Clinical effectiveness and safety of time-lapse imaging systems for embryo incubation and selection in in-vitro fertilisation treatment (TILT): a multicentre, three-parallel-group, double-blind, randomised controlled trial

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Summary

Background Time-lapse imaging systems for embryo incubation and selection might improve outcomes of in-vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) treatment due to undisturbed embryo culture conditions, improved embryo selection, or both. However, the benefit remains uncertain. We aimed to evaluate the effectiveness of time-lapse imaging systems providing undisturbed culture and embryo selection, and time-lapse imaging systems providing only undisturbed culture, and compared each with standard care without time-lapse imaging.

Methods We conducted a multicentre, three-parallel-group, double-blind, randomised controlled trial in participants undergoing IVF or ICSI at seven IVF centres in the UK and Hong Kong. Embryologists randomly assigned participants using a web-based system, stratified by clinic in a 1:1:1 ratio to the time-lapse imaging system for undisturbed culture and embryo selection (time-lapse imaging group), time-lapse imaging system for undisturbed culture alone (undisturbed culture group), and standard care without time-lapse imaging (control group). Women were required to be aged 18–42 years and men (ie, their partners) 18 years or older. Couples had to be receiving their first, second, or third IVF or ICSI treatment and could not participate if using donor gametes. Participants and trial staff were masked to group assignment, embryologists were not. The primary outcome was live birth. We performed analyses using the intention-to-treat principle and reported the main analysis in participants with primary outcome data available (full analysis set). The trial is registered on the International Trials Registry (ISRCTN17792989) and is now closed.

Findings 1575 participants were randomly assigned to treatment groups (525 participants per group) between June 21, 2018, and Sept 30, 2022. The live birth rates were 33·7% (175/520) in the time-lapse imaging group, 36·6% (189/516) in the undisturbed culture group, and 33·0% (172/522) in the standard care group. The adjusted odds ratio was 1·04 (97·5% CI 0·73 to 1·47) for time-lapse imaging arm versus control and 1·20 (0·85 to 1·70) for undisturbed culture versus control. The risk reduction for the absolute difference was 0·7 percentage points (97·5% CI −5·85 to 7·25) between the time-lapse imaging and standard care groups and 3·6 percentage points (−3·02 to 10·22) between the undisturbed culture and standard care groups. 79 serious adverse events unrelated to the trial were reported (n=28 in time-lapse imaging, n=27 in undisturbed culture, and n=24 in standard care).

Interpretation In women undergoing IVF or ICSI treatment, the use of time-lapse imaging systems for embryo culture and selection does not significantly increase the odds of live birth compared with standard care without time-lapse imaging.

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Introduction

One in six adults are affected by infertility worldwide.[1] There has been a steady increase in the number of individuals undergoing assisted reproduction treatments such as in-vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI).[2] The success rates of these treatments have however remained static over the last decade.[2–4] Transfer of embryos with the best implantation potential is key to successful IVF treatment.[5] Current methods of embryo selection have poor predictive accuracy, and there is a need for optimisation of embryo selection.[6–8]

Time-lapse imaging of developing embryos is a relatively new technology available to improve embryo selection.

Research in context

Evidence before this study

There was inconclusive and insufficient high-quality evidence for differences in outcomes with the use of time-lapse imaging systems. A Cochrane review (first published in 2015 and last updated in 2019) including 2955 participants from nine trials assessed the overall effect of the time-lapse imaging system and the relative and independent contributions of undisturbed embryo culture and embryo selection on reproductive outcomes. They reported no differences for either variable for any reproductive outcome. The quality of evidence, however, was judged to be low to very low and adequately powered high-quality trials were recommended.

Added value of this study

The time-lapse imaging trial (TILT) is the first and only adequately powered trial to detect meaningful differences in live birth rates with the use of time-lapse imaging systems and provide a definitive answer to the research uncertainty. The study's unique three-group design allowed us to assess the overall effects of the time-lapse imaging system, and to ascertain whether the observed benefit, if any, was due to both undisturbed culture and embryo selection, or undisturbed culture only, in a single trial. 98·9% of primary outcome data were available, with no more than 2·1% missing data for analysis, no participant withdrawals after random assignment, and only 1·3% allocation non-adherence, meaning that this trial provides very high quality evidence for the outcomes reported.

Implications of all the available evidence

TILT suggests no benefit in the use of time-lapse imaging systems for embryo incubation and selection to improve the odds of live birth in women having in-vitro fertilisation and intracytoplasmic sperm injection treatment. Offering TLI to patients and health-care providers with the expectation of improved outcomes cannot be justified. An assessment of the influence of time-lapse imaging systems on embryology laboratory workflow and efficiency, and a health economic evaluation are required if time-lapse imaging systems are to be introduced into routine practice based on these alternative considerations.

