 (92·7%) of 1731 couples were in their first oocyte retrieval cycle, 95 (5·5%) in their second, and 32 (1·9%) in their third. In total, 1522 (87·9%) of 1731 women had a fresh embryo transfer while 209 (12·1%) women did not, because of the risk of ovarian hyperstimulation syndrome (n=88, 42·1%), poor embryo development (n=38, 18·2%), or no fertilisation or gametes available (n=83, 39·7%).

The 12-month cumulative ongoing pregnancy rate was 50·8% (293 of 577) in the TLE group, 50·9% (295 of 579) in the TLR group, and 49·4% (284 of 575) in the control group (p=0·85; table 2). The corresponding OR was 0·99 (95% CI 0·79–1·25) for TLE versus TLR, 1·06 (95% CI 0·84–1·33) for TLR versus control, and 1·06 (95% CI 0·84–1·33) for TLE versus control. The number of women who had a livebirth after natural conception within the follow-up period was 17 (2·9%) of 577 in the TLE group, ten (1·7%) of 579 in the TLR group, and 11 (1·9%) of 575 in the control group. Detailed clinical results after fresh embryo transfer are shown in table 2 and pregnancy follow-up in the appendix (appendix p 2).

The ongoing pregnancy rate after fresh single embryo transfer in a predefined population with good prognosis was 38·2% (125 of 327) in the TLE group, 36·8% (119 of 323) in the TLR group, and 37·9% (123 of 325) in the control group (p=0·90). The corresponding OR was 1·06 (95% CI 0·77–1·46) for TLE versus TLR, 0·96 (95% CI 0·69–1·31) for TLR versus control, and 1·02 (95% CI 0·74–1·40) for TLE versus control. Consistent results were found for all clinical secondary study outcomes (table 2).

A planned subgroup analysis of the cumulative ongoing pregnancy rate in three female age groups revealed an interaction between female age and treatment group, indicating that the outcome differed between these age groups (p=0·02; table 2). In women aged 39 years and older (n=245), the cumulative ongoing pregnancy rate was 40·0% (32 of 80) in the TLE group, 23·7% (18 of 76) in the TLR group, and 31·5% (28 of 89) in the control group (TLE *vs* TLR: OR 2·10, 95% CI 1·05–4·21 and TLE *vs* control: OR 1·44, 95% CI 0·76–2·71). No significant differences were found for the two other female age groups.

	TLE group (n=577)	TLR group (n=579)	Control group (n=575)
Female age (years)	34·1 (4·1)	34·1 (4·1)	34·3 (4·2)
Female BMI (kg/m²)	24·7 (5·0)	24·4 (4·5)	24·8 (4·8)
Female smoking behaviour			
Yes	59 (10·2%)	69 (11·9%)	70 (12·2%)
No	492 (85·3%)	485 (83·8%)	479 (83·3%)
Unknown	26 (4·5%)	25 (4·3%)	26 (4·5%)
Male smoking behaviour			
Yes	108 (18·7%)	104 (18·0%)	107 (18·6%)
No	405 (70·2%)	416 (71·8%)	405 (70·4%)
Unknown	64 (11·1%)	59 (10·2%)	63 (11·0%)
Pregnancy history			
Previous ongoing pregnancy	191 (33·1%)	151 (26·1%)	169 (29·4%)
Previous miscarriage, abortion, EUG	171 (29·6%)	144 (24·9%)	175 (30·4%)
Reason for IVF or ICSI			
Male factor	232 (40·2%)	245 (42·3%)	224 (39·0%)
Female factor	141 (24·4%)	141 (24·4%)	149 (25·9%)
Male and female factor	36 (6·2%)	36 (6·2%)	37 (6·4%)
Unexplained	154 (26·7%)	146 (25·2%)	154 (26·8%)
Other	14 (2·4%)	11 (1·9%)	11 (1·9%)
Duration of infertility (months)	34·6 (22·3)	31·5 (21·3)	32·7 (26·0)
Total FSH dose	1675·9 (2599·4)	1702·5 (2437·1)	1841·6 (1281·2)
Pre-treatment			
Yes (OAC or progestative)	443 (76·8%)	453 (78·2%)	446 (77·6%)
No	134 (23·2%)	126 (21·8%)	129 (22·4%)
Premature LH surge prevention			
GnRH antagonist	95 (16·5%)	92 (15·9%)	87 (15·1%)
GnRH agonist	478 (82·8%)	485 (83·8%)	486 (84·5%)
Stimulation protocol			
Follitropin alfa	519 (89·9%)	504 (87·1%)	501 (87·1%)
Menotrophin	22 (3·8%)	30 (5·2%)	29 (5·0%)
Follitropin beta	2 (0·4%)	0	1 (0·2%)
Urofollitropin	14 (2·4%)	9 (1·6%)	13 (2·3%)
Other	20 (3·5%)	36 (6·2%)	31 (5·4%)
Fertilisation method			
IVF	312 (54·1%)	293 (50·6%)	316 (55·0%)
ICSI	255 (44·2%)	265 (45·8%)	252 (43·8%)
Both	6 (1·0%)	12 (2·1%)	6 (1·0%)
Oocyte retrieval number			
Oocyte retrieval 1	537 (93·1%)	540 (93·3%)	527 (91·7%)
Oocyte retrieval 2	29 (5·0%)	28 (4·8%)	38 (6·6%)
Oocyte retrieval 3	11 (1·9%)	11 (1·9%)	10 (1·7%)

