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Time-lapse systems for embryo incubation and assessment in assisted reproduction (Review)

Armstrong S, Bhide P, Jordan V, Pacey A, Farquhar C

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Time-lapse systems for embryo incubation and assessment in assisted reproduction.
Cochrane Database of Systematic Reviews 2018, Issue 5. Art. No.: CD011320.
DOI: 10.1002/14651858.CD011320.pub3.

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[Intervention Review]

Time-lapse systems for embryo incubation and assessment in assisted reproduction

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Editorial group: Cochrane Gynaecology and Fertility Group.
Publication status and date: Edited (no change to conclusions), published in Issue 8, 2018.

Citation: Armstrong S, Bhide P, Jordan V, Pacey A, Farquhar C. Time-lapse systems for embryo incubation and assessment in assisted reproduction. *Cochrane Database of Systematic Reviews* 2018, Issue 5. Art. No.: CD011320. DOI: 10.1002/14651858.CD011320.pub3.

ABSTRACT

Background

Embryo incubation and assessment is a vital step in assisted reproductive technology (ART). Traditionally, embryo assessment has been achieved by removing embryos from a conventional incubator daily for quality assessment by an embryologist, under a light microscope. Over recent years time-lapse systems have been developed which can take digital images of embryos at frequent time intervals. This allows embryologists, with or without the assistance of embryo selection software, to assess the quality of the embryos without physically removing them from the incubator.

The potential advantages of a time-lapse system (TLS) include the ability to maintain a stable culture environment, therefore limiting the exposure of embryos to changes in gas composition, temperature and movement. A TLS has the potential advantage of improving embryo selection for ART treatment by utilising additional information gained through continuously monitoring embryo development. Use of a TLS often adds significant extra cost onto an in vitro fertilisation (IVF) cycle.

Objectives

To determine the effect of a TLS compared to conventional embryo incubation and assessment on clinical outcomes in couples undergoing ART.

Search methods

We used standard methodology recommended by Cochrane. We searched the Cochrane Gynaecology and Fertility (CGF) Group trials register, CENTRAL, MEDLINE, Embase, CINAHL and two trials registers on 2 August 2017.

Selection criteria

We included randomised controlled trials (RCTs) in the following comparisons: comparing a TLS, with or without embryo selection software, versus conventional incubation with morphological assessment; and TLS with embryo selection software versus TLS without embryo selection software among couples undergoing ART.

Data collection and analysis

We used standard methodological procedures recommended by Cochrane. The primary review outcomes were live birth, miscarriage and stillbirth. Secondary outcomes were clinical pregnancy and cumulative clinical pregnancy. We reported quality of the evidence for important outcomes using GRADE methodology. We made the following comparisons.

TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment

TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images

TLS utilising embryo selection software versus conventional incubation and assessment

Main results

We included eight RCTs (N = 2303 women). The quality of the evidence ranged from very low to moderate. The main limitations were imprecision and risk of bias associated with lack of blinding of participants and researchers, and indirectness secondary to significant heterogeneity between interventions in some studies. There were no data on cumulative clinical pregnancy.

TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment

There is no evidence of a difference between the interventions in terms of live birth rates (odds ratio (OR) 0.73, 95% CI 0.47 to 1.13, 2 RCTs, N = 440, I^2 = 11% , moderate-quality evidence) and may also be no evidence of difference in miscarriage rates (OR 2.25, 95% CI 0.84 to 6.02, 2 RCTs, N = 440, I^2 = 44%, low-quality evidence). The evidence suggests that if the live birth rate associated with conventional incubation and assessment is 33%, the rate with use of TLS with conventional morphological assessment of still TLS images is between 19% and 36%; and that if the miscarriage rate with conventional incubation is 3%, the rate associated with conventional morphological assessment of still TLS images would be between 3% and 18%. There is no evidence of a difference between the interventions in the stillbirth rate (OR 1.00, 95% CI 0.13 to 7.49, 1 RCT, N = 76, low-quality evidence). There is no evidence of a difference between the interventions in clinical pregnancy rates (OR 0.88, 95% CI 0.58 to 1.33, 3 RCTs, N = 489, I^2 = 0%, moderate-quality evidence).

TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images

No data were available on live birth or stillbirth. We are uncertain whether TLS utilising embryo selection software influences miscarriage rates (OR 1.39, 95% CI 0.64 to 3.01, 2 RCTs, N = 463, I^2 = 0%, very low-quality evidence) and there may be no difference in clinical pregnancy rates (OR 0.97, 95% CI 0.67 to 1.42, 2 RCTs, N = 463, I^2 = 0%, low-quality evidence). The evidence suggests that if the miscarriage rate associated with assessment of still TLS images is 5%, the rate with embryo selection software would be between 3% and 14%.

TLS utilising embryo selection software versus conventional incubation and assessment

There is no evidence of a difference between TLS utilising embryo selection software and conventional incubation improving live birth rates (OR 1.21, 95% CI 0.96 to 1.54, 2 RCTs, N = 1017, I^2 = 0%, very low-quality evidence). We are uncertain whether TLS influences miscarriage rates (OR 0.73, 95% CI 0.49 to 1.08, 3 RCTs, N = 1351, I^2 = 0%, very low-quality evidence). The evidence suggests that if the live birth rate associated with no TLS is 38%, the rate with use of conventional incubation would be between 36% and 58%, and that if miscarriage rate with conventional incubation is 9%, the rate associated with TLS would be between 4% and 10%. No data on stillbirths were available. It was uncertain whether the intervention influenced clinical pregnancy rates (OR 1.17, 95% CI 0.94 to 1.45, 3 RCTs, N = 1351, I^2 = 42%, very low-quality evidence).

Authors' conclusions

There is insufficient evidence of differences in live birth, miscarriage, stillbirth or clinical pregnancy to choose between TLS, with or without embryo selection software, and conventional incubation. The studies were at high risk of bias for randomisation and allocation concealment, the result should be interpreted with extreme caution.

PLAIN LANGUAGE SUMMARY

Time-lapse systems for embryo incubation and embryo assessment for couples undergoing IVF and ICSI

Review question

We wanted to determine whether a time-lapse system (TLS) would improve the chances of a pregnancy and liveborn baby, and reduce the risk of miscarriage and stillbirth.

Background

In vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) are processes whereby a woman's eggs and a man's sperm are combined to achieve fertilisation outside of the body. Embryos are stored in an incubator and replaced into the woman between day 2 and 5 of development. Usually, embryos are removed from a conventional incubator for assessment, under a microscope, of their quality and stage of development. A TLS can take images of embryos at frequent time intervals, which allows assessment without removing the embryos from the incubator. A TLS can also apply a software programme that assists the embryologist in selecting the best quality embryo for replacement, potentially improving the chance of a liveborn baby.

Study Characteristics

The evidence is current to August 2017. We included eight studies (randomised controlled trials) of 2303 women undergoing IVF or ICSI. There were three different study designs: 1) TLS with conventional assessment of still TLS images versus conventional incubation and assessment, 2) TLS utilising embryo selection software versus TLS with conventional assessment of still TLS images, and 3) TLS utilising embryo selection software versus conventional incubation and assessment.

Trials included women undergoing IVF, and ICSI; some trials involve frozen embryo transfer and others fresh; one trial includes women using donor eggs, and the remainder use the woman's own eggs; the day of embryo transfer differs between trials; and in some only one embryo is replaced whereas in others, multiple embryos are replaced. We have taken account of these differences when assessing quality of the evidence. These differences should be seen as reflecting 'real world' practices, where there are variations in practice.

What the review found

TLS with conventional assessment of still TLS images versus conventional incubation and assessment

There is probably no difference between these interventions in live birth rates or pregnancy rates (moderate-quality evidence), miscarriage rates or stillbirth rates (low-quality evidence). The evidence suggests that if the live birth rate associated with conventional incubation and assessment is 33%, the rate with use of TLS with conventional morphological assessment of still TLS images is between 19% and 36%.

TLS utilising embryo selection software versus TLS with conventional assessment of still TLS images

No data were available on live birth or stillbirth. We are uncertain whether TLS utilising embryo selection software influences miscarriage rates, compared with TLS with conventional morphological assessment of still TLS images (very low-quality evidence) and clinical pregnancy rates (low-quality evidence). The evidence suggests that if the miscarriage rate associated with assessment of still TLS images is 5%, the rate with embryo selection software would be between 3% and 14%.

TLS utilising embryo selection software versus conventional incubation and assessment

There is no evidence from well designed studies that TLS utilising embryo selection software improves live birth or pregnancy rates compared to no TLS (very low-quality evidence) or reduces miscarriages (very low-quality evidence). The evidence suggests that if the live birth rate associated with no TLS is 38%, the rate with use of conventional incubation would be between 36% and 58%.

Patients need to be aware that there is no good evidence that TLS is more effective than conventional methods of embryo incubation. Women may wish to take part in RCTs on TLS in order to add to the existing evidence base, and help guide ART patients in the future.

Quality of the evidence

The quality of the evidence ranged from very low to moderate.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [Explanation]

TLS with conventional morphological assessment of still TLS images compared to conventional incubation and assessment for embryo incubation and assessment in assisted reproduction

Patient or population: couples undergoing assisted reproductive technology
Setting: fertility clinic
Intervention: TLS with conventional morphological assessment of still TLS images
Comparison: conventional incubation and assessment

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with conventional incubation and assessment	Risk with TLS with conventional morphological assessment of still TLS images				
Live birth	333 per 1,000	267 per 1,000 (190 to 361)	OR 0.73 (0.47 to 1.13)	440 (2 RCTs)	⊕⊕⊕◯ Moderate[a]	
Miscarriage	37 per 1,000	83 per 1,000 (28 to 222)	OR 2.25 (0.84 to 6.02)	440 (2 RCTs)	⊕⊕◯◯ Low[b]	
Stillbirth	53 per 1,000	53 per 1,000 (7 to 294)	OR 1.00 (0.13 to 7.49)	76 (1 RCT)	⊕⊕◯◯ Low[c]	
Clinical pregnancy	353 per 1,000	310 per 1,000 (204 to 469)	OR 0.88 (0.58 to 1.33)	489 (3 RCTs)	⊕⊕⊕◯ Moderate[d]	

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the mean risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio; **TLS:** time-lapse system

GRADE Working Group grades of evidence

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[a] We downgraded our assessment of the evidence for live birth once for imprecision due to there only being two trials, one in good prognosis patients and the other in poorer prognosis patients, totalling 440 women.

[b] We downgraded our assessment of the evidence for miscarriage twice for imprecision secondary to broad confidence intervals (0.84 to 6.02) and a small number of events (total of 25).

[c] We downgraded our assessment of the evidence for stillbirth twice for imprecision. Although two studies examine this outcome, one had no events in either arm, therefore was removed from meta-analysis in accordance with Cochrane guidance. This leaves a single small study with very broad confidence intervals.

[d] We downgraded our assessment of the evidence for clinical pregnancy once for risk of bias owing to unclear risk of selection bias, performance bias and reporting bias in one study, with lack of details on how allocation was concealed after randomisation, no description of who was blinded, and no access to protocol or response from authors to clarify if all outcomes were published. Additionally, the unblinded embryologist decided how many embryos to transfer in one study.

BACKGROUND

Description of the condition

Embryo incubation is a critical step in all in vitro fertilisation (IVF) procedures. Embryo development within media in culture dishes in an incubator is a dynamic process, moving through the fertilisation stage to cleavage stage and then to the blastocyst stage in some cases. Throughout the incubation period, embryos are usually inspected at specific time points to provide a brief 'snap shot' assessment of the way the embryo is developing (morphological features). Embryologists apply a tiered grading system based on the morphology of the embryo in order to predict the potential for implantation and a successful pregnancy (Cummins 1986; Neuber 2003; Scott 2003; Scott 2003a; Shoukir 1997). A consensus on the minimum data set required for the accurate description of embryo morphology was established by Alpha Scientists in Reproductive Medicine and European Society of Human Reproduction and Embryology (ESHRE) Special Interest Group of Embryology (Alpha & ESHRE SIG 2011). A consensus on timings of observation of fertilised oocytes and embryos was established and deemed critical to the ability to compare results between different laboratories. The recommended checks, in hours, following insemination are:

- a fertilisation check at 17 hours, a syngamy (fusion of gametes) check at 23 hours;
- an early cleavage check at 26 hours post-intracytoplasmic sperm injection (ICSI) or 28 hours post-IVF;
- day 2 embryo assessment at 44 hours;
- day 3 embryo assessment at 68 hours;
- day 4 embryo assessment at 92 hours;
- day 5 embryo assessment at 116 hours.