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The method involves digital imaging of developing embryos within the incubators every 5–15 min from the time of IVF up to embryo transfer, creating a time-lapse sequence of embryo development that can be visualised on an external monitor. Embryos can therefore be assessed remotely without removing them from the incubators. Time-lapse imaging systems are offered for their supposed dual benefits. First, an undisturbed embryo culture minimises the fluctuations in temperature, gas concentrations, pH, and humidity in incubators that might arise from the intermittent removal of embryos for assessment in standard culture. This might positively affect embryonic development, embryo quality, and live birth rates.[9] Second, the morphokinetic variables provided by time-lapse videos are considered to confer advantages over static embryo assessments that might miss transient embryo development events pointing to abnormal embryonic development.[10,11] These morphokinetic variables that are supplied by time-lapse imaging systems are used by embryo selection algorithms, which have been postulated to select the embryos with the best implantation potential.[11] Time-lapse imaging systems when used as a selection tool are postulated to reduce time to pregnancy but cannot improve the cumulative chance of live birth when all embryos have been transferred.

There is insufficient high-quality evidence demonstrating the benefits of time-lapse imaging systems over conventional methods of embryo selection, and whether successful fertility outcomes, if any, are due to the undisturbed culture environment or improved embryo selection. Existing reviews and primary studies, including a recent, large three-group trial,[12] have predominantly reported no differences in outcomes between the use of time-lapse imaging and conventional methods of embryo culture and selection, with most trials deemed to be of low to very low quality.[13]

We evaluated the effects of time-lapse imaging systems that provided an undisturbed culture environment and morphokinetic parameters for embryo selection, and time-lapse imaging systems that only provided undisturbed culture environment. These systems were compared with standard care without time-lapse imaging for embryo culture and selection, primarily on live births, and secondarily on several outcomes for effectiveness and safety in women undergoing IVF treatment.

Methods

Study design

The time-lapse imaging trial (TILT) is a pragmatic, multicentre, three-parallel-group, double-blind, randomised controlled trial conducted across seven IVF centres in the UK (n=1185) and Hong Kong (n=390). The trial was approved by the London Central Research Ethics Committee, the National Health Service Health Research Authority (18/LO/0330), the Chinese University of Hong Kong Ethics Committee, and the Prince of Wales Hospital Clinical Research Ethics Committee (2018.423-T). Medical device approval was not required as the time-lapse imaging systems were used within their licensed application. Trial oversight and monitoring were provided by the Trial Management Group and independent Data Monitoring and Trial Steering Committees. The protocol has been previously published.[14] The trial is registered on the International Trials Registry (ISRCTN17792989) and is now closed.

Participants

We recruited couples undergoing IVF or ICSI. Female individuals were required to be aged between 18 years and 42 years, and male individuals (ie, their partners) at least 18 years of age. The couples were receiving their first, second, or third IVF or ICSI treatment and had at least three two-pro-nuclei (2PN) embryos (a sign of normal fertilisation) available on the day of fertilisation check after the IVF or ICSI procedure. The trial excluded participants concomitantly participating in other interventional trials, those having treatment using donor gametes, or planning pre-implantation genetic diagnosis or screening. All participants provided written informed consent. Re-randomisation was permitted as long as participants continued to meet all trial inclusion criteria. Sex data were collected via trial data collection.

Randomisation and masking

We assigned participants randomly in a 1:1:1 ratio to one of three intervention arms. The first intervention was time-lapse imaging; participants assigned to this group had embryo assessment and selection with morphokinetic variables in addition to standard morphological embryo scoring in undisturbed culture conditions in time-lapse imaging incubators. The second intervention was undisturbed culture; participants assigned to this group had only conventional morphological embryo assessment and selection in undisturbed culture conditions in time-lapse imaging incubators. The third intervention was standard care as a control group; participants in this group received conventional morphological embryo assessment using the light microscope and standard embryo culture in standard incubators. Randomisation was done by trial embryologists who were not involved in participant recruitment but did have other involvement in the trial, using a secure web-based randomisation system. Randomisation was stratified by fertility clinic, minimised by the female participant's age (<35 years, 35–40 years, >40 years), and minimised by the type of planned first embryo transfer (ie, fresh or frozen). The minimisation algorithm was regularly monitored to confirm balance between groups due to medically indicated post-randomisation changes in the type of embryo transfer (ie, fresh or frozen). The trial used a re-randomisation design as detailed in the trial protocol and described by Kahan and colleagues.[15] This design was chosen as it is useful to improve recruitment, appropriate for multi-episode settings, and does not affect the validity of the statistical analysis and results, provided the analysis uses an appropriate approach that can accommodate correlated data.

See Online for appendix

Masking embryologists to the intervention was not possible. All other trial staff, including clinicians performing the embryo transfer procedure and participants (until the end of their participation in the trial) were masked.

Procedures

Participants followed local care pathways for IVF or ICSI, except for variation in the steps involving the trial intervention such as embryo incubation, assessment, and selection for transfer into the womb. Each recruiting centre used the time-lapse imaging system of their choice but the same time-lapse imaging system was used at each site for the time-lapse imaging and undisturbed culture groups. Each recruiting centre laboratory followed their own protocols for consumables, laboratory conditions, and processes, but ensured that variables such as culture media; triple gas concentrations of CO_2, O_2, and N_2; and temperature were the same across all trial groups.