Data are n (%) or mean (SD). EUG=extrauterine gestation. FSH=follicle-stimulating hormone. GnRH=gonadotropin-releasing hormone. ICSI=intracytoplasmic sperm injection. IVF=in-vitro fertilisation. LH=luteinising hormone. OAC=oral anticontraceptive. TLE=time-lapse early embryo viability assessment. TLR=time-lapse routine.

Table 1: **Baseline characteristics of the intention-to-treat population**

The time to pregnancy within the 12-month follow-up period was comparable between the three treatment groups (p=0·96; figure 2).

In the TLE group, the embryologist was asked to identify which embryo would be selected based on morphology only and the embryologist and EEVA agreed on 246 (62%) of 395 fresh embryo transfers.

The embryo utilisation rate was 74·8% (2362 of 3160) in the TLE group, 77·2% (2494 of 3229) in the TLR group, and 74·3% (2390 of 3218) in the control group (p=0·87; appendix p 2). A total of 838 women had a livebirth of which 13 cases were twin deliveries (appendix p 2).

The per-protocol analysis also revealed no significant differences between the treatment groups (appendix p 3). A post-hoc subgroup analysis found no interaction between laboratory site and treatment group (p=0·65) with similar results between the three treatment groups in each laboratory (appendix p 3). A total of ten serious adverse events were reported which were not related to the study procedures (five in TLE, four in TLR, and one in the control group).