Traditionally the checks have been achieved by physically removing embryos from the controlled environment of the incubator to analyse them under a light microscope for assessment of embryo development and quality. This practice exposes the embryos to the potentially suboptimal conditions of the environment outside of the incubator and human handling (Meseguer 2012a). Time-lapse systems (TLSs) have evolved over recent years to increase the frequency of morphological observations whilst minimising the impact of the external environment and human handling on embryo development.

Description of the intervention

A TLS is a device which takes digital images of embryos at set time intervals, for example every 5 to 15 minutes. The system can be installed into an existing embryo incubator or can exist as a combined time-lapse incubation system. The images are compiled using specialist software to create a time-lapse sequence of embryo development. Images can be digitally displayed as a time-lapse sequence on an external monitor to allow embryologists to assess the dynamic morphology of embryos thus negating the need for the embryologist to remove embryos from the incubator. Some TLSs also utilise computer-assisted assessment of developmental milestones of embryos, also known as morphokinetic parameters, to offer a semi-quantitative process of embryo evaluation (Conaghan 2013). These cell-tracking software algorithms utilise data such as the timing of embryonic development events, and have evolved as a non-invasive, non-subjective way of attempting to improve the selection of embryos with the highest implantation potential. Some clinics have developed their own algorithms to adapt the standardised one that comes with the TLS device (Petersen 2016). There are a number of commercially available TLSs developed by various manufacturers. TLSs are available as devices that can be placed within existing conventional incubators, and some exist with an integrated incubator. The integrated TLS combines both the time-lapse cameras and the incubator in one device.

How the intervention might work

There are two potential benefits of a TLS. Firstly, an advantage may lie with the undisturbed nature of the culture conditions, whereby image for embryo assessment can be obtained without removing embryos from the incubator environment for conventional bench-top light microscopy (which usually includes heated microscope stages). This minimises the exposure of embryos to both human handling and changes in air temperature and gas composition. This may lead to improved culture conditions.

A second potential advantage may be owing to the ability of a TLS to accumulate detailed time-lapse images of embryo development at regular time intervals. This includes the timing of cell divisions, intervals between cell cycles, and other development factors (eg. dynamic pronuclei patterns, presence of multinucleation and fragmentation, and blastomere symmetry). Many of these features which are transient events may be missed by using standard morphological assessment at set time intervals. These detailed time-lapse sequences can be utilised with or without cell-tracking software algorithms as an adjunct to standard morphological assessment, to select the embryo with the highest implantation potential for transfer. This is important because there is a clear correlation between embryo morphology and viability (Finn 2010; Neuber 2006). The ability to select the highest quality embryo at an optimal stage of development for replacement first in an assisted reproductive technology (ART) cycle may lead to a reduction in time to pregnancy, and reduced need for subsequent embryo transfers. It is worth noting that the different makes of TLS follow the same basic principles but vary in technical detail such as gas mixture, temperature, group or single culture, dark or light field microscopy.

In order to assess the potential advantage of TLSs (i.e. the stable culture environment, or the time-lapse sequence of images which

can be assessed with cell-tracking algorithms, or both), studies can be grouped into the following three designs.
Trial design 1: TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment

- These studies control for how the embryos are selected for transfer, but the incubation differs. This will help to establish whether the culture conditions of the TLS potentially impact on favourable outcomes such as pregnancy and live birth

Trial design 2: TLS utilizing embryo selection software versus TLS with conventional morphological assessment of still TLS images

- These studies control for the culture environment, with both arms of the trial being incubated in a TLS, but the way in which embryos are selected for transfer is tested. This study design will help to establish whether embryo selection software improves the selection of top-quality embryos, and increases the pregnancy and live birth rate

Trial design 3: TLS utilizing embryo selection software versus conventional incubation and assessment

- These studies aim to establish whether a combination of both the stable culture environment and the embryo selection software, is superior to conventional embryo incubation and assessment at improving pregnancy and live birth.

Why it is important to do this review

New interventions, such as TLSs, should be evaluated by randomised controlled trials to establish their safety, clinical effectiveness and cost-effectiveness (Campbell 2000; Harper 2012). Countering the potential benefits outlined above, a TLS involves exposing embryos to light during image acquisition, at predetermined intervals. Furthermore, the authorities responsible for the regulation of fertility clinics and research involving human embryos have a responsibility to provide impartial and authoritative information to prospective and current patients on fertility treatments to aid them in making informed decisions on their care (ACART; HFEA). Therefore it is vital that up-to-date and thorough systematic reviews, accessible to patients and healthcare workers, are published on the topic. This will enable information on the technology's success rates in terms of live birth or ongoing pregnancy rate, and safety in terms of adverse events, to be accessible and help guide informed decision making.
This is an update of a Cochrane review published under the same title in 2015. The original review included two completed RCTs and interim data from one ongoing RCT. The results of the original review showed that there was insufficient evidence of differences in live birth, miscarriage, stillbirth or clinical pregnancy to choose between TLS and conventional incubation.
This updated review is aimed at establishing whether there is evidence of any overall benefit of culturing embryos in a TLS with or without embryo selection software, over current conventional embryo incubation and assessment.

OBJECTIVES

To determine the effect of a time-lapse system (TLS) compared to conventional embryo incubation and assessment on clinical outcomes in couples undergoing assisted reproductive technology (ART).

METHODS

Criteria for considering studies for this review

Types of studies

Inclusions: any randomised controlled trial (RCT), whether published or not, which in principle could answer questions regarding clinical (post-implantation) outcomes.
Exclusions: quasi-randomised and other concurrently controlled studies were excluded. We excluded trials that randomised oocytes or embryos as it would not be possible to compare clinical outcomes. We excluded cross-over trials as the design is not valid in this context.

Types of participants

Couples of any age undergoing assisted reproduction where embryo incubation was required.

Types of interventions

- Time-lapse system (TLS) with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)
- TLS utilizing embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2)
- TLS utilizing embryo selection software versus conventional incubation and assessment (trial design 3)

Any type of TLS , using any type of embryo selection software and any type of conventional incubator, was eligible.

Types of outcome measures

Primary outcomes

1. Live birth rate per couple randomly assigned
2. Miscarriage, and stillbirth

Secondary outcomes

3. Clinical pregnancy, defined as evidence of a gestational sac, confirmed by ultrasound, per couple randomly assigned
4. Cumulative clinical pregnancy rate, per couple randomly assigned

Search methods for identification of studies

Two review authors (SA and PB) searched, from the inception of the databases to 2 August 2017, for all published and unpublished RCTs of time-lapse systems, without language restrictions and in consultation with the Cochrane Gynaecology and Fertility Group (CGFG) Information Specialist. We used both electronic searches of bibliographic databases and handsearching as described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

Electronic searches

We searched in the following electronic databases, trial registers and websites.

- Cochrane Gynaecology and Fertility Group specialised register, PROCITE platform (searched 2 August 2017) (Appendix 1)
- Cochrane Central Register of Studies Online (CRSO), web platform (searched 2 August 2017) (Appendix 2)
- MEDLINE® In-Process & Other Non-Indexed Citations, OVID platform (searched from 1946 to 2 August 2017) (Appendix 3)
- Embase, OVID platform (searched from 1980 to 2 August 2017) (Appendix 4)
- Cumulative Index to Nursing and Allied Health (CINAHL), EBSCO platform (searched from 1961 to 2 August 2017) (Appendix 5)

For MEDLINE, we used the Cochrane highly sensitive search strategy for identifying RCTs: sensitivity and precision maximizing version (2008 revision), Ovid format (Higgins 2011). The LILACS search strategy was combined with the RCT filter of the IAHx interface.
Other electronic sources of trials (web platforms, all searched August 2017) included the following.

- Trial registers for ongoing and registered trials; the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) portal (www.apps.who.int/trialsearch/) and ClinicalTrials.gov (www.clinicaltrials.gov)
- The Web of Knowledge (wokinfo.com/)
- Proquest Dissertations and Theses (search.proquest.com)
- Grey literature through the System for Information on Grey Literature in Europe 'OpenGrey' (www.opengrey.eu/).

Searching other resources

We attempted to identify additional relevant RCTs by using the following methods:

- contact with authors of all RCTs identified by other methods;
- contact with manufacturers of TLSs;
- handsearching of selected journals in obstetrics, gynaecology and reproductive medicine, as well as conference proceedings (for abstracts) of the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM);
- contacting known experts and personal contacts regarding unpublished materials;
- searching the citation lists of all identified articles for any relevant reference.

Data collection and analysis

Selection of studies

Two authors (SA and PB) independently scanned the titles and abstracts of the articles retrieved by the search. We then obtained full texts of potentially eligible studies and examined these independently for their suitability according to the inclusion criteria. In the case of doubt between the two authors, a third author (CF) was consulted to gain consensus on whether to include the trial or not. We documented the selection process with a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart.

Data extraction and management

Two authors (SA and PB) independently obtained and extracted data. In the case of disagreement between the two authors, they consulted a third author to achieve consensus (CF). They extracted data using a data extraction form designed and piloted by the authors. If studies were reported in multiple publications, we extracted data from the different publications and then combined these into a single data extraction form so no data were omitted. The following characteristics of included studies were included in the data extraction form:

- methods;
- participants;
- interventions;
- outcomes, including adverse events;
- funding source for studies.

Assessment of risk of bias in included studies

Two review authors (SA and PB) independently assessed the risk of bias in included studies using the Cochrane 'Risk of bias' assessment tool. We evaluated all included studies for the following:

adequacy of sequence generation and allocation concealment; adequacy of blinding of couples, providers and outcome assessors; completeness of outcome data; risk of selective outcome reporting; and risk of other potential sources of bias (Higgins 2011). Disagreements between authors were resolved by consensus and consulting a third reviewer (VJ). The results of the assessment of risk of bias are presented in the 'Characteristics of included studies' table and a 'Summary of findings' table.

Measures of treatment effect

For dichotomous data (for example, live birth or not), we calculated Mantel-Haenszel odds ratios (ORs) and the 95% confidence intervals (CIs).

Unit of analysis issues

The data were analysed per couple randomised. Studies randomising oocytes or embryos were excluded.

Dealing with missing data

If relevant data were missing from an included study, we contacted the original investigators of the trial to request the missing data. All original investigators were contacted. In particular, we obtained clinical pregnancy and live birth data from Park 2015, miscarriage and clinical pregnancy data per woman randomised for Goodman 2016, and live birth and stillbirth data from Kahraman 2013. If participants were described as 'lost to follow up' without a specified reason, we assumed the participant did not experience the event or outcome (that is, did not become pregnant).

Assessment of heterogeneity

We considered whether the clinical and methodological characteristics of the included studies were sufficiently similar for meta-analysis to provide a clinically meaningful summary. We assessed statistical heterogeneity by measuring the I^2 statistic. We assumed that there was substantial heterogeneity when I^2 was calculated to be greater than 50% (Higgins 2011).

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases, the authors aimed to minimise their potential impact by ensuring a comprehensive search for eligible studies and by being alert to duplication of data. Within-study reporting bias was assessed, and assessed as low risk if all of the study's prespecified primary outcomes were reported as outlined in the study's protocol.

Data synthesis

Where sufficient data were available, we combined the data for the primary outcomes by using a fixed-effect model in the following comparisons.

- TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)
- TLS utilizing embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2)
- TLS utilizing embryo selection software versus conventional incubation and assessment (trial design 3)

Subgroup analysis and investigation of heterogeneity

Where sufficient data were available, we aimed to conduct the following subgroup analyses to determine the potential causes of heterogeneity for the live birth and clinical pregnancy outcomes:

- donor oocytes (from donors of any age) versus autologous oocytes (from women of any age);
- fresh cycles (where embryos were replaced either at cleavage stage (day 3) or blastocyst (day 5)) versus frozen cycles (where frozen embryos were replaced in an assisted reproductive technology cycle).