Embryos were cultured until day 3–6 based on local embryology practice, where embryos are usually transferred on day 3, 4, or 5 of culture and frozen on day 3, 4, 5, or 6 depending on patient and embryo characteristics. In the time-lapse imaging group and undisturbed culture group embryos were not removed from the incubators until the day of embryo transfer or freezing. In the standard care group, embryos were removed for assessment at variable time points in different recruiting centres. Embryo grading was performed at least on day 3; day 5; and, if required, day 6. All available embryos in all three groups were graded on the basis of morphology, using a standardised grading scheme.[16] For embryos in the time-lapse imaging group, laboratories additionally applied morphokinetic variables and any other information available from the time-lapse imaging to select the best embryos for transfer.

All randomly assigned female individuals had a pregnancy test approximately 2 weeks after embryo transfer. Participants with a positive pregnancy test were followed up at 6–8 weeks, 24 weeks, and 6 weeks post-partum.

Outcomes

The primary outcome was the birth of a live baby. This outcome was for the first embryo transfer, either fresh or frozen, for all randomly assigned participants. Secondary outcomes for clinical effectiveness included biochemical pregnancy, clinical pregnancy, clinical pregnancy per embryo transferred, the use of elective single embryo transfer (e-SET), and embryo utilisation rate. Secondary outcomes for clinical safety included multiple pregnancy, pregnancy loss including miscarriage and stillbirth, major congenital abnormalities, birth weight, gestational age, and ectopic pregnancy (appendix p 5). All serious adverse events and protocol violations were reported according to standard procedure.

Statistical analysis

The sample size calculation was based upon the primary outcome of live birth. With a 5% overall significance level (2·5% for each of the two main treatment comparisons: time-lapse imaging *vs* standard care, and undisturbed

culture *vs* standard care), 514 participants per treatment group were necessary to detect an absolute increase in the primary outcome from 26·5% to 35·25% with 80% power. Allowing for 2% loss to follow-up or withdrawal of consent, 525 participants per treatment group were required (1575 in total). The statistical comparison between experimental treatment groups (time-lapse imaging *vs* undisturbed culture) was planned only in the case of rejection of the null hypothesis for at least one of the primary comparisons planned (time-lapse imaging *vs* standard care, or undisturbed culture *vs* standard care). The hierarchical approach permitted the maintainance of the overall type 1 error rate of 5%. This trial used the re-randomisation design detailed in the trial protocol.[15]

We performed the analyses for the primary outcome based on the intention-to-treat principle and reported the main analysis for all participants with data available for the primary outcome (ie, full analysis set). We compared live births fitting a mixed effects logistic regression model using the GLMMadaptive package in R to account for the correlation derived from data of participants who were re-randomised.[17] Parameters were estimated using an adaptive Gauss-Hermite quadrature approximation. Participants were included as a random intercept. We presented the effect estimate as an adjusted odds ratio (OR), which is widely used as a measure of association for binary outcomes. The simple relationship between the coefficient and the OR is the fundamental reason logistic regression has proven to be a powerful technique.[18] For rare events, the OR can approximate the relative risk. A 97·5% confidence interval and a two-sided p-value were also presented. The unadjusted model only included treatment arm as an independent variable. Adjusted models included stratification, minimisation factors, and pre-specified covariates. The pre-specified covariates in the analyses were treatment attempt number, type of infertility, category of infertility, duration of infertility, BMI, type of time-lapse imaging equipment, method of insemination (IVF *vs* ICSI), number of retrieved oocytes, and number of available embryos. A comparison between the two intervention groups using the hierarchial model was not required.

We analysed all secondary outcomes using logistic, Poisson, or linear mixed effects regression models as appropriate, and presented the point estimates in terms of proportions, rates, and means, respectively. For the secondary outcomes we did not plan correction for multiplicity and have reported effect sizes with their 95% CI. Adjusted estimates are presented with the same independent variables as in the primary outcome.

The pre-specified protocol plan was for subgroup analyses of female participant age group (<35 years, 35–40 years, and >40 years). Due to inadequate numbers, the group of those older than 40 years was merged with the 35–40 years age group to achieve meaningful results from the analysis. Therefore, we performed subgroup analyses for female age groups (ie, <35 years and ≥35 years) and pre-specified subgroup analyses for the method of first embryo transfer (ie, fresh or frozen) by including an interaction effect between these minimisation factors and treatment groups in the regression model. A post-hoc subgroup analysis for female age groups of those younger than 38 years versus those 38 years and older was performed to assess the potential effect of the intervention in age groups with differing prognosis.[12,19] A pre-specified subgroup analysis by hospital was planned.

The pre-specified handling of missing data was based on their proportion and characteristics. Imputation was required for ≥5% missing data; nothing was planned to be done in the case of <5% missing data.