	TLE group	TLR group	Control group	TLE *vs* TLR		TLR *vs* control		TLE *vs* control	
				OR (95% CI)	AD (95% CI)	OR (95% CI)	AD (95% CI)	OR (95% CI)	AD (95% CI)
Cumulative results (intention to treat)									
Cumulative positive hCG rate	357/577 (61·9%)	355/579 (61·3%)	357/575 (62·1%)	1·02 (0·81 to 1·30)	0·56 (–5·78 to 6·89)	0·96 (0·76 to 1. 22)	–0·77 (–7·11 to 5·57)	0·99 (0·78 to 1·26)	–0·22 (–6·56 to 6·13)
Cumulative clinical pregnancy rate	328/577 (56·9%)	336/579 (58·0%)	328/575 (57·0%)	0·95 (0·76 to 1·20)	–1·19 (–7·63 to 5·26)	1·04 (0·82 to 1·32)	0·99 (–5·47 to 7·44)	0·99 (0·78 to 1·25)	–0·20 (–6·66 to 6·26)
Cumulative OPR	293/577 (50·8%)	295/579 (51·0%)	284/575 (49·4%)	0·99 (0·79 to 1·25)	–0·17 (–6·69 to 6·35)	1·06 (0·84 to 1·33)	1·56 (–4·96 to 8·08)	1·06 (0·84 to 1·33)	1·39 (–5·14 to 7·92)
Cumulative livebirth rate	281/577 (48·7%)	280/579 (48·4%)	277/575 (48·2%)	1·01 (0·81 to 1·28)	0·34 (–6·17 to 6·86)	1·01 (0·80 to 1·27)	0·19 (–6·33 to 6·71)	1·02 (0·81 to 1·29)	0·53 (–6·00 to 7·05)
Cumulative miscarriage rate	92/577 (15·9%)	94/579 (16·2%)	100/575 (17·4%)	0·98 (0·71 to 1·34)	–0·29 (–5·13 to 4·55)	0·92 (0·68 to 1·25)	–1·16 (–6·00 to 3·69)	0·90 (0·66 to 1·23)	–1·45 (–6·29 to 3·40)
Fresh embryo transfer results (intention to treat)									
Positive hCG rate	217/577 (37·6%)	213/579 (36·8%)	233/575 (40·5%)	1·03 (0·82 to 1·31)	0·82 (–5·51 to 7·1)	0·85 (0·67 to 1·08)	–3·73 (–10·01 to 2·0)	0·88 (0·70 to 1·12)	–2·91 (–9·26 to 3·43)
Clinical pregnancy rate	199/577 (34·5%)	200/579 (34·5%)	206/575 (35·8%)	1·00 (0·78 to 1·27)	–0·05 (–6·27 to 6·16)	0·94 (0·74 to 1·20)	–1·28 (–7·50 to 4·94)	0·94 (0·74 to 1·20)	–1·34 (–7·56 to 4·89)
OPR	171/577 (29·6%)	170/579 (29·4%)	180/575 (31·3%)	1·01 (0·79 to 1·31)	0·28 (–5·70 to 6·25)	0·91 (0·71 to 1·17)	–1·94 (–4·04 to 7·93)	0·92 (0·72 to 1·19)	–1·67 (–7·65 to 4·32)
Livebirth rate	164/577 (28·4%)	163/579 (28·2%)	175/575 (30·4%)	1·01 (0·78 to 1·31)	0·27 (–5·64 to 6·18)	0·90 (0·69 to 1·15)	–2·28 (–8·20 to 3·64)	0·91 (0·70 to 1·17)	–2·01 (–7·94 to 3·91)
Miscarriage rate	49/577 (8·5%)	46/579 (7·9%)	54/575 (9·4%)	1·08 (0·71 to 1·64)	0·55 (–3·11 to 4·20)	0·80 (0·55 to 1·80)	–1·45 (–5·11 to 2·21)	0·89 (0·60 to 1·34)	–0·90 (–4·56 to 2·76)
Fresh embryo transfer results (good prognosis*)									
Positive hCG rate	155/327 (47·4%)	147/323 (45·5%)	153/325 (47·1%)	1·08 (0·71 to 1·64)	1·89 (–6·79 to 10·57)	0·94 (0·69 to 1·28)	–1·57 (–10·26 to 7·13)	1·01 (0·74 to 1·37)	0·32 (–8·99 to 8·35)
Clinical pregnancy rate	142/327 (43·4%)	137/323 (42·4%)	138/325 (42·5%)	1·04 (0·76 to 1·42)	1·01 (–7·60 to 9·62)	0·99 (0·73 to 1·35)	–0·05 (–8·67 to 8·58)	1·04 (0·76 to 1·41)	0·96 (–7·63 to 9·56)
OPR	125/327 (38·2%)	119/323 (36·8%)	123/325 (37·9%)	1·06 (0·77 to 1·46)	1·38 (–7·05 to 9·82)	0·96 (0·69 to 1·31)	–1·00 (–9·45 to 7·44)	1·02 (0·74 to 1·40)	0·38 (–8·03 to 8·79)
Livebirth rate	119/327 (36·4%)	115/323 (35·6%)	121/325 (37·2%)	1·05 (0·76 to 1·45)	1·10 (–7·27 to 9·47)	0·92 (0·67 to 1·27)	–1·94 (–10·32 to 6·44)	0·96 (0·70 to 1·32)	–0·84 (–9·20 to 7·52)
Miscarriage rate	32/327 (9·8%)	30/323 (9·3%)	30/325 (9·2%)	1·05 (0·62 to 1·77)	0·50 (–4·59 to 5·59)	1·01 (0·59 to 1·72)	0·06 (–5·04 to 5·15)	1·06 (0·63 to 1·79)	0·56 (–4·52 to 5·64)
Cumulative OPR in three age groups†									
Age <35·0 years	182/334 (54·5%)	201/331 (60·7%)	175/312 (56·1%)	0·77 (0·57 to 1·05)	–6·23 (–14·74 to 2·27)	1·20 (0·88 to 1·67)	4·64 (–4·02 to 13·29)	0·94 (0·69 to 1·28)	–1·60 (–10·22 to 7·03)
Age 35·0–38·9 years	79/163 (48·5%)	76/172 (44·2%)	81/174 (46·6%)	1·17 (0·76 to 1·80)	4·28 (–7·84 to 16·40)	0·92 (0·60 to 1·41)	–2·37 (–14·29 to 9·56)	1·08 (0·70 to 1·65)	1·92 (–10·18 to 14·01)
Age ≥39·0 years	32/80 (40·0%)	18/76 (23·7%)	28/89 (31·5%)	2·10 (1·05 to 4·21)	16·32 (–0·20 to 32·83)	0·68 (0·34 to 1·37)	–7·78 (–23·88 to 8·33)	1·44 (0·76 to 2·71)	8·54 (–7·40 to 24·48)