If we detected substantial heterogeneity we hoped to explore this by employing the random-effects model. We aimed to take any statistical heterogeneity into account when interpreting the results, especially if there was any variation in the direction of effect.

Sensitivity analysis

We planned to undertake sensitivity analyses for the review outcomes to determine whether the results were robust to decisions made during the review process. These analyses would have included consideration of whether the review conclusions would have differed if:

- the summary effect measure was relative risk rather than odds ratio;
- eligibility was restricted to studies with low risk of bias for randomisation and allocation concealment.

Overall quality of the body of evidence: 'Summary of findings' table

We prepared 'Summary of findings' tables using GRADEpro GDT (www.gradepro.orgGRADEproGDT 2015) and Cochrane methods in March 2018. These tables evaluate the overall quality of the body of evidence for the main review outcomes (live birth, miscarriage, stillbirth and clinical pregnancy) for the review comparisons:

- TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1);
- TLS utilizing embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2); and
- TLS utilizing embryo selection software versus conventional incubation and assessment (trial design 3).

We assessed the quality of the evidence using GRADE criteria: risk of bias, consistency of effect, imprecision, indirectness and publication bias. Judgements about evidence quality (high, moderate, low or very low) was made by two review authors who worked independently (SA and PB), and resolved disagreements by discussion. Judgements were justified, documented, and incorporated into reporting of results for each outcome.

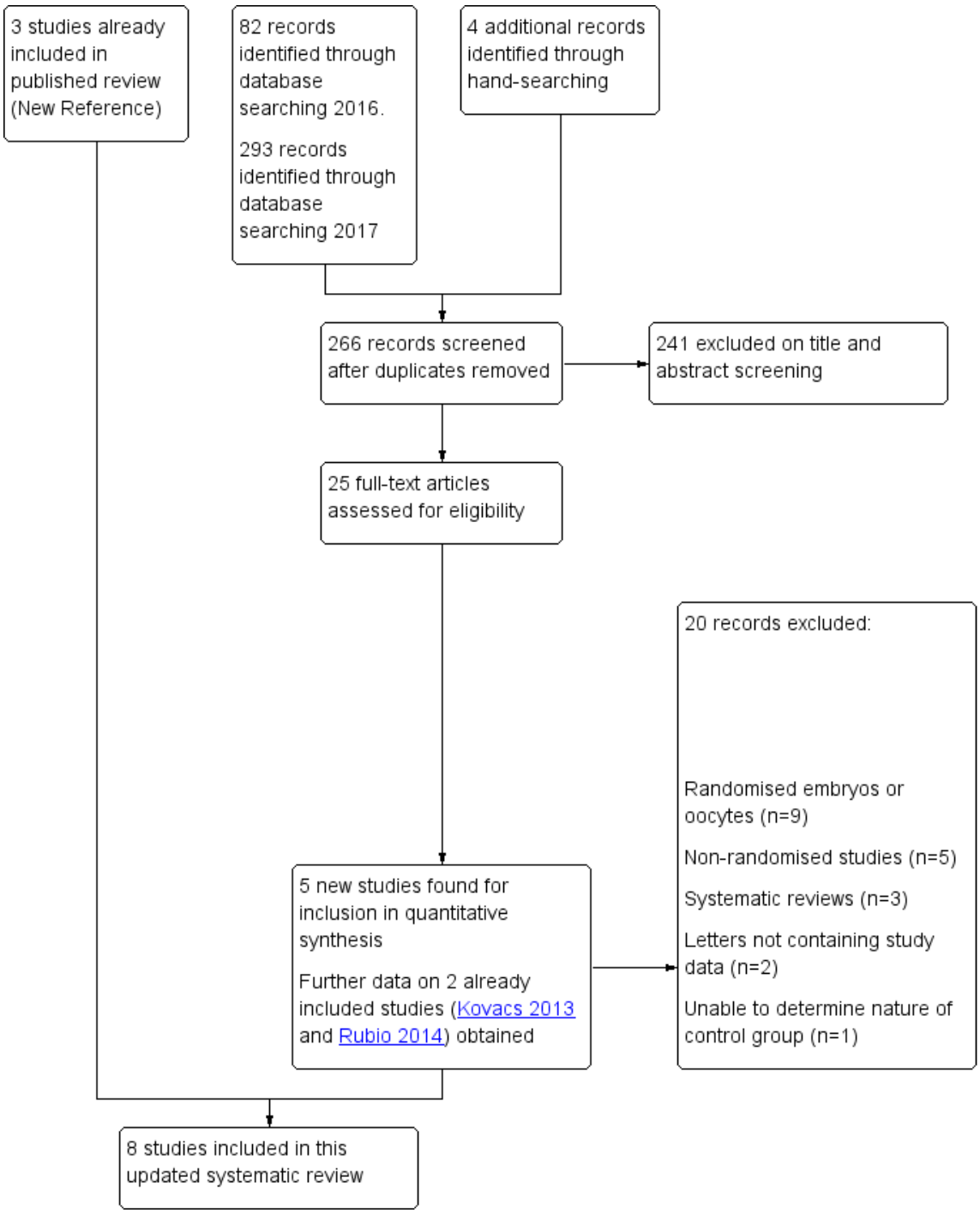
RESULTS

Description of studies

Results of the search

The first iteration of this review included three parallel-design randomised controlled trials (RCTs) from a search which retrieved 33 articles in total (new studies added were Kahraman 2013; Kovacs 2013; Rubio 2014). Two further searches in 2016 and 2017 retrieved 82 and 293 articles respectively. We retrieved a further four articles through handsearching. We screened 266 articles after removing duplicates. Twenty-five full-text articles were potentially eligible and we retrieved these in full text. We identified five new studies which met our inclusion criteria (Goodman 2016; Kaser 2017; Park 2015; Wu 2016; Yang 2017). We excluded the remaining 20 studies for the following reasons: three studies were not RCTs; three were systematic reviews; two were letters; nine randomised embryos or oocytes; two were pseudo-randomised; and in one study we were unable to determine the nature of the control group despite attempts at contacting the authors. (Figure 1, Excluded studies). In total, we included eight RCTs in the quantitative synthesis.

Figure 1. Study flow diagram.



Included studies

Study design and setting

Eight RCTs are included in this review. The largest study is a multi-centre RCT conducted in Spain, which was included in the first iteration of this review (Rubio 2014). Three new single-centre studies added to this review were conducted in the USA (Goodman 2016; Kaser 2017; Wu 2016). Two further new single-centre studies were added; one completed study was undertaken in Sweden (Park 2015), and another ongoing study was undertaken in China, from which we have interim results (Yang 2017). The final two studies were included in the first iteration of this review, one of which was a single-centre RCT conducted in Turkey (Kahraman 2013), and the other is the completed results of a single-centre RCT in Hungary (Kovacs 2013).

Participants

The studies included 2303 infertile couples undergoing assisted reproductive technology (ART). Three studies included couples undergoing intracytoplasmic sperm injection (ICSI) alone (Kahraman 2013; Rubio 2014; Park 2015). Two studies included couples undergoing in vitro fertilisation (IVF) (Goodman 2016; Kovacs 2013). The remaining studies describe including couples undergoing both IVF and ICSI (Kaser 2017; Wu 2016; Yang 2017).

The largest study is Rubio 2014, with 856 participants. The second largest study has 364 participants (Park 2015), followed by the interim results of Yang 2017, with 334 participants. The fourth largest study has 300 participants (Goodman 2016), followed by Kaser 2017, with 163. The sixth largest study is the completed results of Kovacs 2013, with 161 participants. The remaining two studies are relatively small, with 76 and 49 participants (Kahraman 2013; Wu 2016, respectively).

All studies utilised the autologous oocytes of the women randomised into their study with the exception of Rubio 2014, which included couples undergoing ART with autologous or donor oocytes. The proportion of couples receiving donor oocytes in this study is unknown. Most donor oocytes in this study were used in fresh cycles, however some donor oocytes were obtained from an oocyte bank and were therefore vitrified.

All studies included women undergoing fresh embryo transfer, hence no cumulative cycle results are available. The majority of studies undertook single embryo transfer (Kahraman 2013; Kaser 2017; Kovacs 2013; Park 2015; Yang 2017). One study describes replacing between one and three embryos based on published American Society for Reproductive Medicine (ASRM) committee guidance and patient preferences (Goodman 2016). Another study undertook multiple embryo transfer (Rubio 2014), and another did not disclose the number of embryos transferred (Wu 2016).

The reported causes of infertility varied between studies. Some studies specifically described their participants as 'good prognosis patients' (e.g. Rubio 2014; Yang 2017). One study specifically described their participants as 'poor prognosis patients', but gave no further information (Wu 2016). One study described 'tubo-peritoneal factor' as the cause of infertility (Kahraman 2013), and another described male-factor infertility being present in more than 99% of participants in both arms and female-factor infertility being present in approximately 20% of participants in both arms (Park 2015). In Kovacs 2013, various causes of infertility in participants was described ("male, tubal, unexplained etc."). One study described "a combination of anovulation, diminished ovarian reserve, endometriosis, male factor, tubal, unknown, and uterine" as causes of infertility (Kaser 2017). Finally, in Goodman 2016, a range of infertility diagnoses was described, from "unexplained, ovulatory dysfunction, male factor, tubal factor, low ovarian reserve, AMA, endometriosis, mixed factors and other".

Interventions

We have sought to divide studies into three comparisons depending on the nature of the intervention and the control, in order to truly assess if, and where, the benefit of a time-lapse system (TLS) lies.

1) TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)

Three studies undertook this comparison (Kahraman 2013; Park 2015; Wu 2016). All three studies utilised an integrated TLS, and all three had two arms. Embryo transfer (ET) was undertaken at blastocyst in Kahraman 2013, day three in Wu 2016, and day two in Park 2015. It was confirmed on correspondence with the authors of one study that no embryo selection software was utilised in the intervention arm (Kahraman 2013). Embryos were left undisturbed in the TLS in the intervention arm in all three studies. In the control arm, embryos in all studies were assessed by conventional morphology under a benchtop microscope.

2) TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2)

Two studies undertook this comparison (Goodman 2016; Kaser 2017). One study utilised an integrated TLS (Goodman 2016),

and the other utilised a TLS which was placed inside a conventional incubator (Kaser 2017). The embryos in the intervention arms were selected for transfer according to the information obtained from the embryo selection software, however the embryos of the women randomised to the intervention arm in one study were removed from the incubator for conventional benchtop morphology in addition to TLS selection (Kaser 2017). In addition, the embryos in the control arm of this study were assessed with conventional morphological assessment under a benchtop microscope. TLS images were not utilised for the selection of embryos for replacement in the control arm.

One study was a three-arm study (Kaser 2017). There were two intervention arms; both were TLS utilising embryo selection software, but one arm undertook ET on day three and the other undertook ET on day five. The control arm undertook ET on day five. The other study had two arms, and ET was undertaken on day three or day five (Goodman 2016).

We conducted in-depth discussions with the authors of Kaser 2017, and it was decided that trial design 2 was the most appropriate comparison, given that embryo selection software was utilised and the trial design tested the embryo-selection element of the TLS software.

3) TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3)

Three studies undertook this comparison (Kovacs 2013; Rubio 2014; Yang 2017). Two of these utilised a TLS which was placed inside a conventional incubator (Kovacs 2013; Yang 2017); the other study utilised an integrated TLS (Rubio 2014). In Rubio 2014, ET was undertaken on days three and five in both arms; in Kovacs 2013, blastocyst transfer was undertaken in both arms. One study undertook ET on day three in the intervention arm and day five (blastocyst) in the control arm (Yang 2017). We took methodological advice on Yang 2017, and made the decision to keep the study in this review despite the differing days of ET. We gave this study a high 'Risk of bias' rating due to this within-study imbalance.