We performed pre-specified sensitivity analyses to assess the effect of re-randomised participants and allocation non-adherence by excluding these participants from the analysis. Sensitivity analyses for outliers and completed cases were not required. Additionally, we undertook a sensitivity analysis by repeating the main analysis and including all randomly assigned participants; missing data for live birth were assumed considering the worst (all intervention considered negative and control

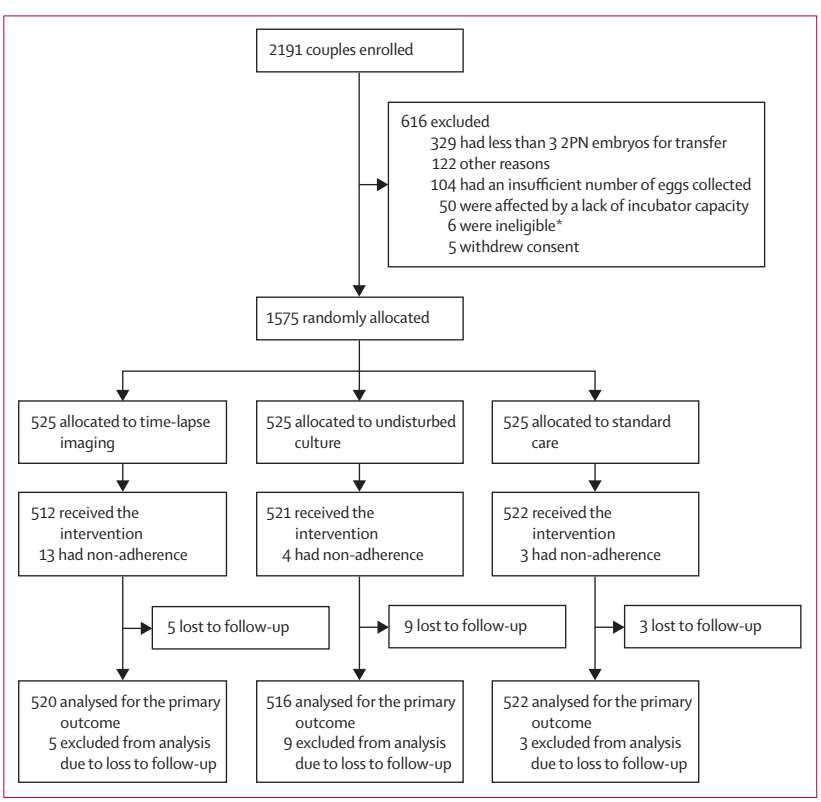

Figure 1: **Trial profile**
Time-lapse imaging: undisturbed embryo culture and embryo selection with morphology and morphokinetic parameters. Undisturbed culture: undisturbed embryo culture and embryo selection with morphology alone. Standard care: standard embryo culture and embryo selection with morphology alone. 2PN=two pro-nuclei. *Six couples provided consent but were ineligible based on inclusion criteria.

	Time-lapse imaging (n=525)	Undisturbed culture (n=525)	Standard care (n=525)
Female age at consent (years)	34·9 (3·7)	35·0 (3·8)	34·9 (3·6)
Type of infertility			
Primary	323 (61·5%)	333 (63·4%)	319 (60·8%)
Primary couple	79 (15·1%)	61 (11·6%)	84 (16·0%)
Secondary	122 (23·2%)	131 (25·0%)	122 (23·2%)
Unknown	1 (0·2%)	0	0
Category of infertility*			
Tubal	92 (17·5%)	73 (13·9%)	80 (15·2%)
Endometriosis	41 (7·8%)	59 (11·2%)	42 (8·0%)
Uterine	10 (1·9%)	15 (2·9%)	18 (3·4%)
Ovulatory	89 (17·0%)	75 (14·3%)	85 (16·2%)
Male	215 (41·0%)	209 (39·8%)	230 (43·8%)
Unexplained	163 (31·1%)	169 (32·2%)	170 (32·4%)
Other[4]	37 (7·0%)	32 (6·1%)	26 (5·0%)
Duration of infertility (months)	37 (27–57)	38 (30–60)	40 (30–60)
Number of previous embryo transfers			
0	446 (85·0%)	430 (81·9%)	425 (81·0%)
1	49 (9·3%)	52 (9·9%)	62 (11·8%)
2	24 (4·6%)	34 (6·5%)	23 (4·4%)
Unknown	6 (1·1%)	9 (1·7%)	15 (2·9%)
Female BMI (kg/m²)			
Mean (SD)	23·6 (3·5)	23·4 (3·2)	23·6 (3·4)
Unknown n (%)	1 (0·2%)	1 (0·2%)	2 (0·4%)
Treatment protocol for controlled ovarian stimulation			
Antagonist	413 (78·7%)	420 (80·0%)	419 (79·8%)
Long agonist	99 (18·9%)	90 (17·1%)	91 (17·3%)
Other[5]	13 (2·5%)	15 (2·9%)	14 (2·7%)
Unknown	0	0	1 (0·2%)
Total dose of follicle stimulating hormone (IU)			
Mean (SD)	2991·9 (1157·3)	2971·6 (1143·5)	2932·2 (1117·2)
Unknown	0	0	1 (0·2%)
Number of eggs collected	12·2 (6·6)	12·3 (6·7)	12·6 (7·3)
Method of insemination			
ICSI	241 (45·9%)	240 (45·7%)	239 (45·5%)
IVF	261 (49·7%)	271 (51·6%)	260 (49·5%)
IVF and ICSI split	23 (4·4%)	14 (2·7%)	25 (4·8%)
Unknown	0	0	1 (0·2%)
Number of 2PN embryos	7·4 (4·4)	7·7 (4·3)	7·6 (4·5)

(Table 1 continues on next page)