Data are n/N (%) unless otherwise specified. Positive hCG was defined as hCG >50 IU/l or positive home pregnancy test 14–17 days after oocyte retrieval. Clinical pregnancy was defined as a gestational sac 5–8 weeks after oocyte retrieval. Ongoing pregnancy was defined as a viable intrauterine pregnancy with fetal heartbeat 10–12 weeks after oocyte retrieval. Livebirth was defined as a delivery resulting in a liveborn child. Miscarriage was defined as pregnancy loss after pregnancy was determined by positive hCG (<21 weeks). Definitions for cumulative pregnancy and miscarriage rates were the same with the addition that at least one pregnancy or miscarriage occurred during the follow-up period of 1 year. Each woman could have multiple pregnancies due to the follow-up period of 1 year. ORs and absolute differences were adjusted for laboratory site and oocyte retrieval number. AD=absolute difference. hCG=human chorionic gonadotropin. OPR=ongoing pregnancy rate. OR=odds ratio. TLE=time-lapse early embryo viability assessment. TLR=time-lapse routine. *Good prognosis was defined as the woman being younger than 40·9 years, day 3 transfer with at least five oocytes and a minimum of four fertilised oocytes. †The planned subgroup analysis of three female age groups revealed interaction between age group and treatment on cumulative OPR (p=0·02).

Table 2: **Cumulative and fresh embryo transfer results**

Discussion

We did the largest double-blind, multicentre RCT on time-lapse monitoring to date and the first with a three-armed design in a single embryo transfer setting to evaluate the performance of the Geri+ time-lapse incubator and EEVA test selection algorithm. The comparison of three study groups and culture of all embryos in the same incubator allowed us to distinguish the effect of the time-lapse-based embryo selection method from the effect of the uninterrupted culture environment. The results of this RCT provide no evidence that the use of a time-lapse monitoring incubator in an IVF laboratory increases the cumulative ongoing pregnancy or livebirth rate compared to standard embryo culture and selection.

The added value of the time-lapse-based embryo selection procedures was analysed by directly comparing the cumulative results achieved in the TLE and TLR group. Embryos were cultured uninterruptedly in both groups and the only difference was the embryo selection method. Time-lapse-based embryo selection did not increase the cumulative ongoing pregnancy rate or time to pregnancy within the follow-up period of 1 year. Whether time-lapse-based embryo selection or any other selection method will ever be able to improve the cumulative ongoing pregnancy or livebirth rate is questionable because most available good quality embryos are transferred at some point thanks to increasingly effective cryopreservation programmes.[12] If embryo selection using time-lapse monitoring is indeed beneficial we would at least expect an increase of the ongoing pregnancy rate after fresh embryo transfers on day 3 and a reduced time to pregnancy in the TLE group, but this was not confirmed by our data. In the TLE group, the embryologist and EEVA agreed which embryo to select for fresh embryo transfer in 62% of the couples who had more than one morphologically good quality embryo, indicating that laboratories might have already established an optimised embryo selection method based on static observations. Three previous RCTs reached similar conclusions regarding embryo selection based on time-lapse parameters when culture conditions were identical in both groups. Kaser and colleagues[13] reported no evidence that the adjunctive use of the EEVA test on day 3 or day 5 improves the ongoing pregnancy rate compared with morphological embryo selection on day 5. However, this pilot RCT was terminated prematurely after including only about 68% of the planned patients. Two other RCTs also found no increased clinical or ongoing pregnancy rates by time-lapse monitoring with the Embryoscope.[14,15] However, the sample sizes of these studies were considerably smaller than ours, no cumulative results were reported, and the study by Ahlström and colleagues[15] terminated inclusions prematurely after reaching 47% of the planned inclusions. The most recent Cochrane review,[7] which included nine RCTs (2955 couples), concluded that there is currently insufficient evidence that time-lapse monitoring is more effective than conventional methods, and that the included studies were at high risk of bias for randomisation and allocation concealment. None of the RCTs included in the Cochrane review provided cumulative results.