Outcomes

All eight studies reported clinical pregnancy rates per couple. Miscarriage data were available for all included studies except for Wu 2016. In the case of Yang 2017, the miscarriage rate was calculated by us using ongoing pregnancy data minus clinical pregnancy data. Miscarriage data are confirmed to be loss of a clinical pregnancy (not biochemical) in the studies by Kahraman 2013; Kaser 2017; Kovacs 2013; Park 2015; and Yang 2017. In two studies the miscarriage data were a mixture of biochemical and clinical pregnancy losses (Goodman 2016; Rubio 2014). Unfortunately the authors of these studies were unable to provide only miscarriage data from clinical pregnancies. In these cases we have taken the pragmatic view to include these data as the majority of the pregnancy losses in these studies are from clinical pregnancies, according to the authors.

We obtained live birth data for three studies following communication with the authors (Kahraman 2013; Kovacs 2013; Park 2015). For Rubio 2014, we obtained data from a related publication and conference abstract pertaining to the same study (Insua 2017; Insua 2015). We obtained stillbirth data from two studies following communication with the authors (Kahraman 2013; Park 2015).

Excluded studies

We excluded 20 studies from the review for the following reasons.

- Three were not RCTs
- Three were systematic reviews
- Two were letters
- Nine randomised embryos or oocytes opposed to women or couples
- In one study we were unable to determine the nature of the control group despite attempts at contacting the authors
- Two studies were pseudo-randomised

Risk of bias in included studies

For details of the 'Risk of bias' assessments, see Figure 2 and Figure 3.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

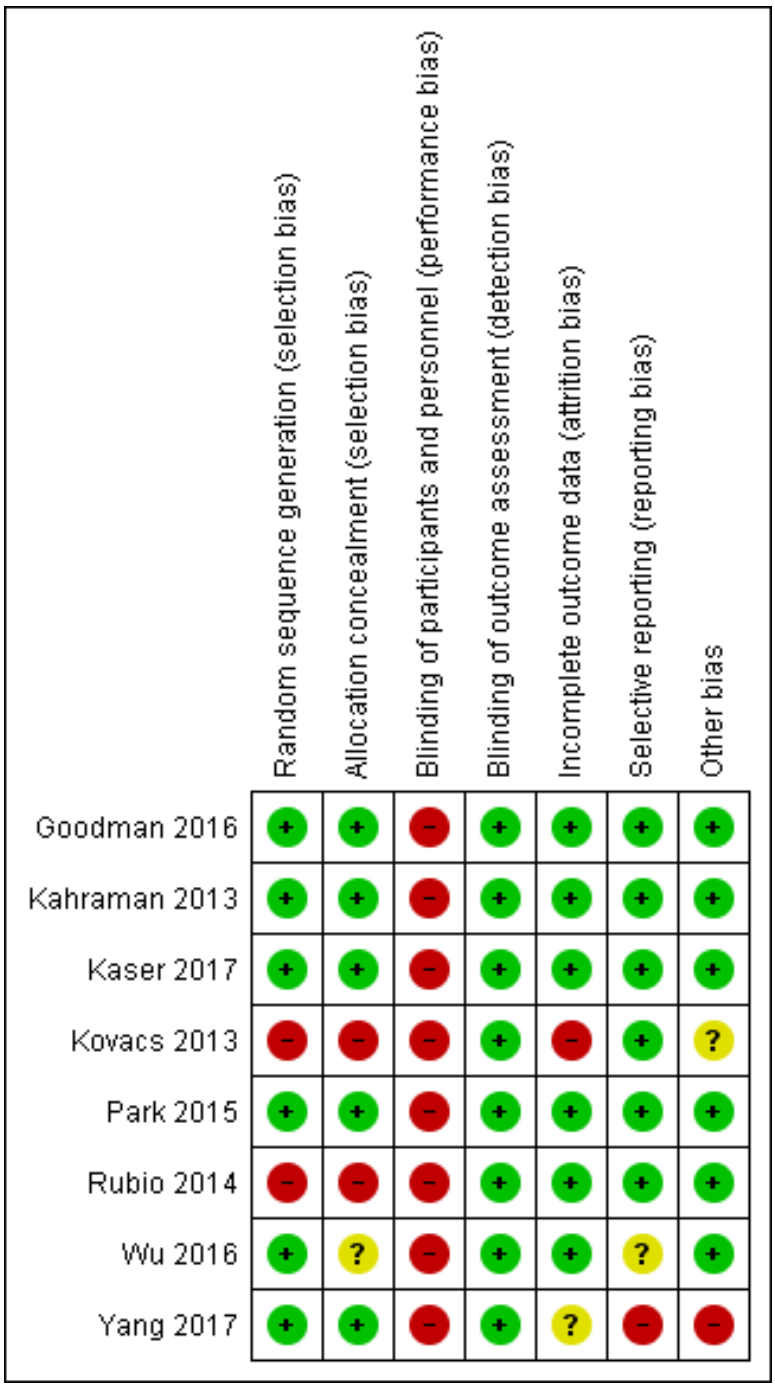
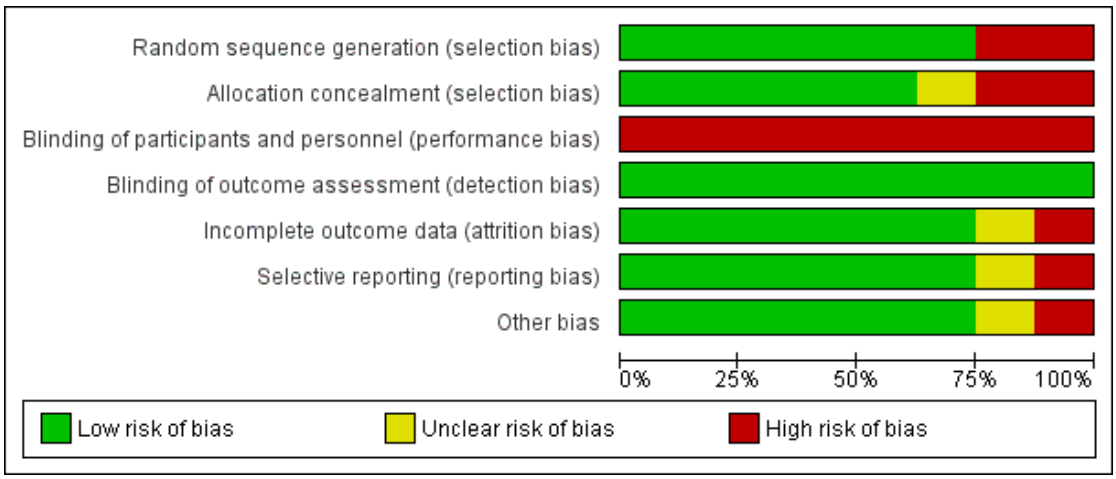


Figure 3. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.



Allocation

Sequence generation

Seven of the eight studies were at low risk of selection bias related to sequence generation. Five used a computer-generated randomisation list (Goodman 2016; Kahraman 2013; Kaser 2017; Park 2015; Wu 2016). One study utilised a random number table (Yang 2017). One study undertook paired randomisation whereby two envelopes containing time-lapse or control group assignments were prepared and the first patient was randomly assigned to one of the groups and the next patient received the other assignment (Kovacs 2013). This was repeated with patient numbers three and four, and so on.

We deemed one study to have high risk of bias in this domain because, although it undertook adequate random sequence generation, some women were able to request the intervention, and in some cases this request was granted (Rubio 2014). The authors of this study assured us that this preferential allocation occurred on a minority of occasions and the vast majority of participants were truly randomised, therefore we have maintained that this is a randomised controlled trial (RCT).

Allocation concealment

Five studies described methods of allocation concealment which were at low risk of selection bias (Goodman 2016; Kahraman 2013; Kaser 2017; Park 2015; Yang 2017). In each of these studies, the randomisation list or numbered opaque sealed envelopes were held and administered by personnel not directly involved in the recruitment of participants.

We deemed two studies to be at high risk of bias for this domain (Kovacs 2013; Rubio 2014). In the case of Kovacs 2013, the randomisation was carried out by the principal investigator who was involved in the study. In the case of Rubio 2014, it was described that in some cases the allocation was non-random.

We judged one study to be at unclear risk of bias in this domain due to the limited description of randomisation (Wu 2016). We understand it was undertaken by a member of the team not associated with the treatment cycle and then subsequently the designation was reported to the embryology staff who processed the participant's oocytes/embryos. However, it is unclear how the randomisation list was stored, at what point the participants were randomised, and whether the person undertaking randomisation was responsible for recruitment.

Blinding

Blinding of participants and personnel (performance bias)

Three studies blinded their couples and this blinding was not broken unless participants withdrew from the study (Goodman 2016;

Kahraman 2013; Park 2015). Clinicians involved in the study were also blinded until after embryo transfer. One study described blinding the embryologist to the Eeva rating for the morphological assessment of embryos (Kaser 2017). The participants and physicians were all blinded to the time-lapse system (TLS) ratings. In addition, the sonographer was blinded in Goodman 2016, and the statistician was blinded in Park 2015.

Three studies did not blind or maintain blinding of their participating couples (Kovacs 2013; Rubio 2014; Yang 2017). In two of these studies the clinical staff were not blinded either (Kovacs 2013; Yang 2017). The gynaecologist and the statistician were blinded in Rubio 2014. We assessed these three studies as being at high risk of this bias.

We deemed one study as having high risk of performance bias as the blinding was not described and it would have been impossible to blind the embryologist (Wu 2016). We have been unable to contact the authors for further clarification.

None of the included studies blinded the embryologists, but this would have been impossible. We considered a lack of blinding of embryologists as a reason for high risk of performance bias. This renders all included studies as having a high risk of performance bias. In some studies, the lack of blinding may have influenced the number or day of transfer. In addition, it is impossible to remove the risk of performance bias when the person selecting the embryo for transfer is unblinded.

Blinding of outcome assessors (detection bias)

We judged all eight studies to be at low risk of detection bias because the outcomes (live birth, clinical pregnancy, miscarriage and stillbirth) are objective, and cannot be influenced by the knowledge of the intervention. Two studies described how those staff undertaking the ultrasounds were blinded to the intervention (Goodman 2016; Rubio 2014). The remaining studies did not blind their outcome assessors, however we still deemed these studies as having low risk of bias due to the reason described above.

Incomplete outcome data

We deemed the following studies to be at low risk of attrition bias:

- Goodman 2016, because we were able to obtain the outcome data from the five women excluded after randomisation;
- Kahraman 2013, because the 12 couples who dropped out after randomisation were accounted for, and the reasons were clearly stated;
- Kaser 2017, because all data were presented in their paper as intention-to-treat;
- Park 2015, because there was only one woman excluded from analysis due to having been accidentally randomised twice;
- Wu 2016, because the small number of patients excluded were accounted for according to pre-determined grounds for exclusion; and
- Rubio 2014, because the 13 couples who were excluded following randomisation were accounted for and were a very small proportion of the total number of couples randomised.

We judged one study to be at high risk of attrition bias because a large proportion of the couples recruited were excluded from the trial (22 out of 161 couples randomised) (Kovacs 2013). On communication with the author it was made clear that these excluded couples were 'dropouts'. Reasons for dropouts were provided, however not all had reasons which tallied with pre-determined exclusion criteria and, with such a high attrition rate, this study is at high risk of attrition bias.

We deemed one study to have unclear risk of attrition bias (Yang 2017). Attrition was mentioned, but reasons were not provided.

We undertook an intention-to-treat analysis on all dichotomous outcomes, using data from those women excluded post-randomisation where possible.

Selective reporting

We considered six studies to be at low risk of reporting bias because they reported and published all outcomes they set out to investigate (Goodman 2016; Kahraman 2013; Kaser 2017; Kovacs 2013; Park 2015; Rubio 2014). This was confirmed on communication with authors and by referencing against online trials registers if they were available.

We considered one study to be at unclear risk of reporting bias because we had no access to their protocol and we couldn't contact the authors to ask if they published all outcomes they set out to assess (Wu 2016).

We deemed one study to be at high risk of reporting bias because on communication with authors, they mentioned a series of outcomes, including implantation rates, twin pregnancy rate (monozygotic twins), and ectopic pregnancy which were never published (Yang 2017). We concede that this is only an interim report and that such reports do not always include all secondary outcomes. However, the fact that this publication is the interim analysis of a full study, and it is not clear from communication with authors that this was a planned analysis, means the study is at risk of selective reporting.