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	Time-lapse imaging (n=525)	Undisturbed culture (n=525)	Standard care (n=525)
Type of time lapse imaging equipment			
Embryoscope	218 (41·5%)	219 (41·7%)	0
Embryoscope Plus	232 (44·2%)	231 (44·0%)	3 (0·6%)
MIRI (ESCO)	65 (12·4%)	65 (12·4%)	0
Other	0	1 (0·2%)	0
Primo Vision	9 (1·7%)	9 (1·7%)	0
Unknown	1 (0·2%)	0	522 (99·4%)
Type of embryo transfer (first after egg collection)			
Freezing of all embryos	195 (37·1%)	197 (37·5%)	189 (36·0%)
Fresh transfer	319 (60·8%)	319 (60·8%)	314 (59·8%)
No embryos available to transfer	11 (2·1%)	9 (1·7%)	22 (4·2%)
Day of embryo transfer (after egg collection)			
2	7 (1·3%)	8 (1·5%)	8 (1·5%)
3	89 (17·0%)	79 (15·0%)	73 (13·9%)
4	0	2 (0·4%)	2 (0·4%)
5	414 (78·9%)	418 (79·6%)	410 (78·1%)
6	4 (0·8%)	9 (1·7%)	9 (1·7%)
Unknown	11 (2·1%)	9 (1·7%)	22 (4·2%)
Number of embryos transferred			
0	20 (3·8%)	17 (3·2%)	29 (5·5%)
1	390 (74·3%)	396 (75·4%)	397 (75·6%)
2	108 (20·6%)	105 (20·0%)	92 (17·5%)
3	0	2 (0·4%)	1 (0·2%)
Unknown	7 (1·3%)	5 (1·0%)	6 (1·1%)

Data are mean (SD), n (%), or median (IQR). Time-lapse imaging: undisturbed embryo culture and embryo selection with morphology and morphokinetic parameters. Undisturbed culture: undisturbed embryo culture and embryo selection with morphology alone. Standard care: standard embryo culture and embryo selection with morphology alone. Female age at consent was a minimisation variable. Primary infertility is where neither partner has had a previous pregnancy. Primary couple infertility is primary infertility for the particular couple or relationship, with either or both individuals having had a previous pregnancy with another partner or in another relationship. ICSI=intra-cytoplasmic sperm injection. IVF=in-vitro fertilisation. 2PN=two pro-nuclei.
*More than one reason for infertility could be present so some percentages do not sum to 100.

Table 1: **Demographic and baseline characteristics of participants**

positive) and best (all intervention considered positive and control negative) case scenarios and also by imputing missing data. A full statistical analysis plan was finalised before data analysis (appendix p 37–85). No interim analyses were performed. All analyses were performed using R software (version 4.3.2). All statistical analyses were based on the statistical analysis plan. The trial data were monitored by an independent data monitoring committee.

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 21, 2018, and Sept 30, 2022, the trial recruited 2191 participants. 1575 were randomly assigned and allocated to either receive time-lapse imaging, undisturbed culture, or standard care (525 participants per group; figure 1; appendix p 7). No participant withdrew consent after random assignment. Allocation non-adherence was reported in 20 (1·3%) of 1575 participants (figure 1).

	Time-lapse imaging	Undisturbed culture	Standard care	Time-lapse imaging *vs* standard care, adjusted OR (CI)	Undisturbed culture *vs* standard care, adjusted OR (CI)
Primary outcome					
Live birth	175/520 (33·7%; 29·6–37·8)	189/516 (36·6%; 32·4–40·8)	172/522 (33·0%; 29·0–37·0)	1·04 (0·73–1·47)	1·20 (0·85–1·70)
Secondary outcomes					
Clinical pregnancy	219/519 (42·2%; 37·9–46·6)	225/518 (43·4%; 39·1–47·8)	212/519 (40·9%; 36·6–45·2)	1·06 (0·82–1·38)	1·11 (0·86–1·46)
Elective single embryo transfer rate	372/518 (71·8%; 67·7–75·7)	370/520 (71·2%; 67·1–75·0)	375/519 (72·3%; 68·2–76·1)	0·96 (0·69–1·34)	0·91 (0·65–1·27)
Pregnancy loss (between clinical pregnancy and 24 weeks gestation)	45/515 (8·7%; 6·4–11·5)	33/513 (6·4%; 4·5–8·9)	42/517 (8·1%; 5·9–10·8)	1·09 (0·66–1·77)	0·76 (0·45–1·29)
Multiple pregnancy rate	11/519 (2·1%; 1·1–3·8)	15/518 (2·9%; 1·6–4·7)	10/519 (1·9%; 0·9–3·5)	1·13 (0·45–2·84)	1·50 (0·63–3·61)
Incidence of major congenital anomalies	3/524 (0·6%; 0·1–1·7)	6/523 (1·2%; 0·4–2·5)	8/523 (1·5%; 0·7–3·0)	0·36 (0·07–2·01)	0·74 (0·21–2·57)

Data are n/N (%; CI); CI is 97·5% for the primary outcome and 95% for secondary outcomes. Ns vary due to missing data. OR was adjusted for the stratification and minimisation factors. Time-lapse imaging: undisturbed embryo culture and embryo selection with morphology and morphokinetic parameters. Undisturbed culture: undisturbed embryo culture and embryo selection with morphology alone. Standard care: standard embryo culture and embryo selection with morphology alone. Clinical pregnancy: at least one intrauterine gestation sac seen at 6–8 weeks of gestation; multiple pregnancies count as one clinical pregnancy. Multiple pregnancy: two or more gestational sacs seen on ultrasound scan at 6–8 weeks. OR=odds ratio.