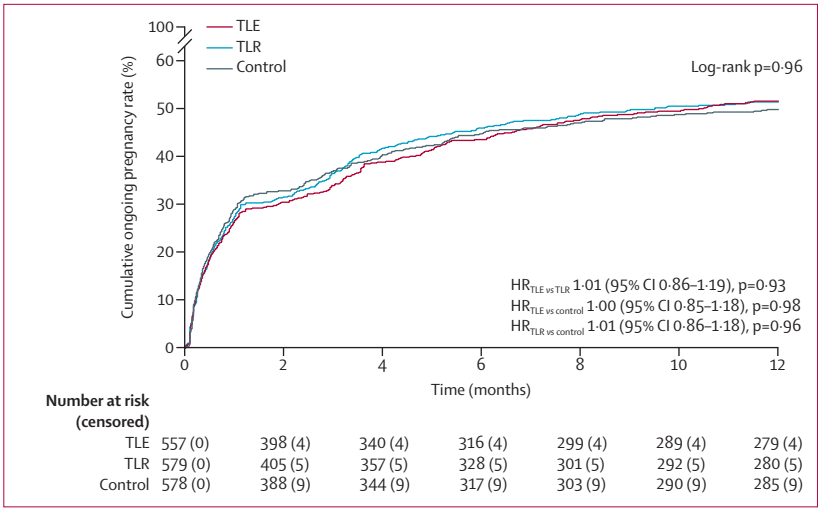

Figure 2: **Time to pregnancy**
Ongoing pregnancy rate over time with numbers at risk. HR=hazard ratio. TLE=time-lapse early embryo viability assessment. TLR=time-lapse routine.

The potential benefit of the uninterrupted culture conditions was tested by comparing uninterrupted embryo culture in the TLR group to interrupted culture in the control group when embryo selection was based only on morphology in both groups. We found no significant differences in terms of cumulative ongoing pregnancy or livebirth rate between uninterrupted and interrupted culture. Our study has the advantage that the same incubator was used for embryo culture in all groups. However, we were only able to study the effect of undisturbed embryo culture between day 1 and day 3. Four other RCTs[16–19] compared uninterrupted embryo culture with traditional culture using a varying number of uninterrupted culture days but used different types of incubators in both groups. Nevertheless, none of these studies found increased pregnancy results for uninterrupted embryo culture between 2 and 5 days. Three RCTs on time-lapse monitoring have not been able to analyse the effect of the time-lapse-based selection method independently from the culture conditions, and therefore should be interpreted with caution.[11,20,21]

We did subgroup analyses of three female age groups to study whether a particular age group would benefit from the application of time-lapse monitoring more than others and found significant interaction between intervention and age group. Women aged 39 years and older were 2·1 times more likely to become pregnant within 12 months using time-lapse-based embryo selection in the TLE group (40·0%) compared with morphological embryo selection in the TLR group (23·7%). However, the difference between the TLE group and control group was not statistically significant for this age group. As this is a subgroup finding, it needs

to be confirmed in future trials with a larger sample size, but these results could also be attributed to chance.

To ensure generalisability of our findings, we applied broad inclusion criteria and enrolled couples from multiple clinical sites. Although each laboratory used the same time-lapse incubator model (Geri+), settings for CO_2 and temperature, as well as choice of culture medium, laboratory protocols, and cryopreservation techniques differed between laboratories. Furthermore, each laboratory used their own conventional dishes for embryo culture in the control group. These differences between the laboratories should be considered a strength of our study as they represent the variation that is present in everyday IVF practice. The clinical results in the TLE group showed some variance between the five IVF laboratories, which questions whether the EEVA test performs equally well under different laboratory conditions since timing parameters might be affected by culture conditions.[22,23] However, no statistically significant differences were found between the three groups per laboratory, which shows similar performance of the interventions under different laboratory circumstances.

Cumulative ongoing pregnancy rate was selected as one of our co-primary endpoints based on a Dutch IVF trial published in 2013 that reported cumulative results.[10] We expected a cumulative ongoing pregnancy rate of 27% in the control group, however, we found an average cumulative ongoing pregnancy rate of 50·4% in all three groups. This discrepancy can be explained by the improvement of cryopreservation methods.

Livebirth rate is predominantly being used as primary endpoint nowadays, but the most appropriate primary outcome measure in reproductive medicine is still subject to debate.[24] We selected cumulative ongoing pregnancy rate as the most relevant endpoint in our study, which also had the advantage of a timely interim analysis after 50% of the inclusions. Furthermore, we observed similar outcomes for cumulative ongoing pregnancy and livebirth rates in all three groups.