Other potential sources of bias

We found no potential sources of within-study bias in Goodman 2016, Kahraman 2013, Kaser 2017, Park 2015, Rubio 2014, and Wu 2016. We assessed these studies as having low risk of this bias.

We deemed one study to have an unclear risk of within-study bias (Kovacs 2013). Data included in this review were obtained from the author directly, however these have not be published. Interim reporting and analysis of results from this study are available in various published sources, with differing results.

We assessed one study, Yang 2017, as having a high risk of within-study bias. This is due to the difference in day of embryo transfer

between arms of study (day three for intervention and day five for control). This difference in maturity of the embryo may have had an impact on the likelihood of an ongoing pregnancy.

Effects of interventions

See: **Summary of findings for the main comparison** TLS with conventional morphological assessment of still TLS images compared to conventional incubation and assessment for embryo incubation and assessment in assisted reproduction; **Summary of findings 2** TLS utilising embryo selection software compared to TLS with conventional morphological assessment of still TLS images for embryo incubation and assessment in assisted reproduction; **Summary of findings 3** TLS utilising embryo selection software compared to conventional incubation and assessment for embryo incubation and assessment in assisted reproduction

1. Time-lapse system (TLS) with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)

Three studies undertook this comparison (Kahraman 2013; Park 2015; Wu 2016), with a total of 489 participants.

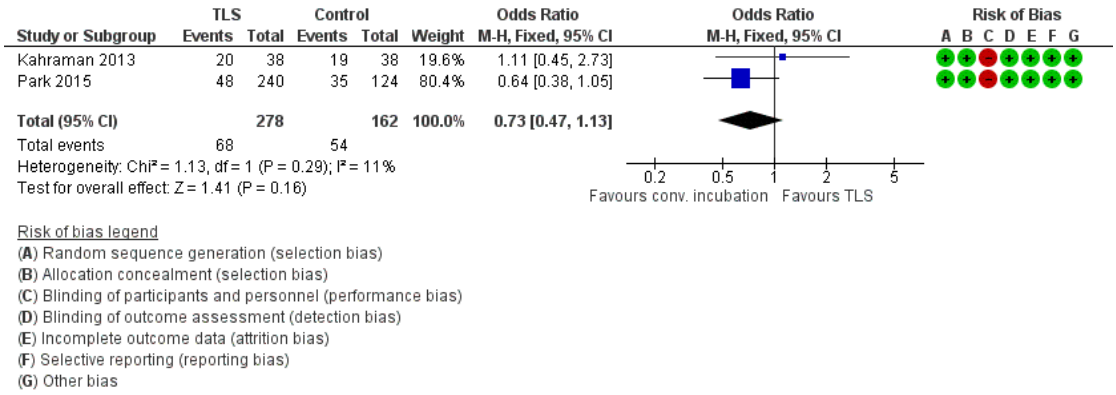
Primary outcomes

1.1 Live birth

Two studies provided live birth data following correspondence with their authors (Kahraman 2013; Park 2015; N = 440). Sixty-eight live births were reported in the TLS arm from the 278 women randomised to that arm. There were 54 live births from the 162 women randomised to the control arm (conventional incubation and embryo assessment).

There is probably no difference between the interventions in live birth rates (odds ratio (OR) 0.73, 95% confidence interval (CI) 0.47 to 1.13, 2 RCTs, N = 440, I^2 = 11% , moderate-quality evidence, Analysis 1.1; Figure 4). The evidence suggests that if the live birth rate associated with conventional incubation and assessment is 33%, the rate with use of TLS with conventional morphological assessment of still TLS images is between 19% and 36%.

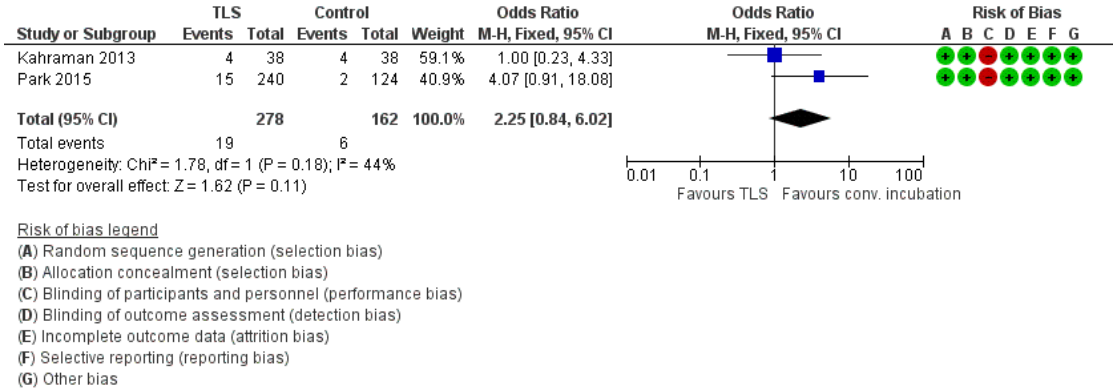
Figure 4. Forest plot of comparison: 1 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1), outcome: 1.1 Livebirth.



1.2 Miscarriage and stillbirth

Two studies provided both miscarriage and stillbirth data (Kahraman 2013; Park 2015; N = 440). The stillbirth data were available following communication with the authors of Park 2015. There also may be no difference between the interventions in miscarriage rates. Out of 278 women randomised to the intervention arm, 19 women experienced a miscarriage; out of the 162 randomised to the control arm, 6 experienced miscarriage (OR 2.25, 95% CI 0.84 to 6.02, 2 RCTs, N = 440, I^2 = 44%, low-quality evidence, Analysis 1.2; Figure 5). The evidence suggests that if the miscarriage rate with conventional incubation is 3%, the rate associated with TLS with conventional morphological assessment of still TLS images would be between 3% and 18%.

Figure 5. Forest plot of comparison: 2 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment: miscarriage.



Regarding stillbirth, there were two stillbirths out of 38 women randomised to the intervention arm and two out of the 38 women randomised to the control arm in the study by Kahraman 2013. There were no stillbirths recorded in either arm of the study in Park 2015, meaning that its result is inestimable. In accordance with Cochrane methodological guidance, we have removed Park 2015 from meta-analysis. Results from this solitary study (not meta-analysis) suggest that there may be no difference between the interventions in rates of stillbirth (OR 1.00, 95% CI 0.13 to 7.49, 1 RCT, N = 76, low-quality evidence, Analysis 1.3).

Secondary outcomes

1.3 Clinical pregnancy

All three studies provided clinical pregnancy data (Kahraman 2013; Park 2015; Wu 2016; N = 489). Of the 302 women randomised to the intervention arm there were 92 clinical pregnancies, and from the 187 women randomised to the control arm there were 66. The moderate-quality evidence suggests that there is probably no difference between TLS with conventional morphological assessment of still TLS images and conventional incubation and assessment (OR 0.88, 95% CI 0.58 to 1.33, 3 RCTs, N = 489, I^2 = 0%, moderate-quality evidence Analysis 1.4).

2. TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2)

Two studies undertook this comparison (Goodman 2016; Kaser 2017), with a total of 463 participants. It is worth noting that in Kaser 2017, there were two intervention groups; one involved day three embryo transfer, and the other involved day five embryo transfer. The two intervention groups are represented as separate

entities at meta-analysis and the single control group has been split to share between the two intervention groups in order to avoid artificially doubling the effect of the control group.

Primary outcomes

2.1 Live birth

Live birth data were not collected by either study. This was confirmed on correspondence with the authors of both studies.

2.2 Miscarriage and stillbirth

Stillbirth data were not collected by either study.

We obtained miscarriage data for all women randomised following correspondence with the authors of both studies. For Goodman 2016, the miscarriage data include a combination of biochemical and clinical pregnancy losses. Unfortunately these data could not be separated for this review. For Kaser 2017, the data include miscarriages from clinical pregnancy losses.

There were 18 miscarriages out of 260 women randomised to the intervention arm, and 11 out of 203 women randomised to the control arm. We are uncertain whether TLS utilising embryo selection software influences miscarriage rates (OR 1.39, 95% CI 0.64 to 3.01, 2 RCTs, N = 463, I^2 = 0%, very low-quality evidence, Analysis 2.1). The evidence suggests that if the miscarriage rate associated with assessment of still TLS images is 5%, the rate with embryo selection software would be between 3% and 14%.

Secondary outcomes

2.3 Clinical pregnancy

Both studies reported this outcome. There were 132 clinical pregnancies from the 260 women randomised to the intervention group and 109 pregnancies from the 203 women randomised to the control group. There may be no difference between the interventions in clinical pregnancy rates (OR 0.97, 95% CI 0.67 to 1.42, 2 RCTs, N = 463, I^2 = 0%, low-quality evidence, Analysis 2.2).

3. TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3)

Three studies undertook this comparison (Kovacs 2013; Rubio 2014; Yang 2017), with a total of 1351 participants.

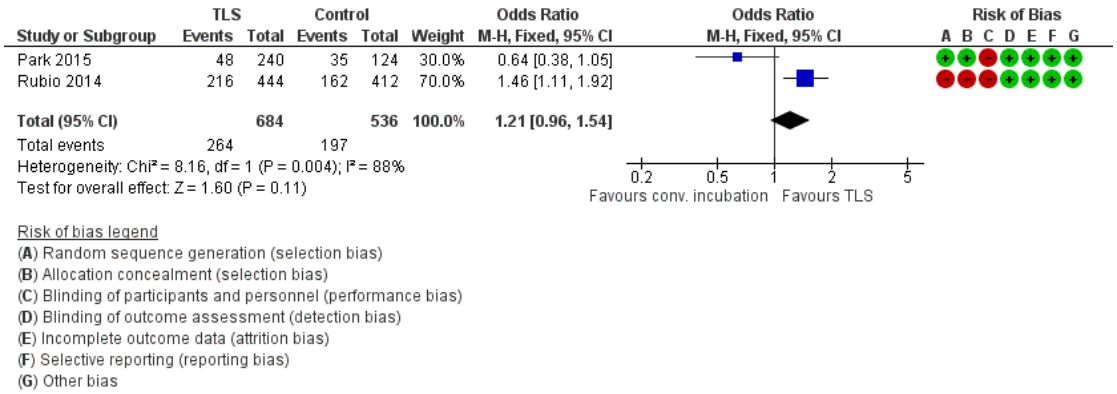
Primary outcomes

3.1 Live birth

Live birth data were available for two studies (Kovacs 2013; Rubio 2014). For Kovacs 2013, live birth data were provided following a request via correspondence. For Rubio 2014, we obtained data from a recently published paper and a published conference abstract (the references for these are provided as sub-references under Rubio 2014).

There were 250 live births from the 524 women randomised to the intervention arm, and 188 live births from the 493 women randomised to the control arm. There is very low-quality evidence that TLS utilising embryo selection software may improve live birth rates compared to conventional incubation and assessment (OR 1.21, 95% CI 0.96 to 1.56, 2 RCTs, N = 1017, I^2 = 0%, Analysis 3.1 Figure 6). The evidence suggests that if the live birth rate associated with no TLS is 38%, the rate with use of conventional incubation would be between 44% and 73%.

Figure 6. Forest plot of comparison: 3 TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3), outcome: 3.1 Livebirth.



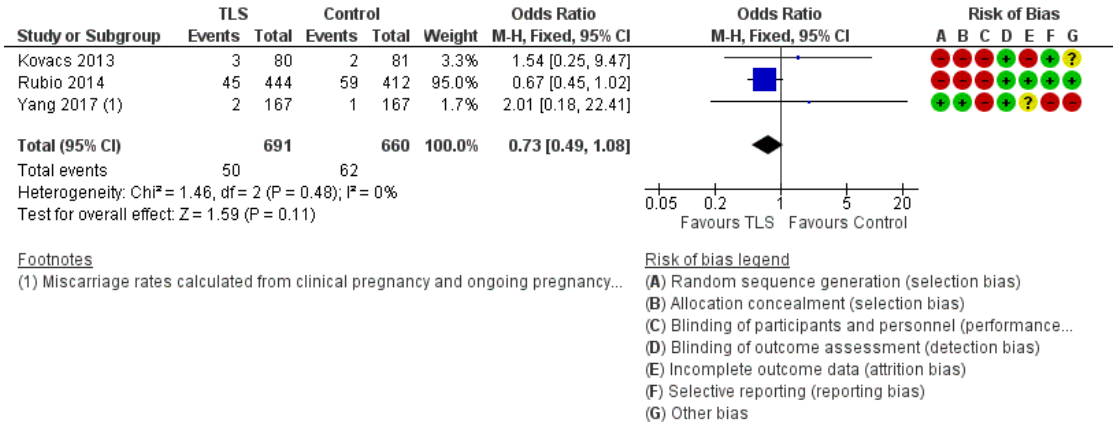
3.2 Miscarriage and stillbirth

Stillbirth data were not collected by any study.