Table 2: **Primary and secondary outcomes**

Baseline demographic and clinical characteristics of participants were similar in the three groups (table 1; appendix pp 8–17). As less than 5% of data for primary and secondary outcomes were missing, we did not impute missing data for the main analysis. Minor deviations due to data characteristics retained statistical integrity and did not alter outcomes.

Primary outcome data were available for 1558 (98·9%) of 1575 participants. No more than 2·1% of any outcome or covariates data were missing for any analysis in the study, meaning that missing data did not need to be imputed for the main analysis (appendix pp 18–19). The live birth rates were 33·7% (175 of 520) in the time-lapse imaging group, 36·6% (189 of 516) in the undisturbed culture group, and 33·0% (172 of 522) in the standard care group. There were no significant differences in live birth rates between the time-lapse imaging and standard care groups (adjusted OR 1·04, 97·5% CI 0·73 to 1·47) or between the undisturbed culture and standard care groups (1·20, 0·85 to 1·70; table 2). The risk reduction for the absolute difference was 0·7 percentage points (97·5% CI –5·85 to 7·25) between the time-lapse imaging and standard care groups and 3·6 percentage points (–3·02 to 10·22) between the undisturbed culture and standard care groups. The findings were similar in the sensitivity analyses that assessed the effect of missing data, exclusion of re-randomised participants, or allocation non-adherence (appendix pp 20–29).

The clinical pregnancy rates were 42·2% (219 of 519) in the time-lapse imaging group, 43·4% (225 of 518) in the undisturbed culture group, and 40·9% (212 of 519) in the standard care group. There were no significant differences between the time-lapse imaging and standard care groups (adjusted OR 1·06, 97·5% CI 0·82–1·38) or undisturbed culture and standard care groups (1·11, 0·86–1·46). The rates of pregnancy loss between clinical pregnancy and 24 weeks' gestation were not statistically different between time-lapse imaging (45 [8·7%] of 515) and standard care (42 [8·1%] of 517; adjusted OR 1·09, 95% CI 0·66–1·77) or between undisturbed culture (33 [6·4%] of 513) and standard care (adjusted OR 0·76, 95% CI 0·45–1·29). None of the other secondary outcomes for clinical effectiveness and safety showed significant differences between the intervention and control groups (table 2, appendix pp 30–32, 34–35).

The trial reported 79 serious adverse events in 67 (4·3%) participants (28 in the time-lapse imaging

	Hospital admission or prolongation of hospital stay	Life-threatening event (including death)	Other important medical event	Congenital abnormality
Time-lapse imaging (n=28)				
Baby	10 (35·7%)	0	0	11 (39·3%)
Mother	6 (21·4%)	0	1 (3·6%)	NA
Undisturbed culture (n=27)				
Baby	9 (33·3%)	0	0	11 (40·7%)
Mother	6 (22·2%)	0	1 (3·7%)	NA
Standard care (n=24)				
Baby	2 (8·3%)	1 (4·2%)	0	15 (62·5%)
Mother	5 (20·8%)	1 (4·2%)	0	NA

Time-lapse imaging: undisturbed embryo culture and embryo selection with morphology and morphokinetic parameters. Undisturbed culture: undisturbed embryo culture and embryo selection with morphology alone. Standard care: standard embryo culture and embryo selection with morphology alone.

Table 3: **Serious adverse events**

group, 27 in the undisturbed culture group, and 24 in the standard care group). All events were considered unrelated to the trial. There were 37 congenital abnormalities reported in the fetuses of 36 (2·3%) participants (11 in the time-lapse imaging group, 11 in the undisturbed culture group, and 15 in the standard care group); of these, 17 were major and 20 were minor (table 3). The trial reported 33 protocol violations, excluding allocation non-adherences (17 in the time-lapse imaging group, 11 in the undisturbed culture group, and five in the standard care group; appendix pp 35–36). Minor deviations (appendix pp 3–5) from the statistical analysis plan due to data characteristics retained statistical integrity and did not alter outcomes.

Pre-specified subgroup analyses showed no significant interaction between minimisation factors and treatment group. We did not find significant subgroup differences by women's age (<35 years *vs* ≥35 years) or by the method of embryo transfer (fresh *vs* frozen) for live birth rate or any secondary outcomes. The post hoc subgroup analysis done with the female age cut off at 38 years showed no differences in live birth rate or any secondary outcome (figure 2). The pre-specified subgroup analysis by hospital was not performed due to small participant numbers at some centres.

Discussion

In female individuals undergoing assisted reproduction with IVF or ICSI treatment, the use of time-lapse imaging systems either for undisturbed embryo culture and embryo selection, or for undisturbed culture alone, did not result in increased rates of live birth compared with conventional methods of embryo culture and selection. There were no differences in the rates of biochemical and clinical pregnancies and pregnancy losses between the use of time-lapse imaging systems and standard care. The findings were similar irrespective of the female participant's age and use of fresh or frozen embryo replacement, for both primary and secondary outcomes.