A possible limitation of our study is that EEVA test results were only applied for the selection of embryos with good morphology in the TLE group, while it may be more meaningful to apply EEVA test results for all embryos of a patient. However, such an embryo selection strategy would not be considered ethical because of the known correlation of embryo morphology and implantation. Additionally, the EEVA test was designed to be used in adjunction with morphology. All good quality embryos that had at least six cells and less than 20% fragmentation on day 3 were reviewed in the TLE group to check whether the system identified each cleavage event correctly and a manual update was done if necessary. Although abnormal (ie, direct) cleavage patterns were identified, reverse cleavage events could not be detected this way.

We were only able to study the performance of one time-lapse system, which questions whether our results are generalisable to other time-lapse systems, because the culture system and the methods for analysing embryos differ between systems. Further development, such as the addition of artificial intelligence in the algorithms that analyse the images and select embryos, is an ongoing process, while the planning, execution, and follow-up of RCTs could take a long time. Therefore, systems could be considered outdated when results from RCTs become available. Whether other time-lapse selection models, algorithms, or artificial intelligence will result in similar outcomes is unknown, but new methods cannot automatically be expected to perform better, especially in terms of cumulative results. Despite the details differing, the underlying principles of time-lapse monitoring remain broadly the same. Although new time-lapse systems could be more accurate in evaluating embryos, it is unlikely that this will lead to any relevant differences for embryo selection from the cohort of available embryos per patient. Our study has shown that the additional information from video recordings and the stable culture conditions that are part of the system do not lead to improved clinical results. If sufficient preclinical evidence is present, adequately designed and powered RCTs such as our study must be done with proof of efficacy before clinical application of new time-lapse systems can be considered.[25]

The introduction of innovations in routine clinical practice of reproductive medicine often precedes the RCTs that should evaluate them.[25] IVF laboratories around the world hope to increase pregnancy rates by offering time-lapse-based embryo selection or embryo culture until day 5 to enable self-selection of viable embryos. Whether extended culture can indeed increase cumulative pregnancy results is uncertain,[26] while blastocyst transfers have been associated with more premature births.[26,27] Our data also show that time-lapse-based embryo selection using the EEVA selection method and uninterrupted culture conditions cannot improve cumulative pregnancy and livebirth rates or the time to pregnancy. Although time-lapse monitoring could potentially have other benefits, the high costs of a time-lapse incubator and disposables must be taken into consideration in the absence of a clinical benefit. Furthermore, time-lapse annotations can result in additional workload if they are not automated or need to be reviewed by laboratory personnel. For these reasons, a cost effectiveness analysis is currently being done on our data. In conclusion, our results show that the widespread application of time-lapse monitoring with the promise of improved clinical outcomes should be questioned for the general IVF population. Moreover, the practice to financially charge patients extra for the use of time-lapse monitoring can no longer be justified.

Contributors

DCK was responsible for study design, seeking ethical approval, overall logistics, data analysis, and writing of the manuscript as project leader and principal investigator. MvW was the study methodologist and responsible for study design, discussion, statistical power calculations, and data analysis. DCK and MvW did the data analysis. CBL was the coordinating investigator and responsible for study design, logistics,

discussion, and drafting of the manuscript. CGV, LR, MHJMC, ES, EHK, MHECP, DC, FB, BJC, JMJS, and SM were principal investigators and were responsible for the design of the study. All authors contributed to the execution of the study, review of the text, and final approval of the manuscript.

Declaration of interests

DCK received the Fertility Society of Australia exchange award. MHJMC reports an unrestricted grant for implementing Value Based Healthcare paid to their institution and a personal speakers fee from Merck (Netherlands). FB reports a research support grant from Merck (Netherlands), Health Care Efficiency Research program grant from the Netherlands Organisation for Health Research and Development, speaker fees and scientific dinner symposium from Besins Healthcare Monaco, and is a member of the advisory board for Merck (Netherlands) and Ferring (Netherlands). CHdK reports a donation from Merck (Netherlands) for the European Society of Human Reproduction and Embryology annual meeting 2022 in Milan. MvW is coordinating editor of the Cochrane Gynecology and Fertility Group. CBL is Editor-In-Chief for Human Reproduction. All other authors declare no competing interests.

Data sharing

Restricted access to the study data can be arranged on request to the corresponding author. Written proposals will be assessed by the SelecTIMO study group. A data sharing agreement including terms and conditions for authorship and publication will have to be signed before making the data available.

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