Miscarriage data are losses of clinical pregnancies in two studies (Kovacs 2013; Yang 2017). The other study has a combination of biochemical and clinical pregnancy losses (Rubio 2014).

There were 50 miscarriages from 691 women randomised to the intervention arm, and 62 miscarriages from 660 women randomised to the control arm. We are uncertain whether TLS utilising embryo selection software influences miscarriage rates (OR 0.73, 95% CI 0.49 to 1.08, 3 RCTs, N = 1351, I^2 = 0%, very low-quality evidence, Analysis 3.2; Figure 7). The evidence suggests that if miscarriage rate with conventional incubation is 9%, the rate associated with TLS would be between 4% and 10%.

Figure 7. Forest plot of comparison: 3 TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3), outcome: 3.2 Miscarriage.

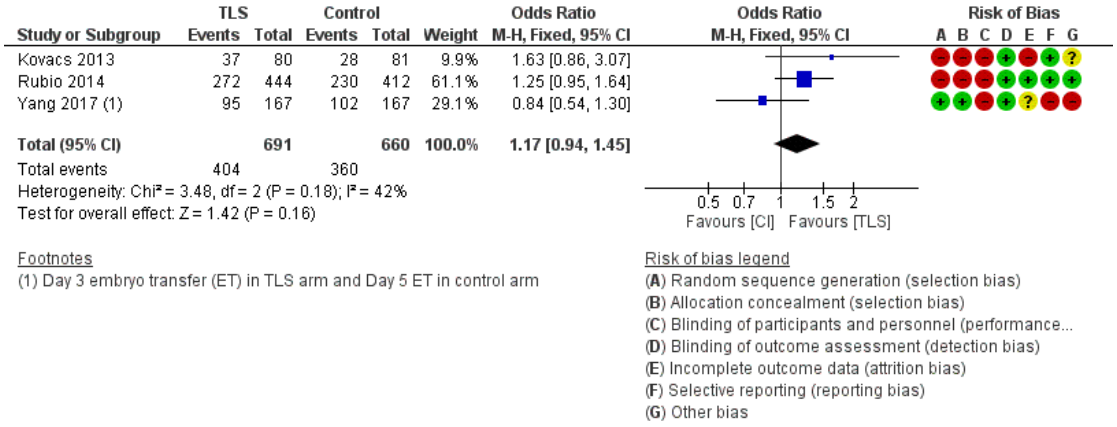


Secondary outcomes

3.3 Clinical pregnancy

All three studies reported this outcome. There were 404 clinical pregnancies from the 691 women randomised to the intervention arm, and 360 pregnancies from the 660 women randomised to the control arm. We are uncertain whether TLS utilising embryo selection software influences clinical pregnancy rates (OR 1.17, 95% CI 0.94 to 1.45, 3 RCTs, N = 1351, I^2 = 42%, very low-quality evidence, Analysis 3.3 Figure 8).

Figure 8. Forest plot of comparison: 3 TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3), outcome: 3.3 Clinical pregnancy.



It is worth noting that one study, Yang 2017, is very different in design to the other two included studies owing to the fact that it has differing days of embryo transfer in the intervention and the control arm of the study. When we removed this study in a sensitivity analysis, the pooled effect changed, revealing very low-quality evidence of an improvement in clinical pregnancy rates (OR 1.30, 95% CI 1.02 to 1.67, 2 RCTs, N = 1017, I^2=0%, very low-quality evidence).

Subgroup and sensitivity analysis

We did not perform any planned subgroup or sensitivity analyses as there were insufficient number of included studies within the meta-analyses.

ADDITIONAL SUMMARY OF FINDINGS [Explanation]

TLS utilising embryo selection software compared to TLS with conventional morphological assessment of still TLS images for embryo incubation and assessment in assisted reproduction

Patient or population: couples undergoing assisted reproductive technology
Setting: fertility clinic
Intervention: TLS utilising embryo selection software
Comparison: TLS with conventional morphological assessment of still TLS images

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with TLS with conventional morphological assessment of still TLS images (trial design 2)	Risk with TLS utilizing embryo selection software				
Live birth	0 per 1000	0 per 1000	not estimable	0 RCTs		
Miscarriage	54 per 1,000	74 per 1,000 (35 to 147)	OR 1.39 (0.64 to 3.01)	463 (2 RCTs)	⊕◯◯◯ Very low[a]	
Stillbirth	0 per 1000	0 per 1000	not estimable	0 RCTs		
Clinical pregnancy	537 per 1,000	529 per 1,000 (437 to 622)	OR 0.97 (0.67 to 1.42)	463 (2 RCTs)	⊕⊕◯◯ Low[b]	

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the mean risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: Confidence interval; **OR:** Odds ratio; **TLS:** time-lapse system

GRADE Working Group grades of evidence

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[a] We downgraded our assessment of the quality of evidence for miscarriage three times: once for risk of bias, once for indirectness and once for imprecision. The risk of bias is secondary to performance bias due to varying days and numbers of embryos transferred, decided upon by an unblinded embryologist. There is heterogeneity between the study designs leading to indirectness; one included study involved removing embryos for bench-top microscopy daily in both the intervention and control arms, whereas the other left embryos in the intervention and control arms undisturbed. The imprecision is secondary to broad confidence intervals.

[b] We downgraded our assessment of the quality of evidence for clinical pregnancy twice: once for risk of bias and once for indirectness, for the same reasons as outlined above.

TLS utilising embryo selection software compared to conventional incubation and assessment for embryo incubation and assessment in assisted reproduction

Patient or population: couples undergoing ART
Setting: fertility clinic
Intervention: TLS utilising embryo selection software
Comparison: conventional incubation and assessment

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with conventional incubation and assessment	Risk with TLS utilising embryo selection software				
Live birth	381 per 1,000	461 per 1,000 (365 to 586)	OR 1.21 (0.96 to 1.54)	1017 (2 RCTs)	⊕◯◯◯ Very low[a]	
Miscarriage	94 per 1,000	70 per 1,000 (48 to 101)	OR 0.73 (0.49 to 1.08)	1351 (3 RCTs)	⊕◯◯◯ Very low[b]	
Stillbirth	0 per 1000	0 per 1000	not estimable	0 RCTs		
Clinical pregnancy	545 per 1,000	584 per 1,000 (530 to 635)	OR 1.17 (0.94 to 1.45)	1351 (3 RCTs)	⊕◯◯◯ Very low[c]	

***The risk in the intervention group** (and its 95% confidence interval) is based on the mean risk in the comparison group and the relative effect of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio; **TLS:** time-lapse system

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[a] We downgraded our assessment of the quality of evidence for live birth twice for risk of bias and once for indirectness. All included studies are at high risk of performance bias owing to lack of blinding or incomplete blinding. There was also high risk of selection bias. In one study, the randomisation of participants was undertaken by the principal investigator and allocation concealment was not described. In another study, some patients could request the intervention and this request was granted. The indirectness was due to one included study undertaking multiple embryo transfers per woman, and included women receiving donor oocytes from younger women.

[b] We downgraded our assessment of the quality of evidence for miscarriage twice for risk of bias, as outlined above, and once for indirectness secondary to one included study including miscarriages of biochemical as well as clinical pregnancies. These miscarriage data could not be separated by the authors of the study.

[c] We downgraded our assessment of the quality of evidence for clinical pregnancy twice for risk of bias and once for indirectness secondary to the day of embryo transfer being variable between studies. One study had blastocyst transfers, one had varied days of transfer and one had day-three transfer for the intervention arm and day-five transfer for the control arm.

DISCUSSION

Summary of main results

Trial design 1

The comparison 'Time-lapse system (TLS) with conventional morphological assessment of still TLS images versus conventional incubation and assessment', aims to assess the potential advantages of a stable incubator environment. The embryo selection software is not utilised and the embryos are left undisturbed until transfer. The three relevant studies included participants with a variety of infertility diagnoses. One described its participants as 'poor prognosis', with no further details (Wu 2016). Another described women with 'tubo-peritoneal factor' (Kahraman 2013), and the third described over 99% male-factor infertility, with 20% female-factor in both arms (Park 2015). This variety adds to the broad applicability of results to common clinical practice. Two studies undertook embryo transfer at day two or three (Park 2015; Wu 2016), whereas the third study undertook blastocyst transfer (Kahraman 2013). All oocytes were autologous.

There is moderate-quality evidence that there is probably no difference between the interventions in live birth rate or clinical pregnancy rates. There is also low-quality evidence of no difference between the interventions in the rates of miscarriage or stillbirth per couple randomly assigned.

Trial design 2

The comparison 'TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images' aims to assess the potential advantages of the embryo selection software over conventional morphology. In this comparison, both arms of the study are housed in a TLS, but the embryo selection software is only utilised in one arm. Therefore the incubator environment is identical in both arms. Two studies were eligible for this comparison. One had two intervention arms; embryo transfer on day three, and embryo transfer on day five (Kaser 2017). The control arm had embryo transfer on day five only. The other study, Goodman 2016, undertook a combination of embryo transfer on day three or five. It is worth noting that the embryos were left undisturbed in Goodman 2016, however in Kaser 2017, the embryos in both the intervention arms and the control arms underwent daily conventional morphological assessment in addition to the application of embryo selection software in the intervention arms. There was a broad variety of infertility diagnoses in both studies, which adds to the overall applicability of results to broad clinical practice in reality.

We are uncertain whether there is a difference between the interventions in terms of miscarriage rates as the evidence is very low-quality. There is low-quality evidence suggesting that there may be no difference in clinical pregnancy rates. No evidence for live birth or stillbirth was available.

Trial design 3

The comparison 'TLS utilising embryo selection software versus conventional incubation and assessment' aims to assess the potential advantages of a combination of the stable incubator environment*and* the embryo selection software versus conventional incubation and assessment. Three studies undertook this comparison. One of these utilised a combination of autologous and donor oocytes, the proportion of each are unknown (Rubio 2014). The remaining two studies used autologous oocytes. One study undertook embryo transfer on day three in the intervention group and day five in the control group (Yang 2017). Another study undertook transfer on day five (Kovacs 2013). In Rubio 2014, there was a combination of transfer on day three and day five. A variety of infertility diagnoses were recorded in the women in these studies. Two studies described their participants as 'good prognosis' (Rubio 2014; Yang 2017).

The meta-analysis revealed very low-quality evidence of an improvement in live birth rate when TLS utilising embryo selection software was used versus conventional incubation and assessment, however it is important to reflect on reasons to be cautious when accepting this result. Firstly, one study, Yang 2017, is an incomplete study and has not yet contributed live birth rates to the analysis. It is highly likely that the live birth result will change when these data are added, given that they observed lower clinical pregnancy rates and higher miscarriage rates in the intervention arm. Secondly, it is worth reflecting on the inconsistency of results across the three trial designs. The pooled estimates from trial design 1 report lower success with the incubator aspect of TLS; likewise trial design 2 report lower success with the cell tracking software element of TLS; however trial design 3 reports higher success when using both. From a scientific point of view, it is difficult to reason why there should be this discrepancy in findings, especially between trial design 2 and 3.

We are uncertain whether there is a difference between TLS utilising embryo selection software versus conventional incubation and assessment in miscarriage rates or clinical pregnancy rates, as the evidence is very low-quality. Stillbirth was not examined by these studies.

Overall completeness and applicability of evidence

This updated systematic review on time-lapse systems now includes eight RCTs, and includes additional data from two studies included in the first iteration of the review (Kovacs 2013; Rubio 2014). Data from 2303 women has gone towards formulating the findings of this review, however there are some comparisons which are better informed than others.