To our knowledge, TILT is the only trial adequately powered to detect meaningful differences in live births, the most important outcome for couples with infertility and health-care providers of IVF.[20] The required sample size and power calculation for the trial were prespecified and registered prospectively; this prespecified sample

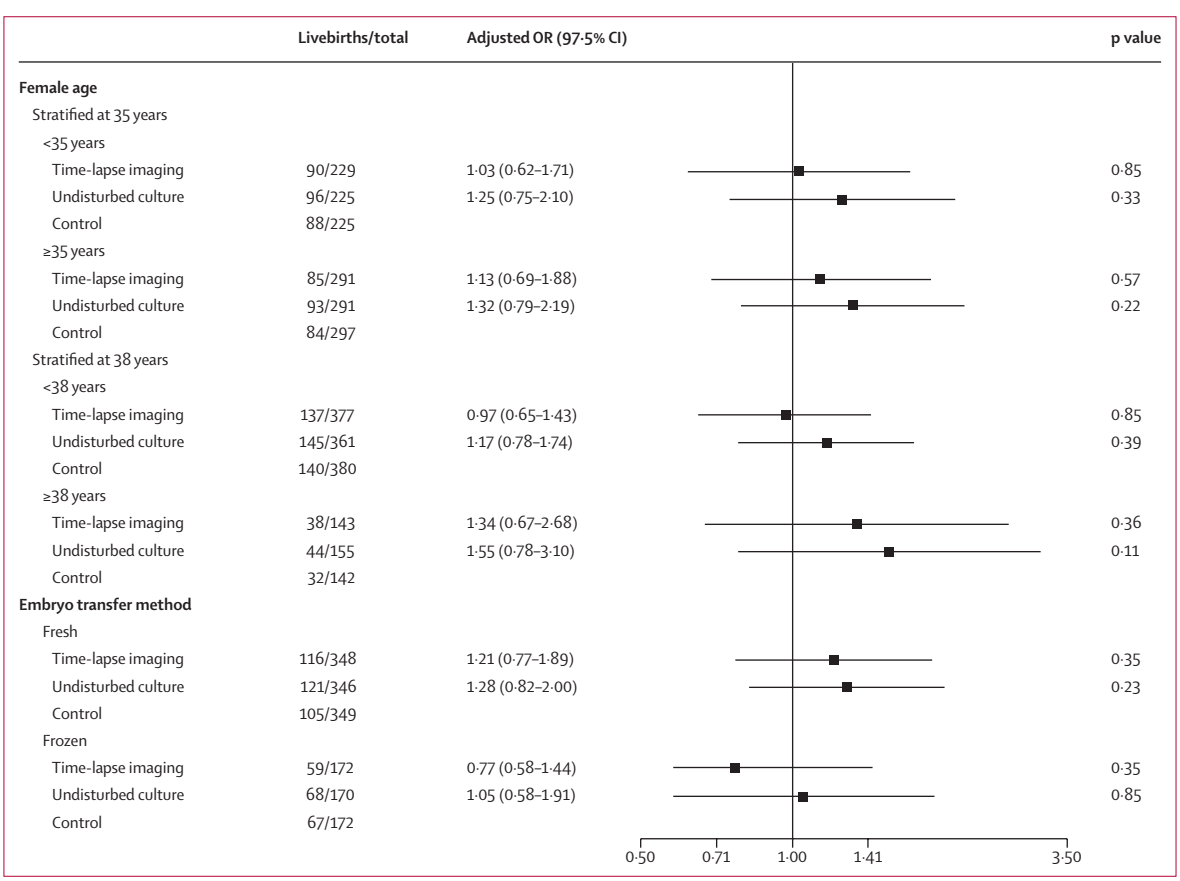

Figure 2: **Subgroup analyses for live birth**

Time-lapse imaging: undisturbed embryo culture and embryo selection with morphology and morphokinetic parameters. Undisturbed culture: undisturbed embryo culture and embryo selection with morphology alone. Standard care: standard embryo culture and embryo selection with morphology alone. Adjusted ORs are adjusted for the stratification and minimisation factors (appendix pp 37–85). OR=odds ratio.

size was reached. The independent trial data monitoring committee monitored the results throughout the trial. The results should be interpreted within the entire range of the width of the confidence intervals around the point estimates of the effects reported. The unique three-group design allowed us to not only assess the overall effects of the time-lapse imaging system, but also ascertain whether the observed benefit would have been due to both undisturbed culture and embryo selection, or undisturbed culture only, in a single trial. The pragmatic trial design allowed centres to use time-lapse imaging systems and IVF protocols of their choice, reflecting real-life practice. The broad eligibility criteria included women undergoing elective freeze-all cycles, which are now increasingly used in practice.[2] The generalisability of the results was ensured by the international multicentre recruitment. The reported participant characteristics and outcomes were similar to those reported by national and international registries, further supporting generalisability. The trial's design could be argued as introducing bias, as several demographic, baseline, and treatment variables such as age, IVF protocols, duration of embryo culture, and the number of embryos transferred might vary across the trial. However as in any randomised controlled trial, the participants and hence these baseline variables are randomly distributed between the trial groups; the small differences that exist in all randomised trials do not introduce bias as they are randomly distributed. To further reduce the risk of bias, we stratified the randomisation by centre and used female age and type of planned embryo transfer as minimisation variables. Analyses in regression models were also adjusted for minimisation and stratification factors and additional selected covariates.