For example, approximately 59% of participants were included in trials that assessed TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3). This is mainly due to the largest trial undertaking this comparison (Rubio

2014). Trial designs 1 and 2 (TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment, and TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images) include the remaining 21% and 20% of participants respectively, but there are no women available to inform live birth findings in trial design 2, meaning there are profound gaps in evidence for TLS in this comparison. In addition, there were no stillbirth data for trial designs 2 and 3. This may be because stillbirth is so rare that it is not considered to be an important outcome, but it is important that future trials report this outcome, as it is a measure of safety.

Trial designs 1 and 2 included 489 and 463 women respectively. This is in comparison to the 1351 women who were included in trial design 3. Despite the additional information from previous and newly incorporated trials, the results of this review remain unclear. Further trials of each design are required to bolster participant numbers and to interrogate the robustness of the finding of an improvement of live birth with TLS versus control in trial design 3. The largest trial that informs trial design 3 has a number of biases, arising from the non-randomised approach for some participants, the subsequent lack of blinding, the use of donor oocytes in a number of women, and the routine use of multiple embryo transfer.

There was heterogeneity between trials in the diagnosis of infertility, the day of embryo transfer, the use of IVF or ICSI and the make and model of TLS. All of these factors help to make the results of this review more applicable to clinical practice in the real world, where there is naturally this variation in clinical practices.

All studies excluded women who underwent frozen embryo transfer, except Kahraman 2013, whose investigators were able to provide data for these women. For Rubio 2014, the investigators were unable to provide data specifically for women who underwent donor oocyte IVF/ICSI. Therefore, in order to subgroup autologous, donor and frozen oocytes, future studies will need to present their results under these subgroups and state explicitly how many couples underwent these interventions.

Elective single embryo transfer was undertaken in most studies (Kahraman 2013; Kaser 2017; Kovacs 2013; Park 2015; Yang 2017), however two studies undertook multiple embryo transfers (Goodman 2016; Rubio 2014;). We were unable to obtain figures from the authors of Rubio 2014, on exactly what proportion of couples received multiple embryo transfer in each arm of the study. Given that this study contributed a large proportion of the data in trial design 3, it is important to recognise that the results presented here may reflect rates of clinical outcomes in keeping with multiple embryo transfer as opposed to single embryo transfer. One study did not disclose the number of embryos transferred per woman (Wu 2016).

Quality of the evidence

The quality of the evidence ranged from very low to moderate. The main limitations were risk of bias, imprecision and indirectness. Risk of bias was commonly associated with lack of blinding of participants or those involved in the study, attrition rates following randomisation, reporting of interim results and variation in number and day of embryos transferred between arms of the study.

The quality of the evidence for trial design 1 (TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment) is low to moderate. The evidence for live birth was downgraded in GRADE for imprecision owing to there only being 2 trials, totaling 440 women. The evidence for miscarriage and stillbirth was downgraded twice each for imprecision secondary to broad confidence intervals and a small number of events. The evidence for clinical pregnancy was downgraded once for risk of bias owing to unclear selection and performance bias (Summary of findings for the main comparison).

The quality of evidence for trial design 2 (TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images) is very low to low. The quality of evidence for miscarriage was very low, and was downgraded once for risk of bias, once for indirectness and once for imprecision. This was owing to varying days and numbers of embryos transferred, decided upon by an unblinded embryologist, and secondary to heterogeneity between the study designs. One included study involved removing embryos for bench-top microscopy daily in both the intervention and control arms, whereas the other left embryos in the intervention and control arms undisturbed. Also, there were broad confidence intervals of the two included studies which indicates imprecision. The quality of evidence for clinical pregnancy was low and was downgraded in GRADE once for the heterogeneity in study designs mentioned above (Summary of findings 2).

The quality of the evidence for trial design 3 (TLS utilising embryo selection software versus conventional incubation and assessment) is of very low quality. Live birth was downgraded in GRADE twice for risk of bias and once for indirectness. All included studies are at high risk of performance bias owing to lack of blinding or incomplete blinding. There was also high risk of selection bias. In one study, the randomisation of participants was undertaken by the principal investigator and allocation concealment was not described. In another study, some patients could request the intervention and this request was granted. The indirectness was due to one included study undertaking multiple embryo transfers per woman, and included women receiving donor oocytes from younger women. Likewise, miscarriage was downgraded in GRADE twice for risk of bias as mentioned above and once for indirectness secondary to one included study including miscarriages of biochemical as well as clinical pregnancies. These miscarriage data could not be separated by the authors of the study. Finally, clinical pregnancy was downgraded twice for risk of bias and once for indirectness secondary to the day of embryo transfer

being variable between studies. One study had blastocyst transfers, one had varied days of transfer and one had day-three transfer for the intervention arm and day-five transfer for the control arm. (Summary of findings 3).

It should be noted that despite all studies being at high risk of performance bias owing to the lack of blinding of embryologists, we have not downgraded any studies for this unless other aspects of performance bias were lacking, for example, if participants were unblinded, or if the day or number of embryos transferred was decided by the unblinded embryologist.

Potential biases in the review process

We aimed to identify all eligible studies for inclusion in this review, and we contacted the included study authors on many occasions to seek as much information for inclusion as possible. Authors of most studies have been forthcoming with further study information, which has helped us to accrue a full picture of the study outcomes, as well as providing information needed to assess and establish the risk of bias.

Agreements and disagreements with other studies or reviews

To date, there are four published systematic reviews which have included the same inclusion and exclusion criteria on the topic of TLS versus conventional incubation (Chen 2017; Polanski 2014; Kaser 2014; Pribenszky 2017). Two of these are now out of date and new studies have been published since then (Polanski 2014; Kaser 2014). Both reviews report no evidence of a difference between TLS and control.

One systematic review, Kaser 2014, included 13 eligible studies after systematic searching, however none of the studies were RCTs and the majority were retrospective cohort studies. This review concludes that there is currently limited evidence to support the routine clinical use of TLS for selection of human pre-implantation embryos.

Six eligible studies were included in Chen 2017, but it missed out two further eligible RCTs that are included in this review. It does not include all the potential live birth data, including data from Kahraman 2013; Kovacs 2013; Park 2015. It concludes that there is currently "insufficient evidence to support that time-lapse imaging is superior to conventional methods for embryo incubation and selection".

In Pribenszky 2017, the authors undertook a systematic review of TLS utilising TLS embryo selection software. They concluded that TLS using embryo selection software was associated with a significantly higher ongoing pregnancy rate, a significantly lower early pregnancy loss and a significantly higher live birth rate in comparison to control. However, we have detected a number of problems with this review which have been published as a letter (Armstrong 2018). The issues outlined are as follows.

- They have combined trials with different intervention and control arms. For example, three of the five included trials are study design 3, but one is study design 1 and one is study design 2.
- They have also included a trial that describes itself as a prospective cohort study, not an RCT. On closer investigation, this trial is pseudo-randomised (randomisation based on patient record number). This is not considered methodologically sound for systematic reviews of RCTs.
- The authors describe applying an intention-to-treat analysis (which is considered the gold standard in fertility research), however the early pregnancy loss, live birth and stillbirth data are analysed per woman that became pregnant. This is known to skew the results toward showing a larger intervention effect.
- It appears that full data from the included trials have not been entered into the review. For example, live birth data is not included from Rubio 2014, despite being published as an abstract in 2015.
- We note that all three authors declared in this review that they work for Vitrolife, a biotechnology company that manufactures and promotes TLS.

AUTHORS' CONCLUSIONS

Implications for practice

Overall, there is insufficient evidence of differences in live birth, miscarriage, stillbirth or clinical pregnancy to choose between time-lapse system (TLS), with or without embryo selection software, and control. In one comparison (TLS utilising embryo selection software versus conventional incubation and assessment) there was very low-quality evidence of an improvement in live birth with TLS, however, there was no associated improvement in clinical pregnancy or reduction in miscarriage, therefore the result should be interpreted with extreme caution. Additionally, the two trials which inform this outcome are at high risk of bias.

Patients need to be aware, especially in view of the cost of TLS, that there is no good evidence that TLS with or without embryo selection software is more effective than conventional methods of embryo incubation and assessment. Women may wish to take part in randomised controlled trials (RCTs) on TLS in order to be able to add to the existing evidence base, and help guide assisted reproductive technology patients of the future.

Implications for research

RCTs which randomise couples or women, not embryos or oocytes, to either TLS or conventional incubation should be designed and conducted to add to the currently limited RCT evi-

dence. These studies should be large enough to answer the clinical questions that are important in fertility research, such as live birth, clinical and ongoing pregnancy, and adverse events. Cumulative clinical pregnancy rates should be reported in future studies in order to determine the impact of a TLS on embryo selection.

Suggested designs of RCTs which seek to differentiate the unique advantages of TLS are as follows.

- Trial design 1) TLS utilising routine morphological assessment of TLS images versus conventional incubation and assessment

- Trial design 2a) TLS utilising embryo selection software versus TLS utilising routine morphological assessment of TLS images

- Trial design 2b) TLS utilising one type of embryo selection software versus TLS utilising a different type of embryo selection software

- Trial design 3) TLS utilising embryo selection software versus conventional incubation and assessment

These study designs will help to differentiate between: the potential advantages of the stable culture environment TLS provides (trial design 1); the potential advantage of embryo selection software (trial design 2); and the potential advantage of TLS in its entirety utilising embryo selection software versus conventional incubation and assessment (trial design 3).

In addition, it would be useful for future trials to include a cost analysis element, which may help patients to balance the costs and benefits of using this technology. It may also be helpful to explore patient satisfaction and quality of life with TLS versus control. Some clinics are sharing TLS images with patients during the incubation period. It would be useful to explore whether this helps or worsens treatment anxiety.

ACKNOWLEDGEMENTS

Our heartfelt thanks go to:

- Marian Showell for her assistance with search strategies and re-running searches;

- Helen Nagels for her editorial advice and assistance;

- the editorial review team who dedicated their valued time and effort to this review;

- the women/couples who joined the RCTs included here, whose altruism has helped to guide evidence;

- Ms Nicola Arroll and Dr Lynsey Cree who contributed to the first iteration of this review.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Goodman 2016

Methods	Study: completed single-centre RCT of couples with infertility undergoing IVF Country: USA Cause and length of infertility: infertility diagnosis ranged from unexplained, ovulatory dysfunction, male factor, tubal factor, low ovarian reserve, AMA, endometriosis, mixed factors and other. Mean length of infertility in both groups was approximately 31.5 months Oocytes: autologous oocytes Embryo transfer: between 1 and 3 fresh embryos on day 3 or day 5. The number of embryos transferred was based on published ASRM committee guidance and patient preferences Informed consent: yes Total study duration: March 2014 to May 2015 (14 months) Funding sources: quote: "no external funding for the study"
Participants	A total of 300 couples with infertility undergoing IVF with autologous oocytes were recruited: 150 randomised to TLS selection (cell-tracking algorithm of TLS utilised) and 150 randomised to conventional selection (TLS with conventional once-daily morphologic embryo screening) 5 couples did not receive the allocated intervention, 2 from the time-lapse selection arm due to lack of fertilisation, and 3 from the conventional selection group, 2 due to no fertilisation and 1 due to no sperm Age (years, mean ± SD, time-lapse selection versus conventional selection): 33.6 ± 4.0 versus 33.2 years ± 3.9 years BMI (kg/m^2, mean ± SD, time-lapse selection versus conventional selection): 26.3 ± 6.7 versus 26.9 ± 7.4 Ethnicity: combination of white, black, Asian, Middle Eastern and other Inclusion criteria: • aged 18-43 years; • undergoing autologous IVF cycle between March 2014 and May 2015; • plan for fresh embryo transfer. Exclusion criteria: • did not undergo fresh transfer owing to previously unforeseen reasons; • women with only 1-3 zygotes.
Interventions	TLS utilising cell-tracking algorithm (intervention) TLS with conventional assessment of morphological parameters from still TLS images (control)
Outcomes	Clinical pregnancy rate per couple randomised Adverse events: miscarriage per couple randomised
Notes	Data on clinical pregnancy from women excluded following randomisation, and miscarriage data were obtained following communication with the authors Live birth and stillbirth data were requested, but not available