Participants were randomly assigned at a specific time point after fertilisation check, closest to the start of the intervention, to minimise the number of participant withdrawals and protocol deviations without compromising the integrity of the intervention. This method ensured the robustness of the results, which were analysed using the intention-to-treat principle as compared with previous trials where substantial differences between intention-to-treat and per protocol populations were observed. Data collection and follow-up were rigorous, with less than 5% of participants having missing data for outcomes or covariates used in the analysis.

Cumulative live birth rate, for which live birth following sequential use of all or several embryos resulting from a single oocyte retrieval is assessed, has been increasingly advocated as a preferred outcome in IVF trials rather than live birth from a single embryo transfer. We consider this outcome to be inappropriate for time-lapse imaging as this intervention is directed to selecting the best embryo from a cohort and aims to improve the live birth rate for the first embryo transfer. Although trial recruitment was prolonged by the SARS-CoV-2 pandemic, we do not believe that this affected the results of the trial as our patient population, clinical care pathways, and time-lapse technology remained similar throughout the course of the trial. These findings are applicable for time-lapse imaging systems currently in practice, although they might change if the technology changes. We are not aware of substantial new time-lapse imaging technology that has been introduced or surpassed the existing time-lapse imaging systems that were evaluated.

Congenital anomalies were patient reported and confirmed through medical records. Anomalies could have been under-reported but their random distribution would not affect comparisons between trial groups.

Our results support the findings of several previous trials that assessed the effect of time-lapse imaging systems.[13,21–24] However, none of these were adequately powered to detect differences in live birth rate or were able to provide high quality evidence. A single large trial that reported benefit with the use of time-lapse imaging systems was judged to be of very low quality and subject to significant bias.[25] A recently published three-group trial reported similar outcomes but was powered to only detect differences in cumulative ongoing pregnancy rate, used a single time-lapse imaging system, and transferred all embryos on day 3 of culture.[12]

In this trial, 80% of all embryo transfers were done on day 5 of embryo culture, in line with current practice. This extended culture likely acts as a natural selection tool, providing better success rates compared with transfer of embryos on day 3, irrespective of the use of time-lapse imaging or conventional systems.

The higher incidence of aneuploidy with increasing maternal age is often listed as a major reason for age-dependent success rates in IVF and ICSI treatments.[26] The use of time-lapse imaging selection algorithms have been suggested as a non-invasive technique for assessing embryonic aneuploidy with increased live births reported in women older than 38 years.[12] We did not find any differences in the direction or magnitude of effect between time-lapse imaging systems and standard care for primary and secondary outcomes in the subgroup analyses for women's ages, stratified at 35 years and 38 years. Although this trial used the same culture media and conditions across groups, there remains uncertainty regarding the effect of sequential and single step media on outcomes.

Individual participant data meta-analysis using larger datasets might be able to provide definitive evidence for subgroups of women based on age or to detect significant differences in secondary outcomes such as pregnancy loss. Considerable heterogeneity is seen in the delivery of the time-lapse imaging intervention across trials with respect to the duration of undisturbed culture and day of embryo transfer. Analysis of subgroups based on differences in intervention in larger datasets might elucidate the optimal technique for the use of time-lapse imaging systems. An

assessment of the influence of time-lapse imaging systems on embryology laboratory workflow and efficiency, and a health economic evaluation are required if time-lapse imaging systems are to be introduced into routine practice based on these considerations.

This trial suggests no benefit in the use of time-lapse imaging systems for embryo incubation and selection to improve the odds of live birth in women having IVF and ICSI treatment.

Contributors

PB, DYLC, DL, JD, TP, KSK, and ST were responsible for conception and design. PB, DYLC, DL, DB, JH, DP, YaC, MCP, and TP were responsible for trial management. PB, DYLC, OA, YiC, JPWC, BC, LC, SD, and AR acquired study data. TP, MCP, FGC, AW, EB, PB, KSK, and ST provided statistical input and interpreted the data. EB, MCP, and TP accessed and verified the data and performed the data analysis. PB wrote the manuscript as project leader and chief investigator. All authors had full access to all the data from the trial, contributed to the execution of the study, reviewed the manuscript for content, and gave final approval for submission of the manuscript.

Declaration of interests

PB declares support for the current trial and manuscript from Barts Charity (MGU0374) and Pharmasure Pharmaceuticals through Queen Mary University of London. DYLC declares support for the current trial and manuscript from Hong Kong OG trust fund (reference number 6904985), Hong Kong Health and Medical Research Fund (07180566), and Hong Kong Matching Fund (RMG01-8601386) through the Chinese University of Hong Kong. YiC declares grants from the National Institute for Health and Care Research and Medical Research Council through the University of Southampton, honoraria for lectures through Ferring Pharmaceuticals and Merck, and being a minor shareholder for Complete Fertility. All other authors declare no competing interests.

Data sharing

Anonymised participant data will be made available after publication of the primary trial results upon request to the corresponding author with a mutually agreed data sharing agreement.

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