Goodman 2016 (*Continued*)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Quote: "Patients were randomised 1:1 to conventional embryo selection versus Embryoscope time-lapse morphokinetic selection with the use of a computer-generated random number sequence"
Allocation concealment (selection bias)	Low risk	Quote: "The list was housed in the laboratory, where it was accessible only by research personnel not involved with the recruitment of patients"
Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "Patients, physicians and staff, and sonographers were blinded to how embryos were selected". However the embryologist who was responsible for deciding on day of embryo transfer (day 3 or day 5) was unblinded, therefore deemed high risk
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "sonographers were blinded".
Incomplete outcome data (attrition bias) All outcomes	Low risk	We have obtained from the authors all relevant data from women who were excluded post randomisation
Selective reporting (reporting bias)	Low risk	We confirmed with authors that all outcomes the study set out to assess were published
Other bias	Low risk	No other sources of bias identified

Kahraman 2013

Methods	Study: completed single-centre RCT of couples with infertility undergoing ICSI Country: Turkey Cause and length of infertility: tubo-peritoneal factor. Length of infertility not reported Oocytes: autologous oocytes Embryo transfer: single embryo transfer at blastocyst Informed consent: yes Total study duration: December 2011 to June 2012 (6 months) Funding sources: none
Participants	A total of 76 couples with infertility undergoing ICSI with autologous oocytes were recruited: 38 randomised to TLS and 38 were randomised to conventional incubation In all, 12 couples withdrew from the study: 7 in the conventional incubation arm and 5 from the TLS arm Reasons for withdrawal were documented and outcomes, such as live birth, adverse events and clinical pregnancy for these couples were included in the data in this review Age (years, mean ± SD, TLS versus conventional incubation): 28.5 ± 3.32 versus 28.5 years ± 3.72 years; P = 0.83 BMI (kg/m^2, mean ± SD, TLS versus conventional incubation): 23.92 ± 3.79 versus 23.92 ± 4.42; P = 0.77 Ethnicity: not reported Inclusion criteria: • first or second treatment cycle; • age < 35 years, BMI < 28 kg/m^2; • ≥ 8 oocytes retrieved. Exclusion criteria: • recurrent spontaneous abortions; • severe endometriosis; • PCOS; • hydrosalpinx; • uterine pathology; • severe male factor (< 5 million motile sperm in total ejaculate); • very severe morphological sperm defects (dominantly globozoospermic or macrocephalic samples).
Interventions	TLS with conventional morphological assessment of still TLS images (intervention) Conventional incubation and assessment (control)
Outcomes	Live birth rates per couple randomised Clinical pregnancy rate per couple randomised Adverse events: stillbirth and miscarriage per couple randomised
Notes	Live birth information was available following communication with the author and was not published

Risk of bias

Bias	Authors' judgement	Support for judgement

Random sequence generation (selection bias)	Low risk	Quote: "Computer based randomisation list" Quote: "Randomisation was done according to a list generated on random.org"
Allocation concealment (selection bias)	Low risk	Communication with author. Quote: "Randomization list was held by one of the investigators who was not involved clinically with the patients. Also, he was not routinely working in the embryology laboratory. The randomization from random.org was printed out into sequentially numbered lists where the groups were masked and not revealed until the recruitment of each patient"
Blinding of participants and personnel (performance bias) All outcomes	High risk	Communication with author. Quote: "Clinicians were blinded in the study up to the point after the embryo transfer was performed. Also the patients did not know to which group they were allocated. Only the discontinued patients received information about the incubation process once the drop-out decision was made (Due to the need to inform the patients about their early/cancelled transfers)". The embryologist was impossible to blind, therefore performance bias deemed high risk
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Communication with author. Quote: "Clinicians, those assessing the outcome were not necessarily blinded to the intervention as some of our ART patients prefer to have those controls outside our clinic and report the outcomes to us" The outcomes are objective and are therefore unlikely to be influenced by knowledge of the intervention, therefore we graded this as low risk
Incomplete outcome data (attrition bias) All outcomes	Low risk	A total of 12 couples following randomisation discontinued the trial secondary to adverse events which were not reported as adverse events or analysed within the main publication. However, on communication with the author, the numbers of discontinued participants in each arm was disclosed, alongside reasons for dropouts. Quote: "embryos transferred day 3, 4 and

Kahraman 2013 (*Continued*)

		5 with single blastocyst developed; total freezing because of ovarian hyperstimulation syndrome (OHSS) risk"
Selective reporting (reporting bias)	Low risk	Communication with author. Quote: "As reported in our article, we have published all of the outcomes we aimed to assess. Unfortunately, we do not formally prepare a study protocol" On contacting the author, information on live birth and adverse events was offered, although these weren't published
Other bias	Low risk	None detected

Kaser 2017

Methods	Study: completed RCT of couples with infertility undergoing a fresh SET Country: USA Cause and length of infertility: a combination of anovulation, diminished ovarian reserve, endometriosis, male factor, tubal, unknown, uterine and other Oocytes: autologous oocytes Embryo transfer: single embryo transfer Informed consent: yes Total study duration: August 2014 to February 2016 (18 months) Funding sources: Progyny, Inc.
Participants	A total of 163 couples with infertility undergoing ART with autologous oocytes were recruited: • 56 were randomised to TLS and day 3 embryo transfer (ET). • 54 were randomised to TLS and day 5 ET. • 53 were randomised to incubation within the TLS and conventional morphology with day 5 ET (control). In all 13 couples did not receive the allocated intervention: • 7 in the TLS and day 3 ET arm (1 due to freeze-all for OHSS risk, 2 embryos transferred in one woman, in one woman the TLS algorithm wasn't followed and 4 women elected to have a day 5 ET). • 2 from the TLS and day 5 ET arm (2 women had freeze-all for OHSS risk). • 4 from the control arm (3 women had freeze-all for OHSS risk, and one woman had two embryos transferred). Age (years, mean ± SD): Day 3 + TLS 34.6 ± 3.1, Day 5 + TLS 33.7 ± 3.4, Day 5 control 34.1 ± 3.1 BMI (kg/m^2, mean ± SD): Day 3 + TLS 26 ± 6.9, Day 5 + TLS 25.5± 6.1, Day 5 control 25.5± 6.5 Ethnicity: a combination of white, Asian, black, Hispanic and 'other' ethnicities Inclusion criteria: • patients with a planned fresh SET; • aged 18 to 40 years;

	• can only be randomised if fertilisation occurs. Exclusion criteria: • use of donor oocytes; • more than 3 prior retrievals without an intervening clinical pregnancy; • *in-vitro* maturation; • gestational carrier; • preimplantation genetic diagnosis or screening; • presence of an uninterrupted hydrosalpinx; • history of intrauterine adhesions; • all embryos frozen due to ovarian hyperstimulation risk prior to randomisation; • less than 4 zygotes and therefore a risk of no blastocyst development.
Interventions	TLS utilising conventional benchtop morphology*and* embryo selection software (two intervention arms; day 3 and day 5 embryo transfer) TLS with conventional benchtop morphology (control). Embryo selection software or time-lapse photography was not utilised
Outcomes	Clinical pregnancy rate per couple randomised Miscarriage rate per couple randomised (data obtained from authors)
Notes	Wrote to authors August 2017 for further information Note differing days of embryo transfer Control group split between two intervention groups for purposes of this review

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Quote: "Subjects were blocked according to age (<35, 35-37, 38-40 years) and randomized 1:1:1 at the fertilization check by an embryologist using computer-generated, random number sequence cards enclosed in opaque, serially numbered envelopes"
Allocation concealment (selection bias)	Low risk	Quote: "random number sequence cards enclosed in opaque, serially numbered envelopes"
Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "Embryologists were blinded to the Eeva (time lapse) ratings at the conventional morphology evaluation (i.e. one embryologist performed conventional morphology and a different embryologist reviewed the Eeva ratings, and patients and physicians were blinded to the Eeva ratings until a negative pregnancy test of the

		primary endpoint was reached". Ultimately the embryologist was unblinded to the allocation therefore high risk of performance bias
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Patients and physicians were blinded to the Eeva ratings. Correspondence with author. Quote: "As patients were randomized to day 3 or day 5 transfer, blinding was not possible between groups 1 vs. group 2/3 (as the patient and physician knew which day the transfer was happening). For patients randomized to groups 2 or 3, both patients and physicians were blinded to study arm (so they knew a day 5 transfer was happening, but not how the embryo was selected for transfer)". The outcomes are objective and are therefore unlikely to be influenced by knowledge of the intervention, therefore we graded this as low risk
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data presented as intention to treat and 'as treated'.
Selective reporting (reporting bias)	Low risk	Communication with authors. Quote: "All outcomes published".
Other bias	Low risk	

Kovacs 2013

Methods	Study: completed multi-centre RCT of couples with infertility undergoing IVF Country: Hungary Cause and length of infertility: various causes (male, tubal, unexplained etc.) of at least one year's duration Oocytes: autologous Embryo transfer: single embryo transfer at blastocyst Informed consent: yes Total study duration: July 2012 to April 2015 (33 months) Funding sources: none
Participants	161 couples with infertility undergoing IVF with single embryo transfer at blastocyst 80 couples were randomised to TLS and 81 were randomised to conventional incubation 22 couples dropped out of the study after randomisation: 12 from the TLS arm (2 dual embryo transfer requested; 1 no fertilisation; 7 less than 3 good embryos on day 3; 2 elective cryopreservation for OHSS risk). 10 dropped out from the control arm (1 no fertilisation; 8 less than 3 good embryos on day 3; 1 elective cryopreservation for OHSS risk)

Kovacs 2013 (*Continued*)

	Age: (years, mean ± SD, TLS versus conventional incubation): 31.2 ± 2.7 versus 32.1 years ± 2.5 years BMI: (kg/m^2, mean ± SD, TLS versus conventional incubation): 22.3 ± 3.3 versus 22.2 ± 3.0 Ethnicity: Caucasian Inclusion criteria: • age < 36 yrs; • baseline FSH < 10 IU/l; • regular 25 to 35 day cycles; • less than 2 previous failed IVF cycles (first or second cycle); • intact uterus; • an indication for IVF; • BMI < 18 to < 30 kg/$m^{2;}$ • acceptance of single embryo transfer; • normal ovarian reserve; • at least 3 good embryos on day 3. Exclusion criteria: • PCOS; • sperm obtained by surgical extraction; • chromosome abnormality; • presence of hydrosalpinx; • stage III/IV endometriosis; • less than 3 good quality day 3 embryos; • lack of consent.
Interventions	TLS utilising cell-tracking algorithm (intervention) Conventional incubation and assessment (control)
Outcomes	Clinical pregnancy rate per couple Adverse events (miscarriage) Live birth
Notes	Unpublished data obtained on correspondence with authors

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	On communication with author, paired randomisation sequence was explained: Quote: "Two envelopes containing time-lapse or control group assignment were prepared. The first patient was randomly assigned to one of the groups and the next patient received the other assignment. This was repeated with patient number 3 and 4 and so on"

Kovacs 2013 (*Continued*)

Allocation concealment (selection bias)	High risk	On communication with author: Quote: "The randomization is carried out by the principal investigator who is involved in the study"
Blinding of participants and personnel (performance bias) All outcomes	High risk	Communication with author. Quote: "There was no blinding".
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Communication with author. Quote: "There was no blinding". The outcomes are objective and are therefore unlikely to be influenced by knowledge of the intervention, therefore we graded this as low risk
Incomplete outcome data (attrition bias) All outcomes	High risk	Dropouts following randomisation and not included in intention to treat: 161 patients were randomised (80 time-lapse versus 81 standard monitoring), 22 dropped out. Reasons for dropouts were provided, however reasons provided were not all pre-determined exclusion criteria and with such a high attrition rate, this study is at high risk of attrition bias
Selective reporting (reporting bias)	Low risk	None detected
Other bias	Unclear risk	Data obtained from author directly. Interim reporting and analysis of results available in various published sources with differing results

Park 2015

Methods	Study: single centre RCT, couples undergoing ICSI Country: Sweden Cause and length of infertility: male factor infertility was present in > 99% of participants in both arms of the study. Female factor infertility was present in approximately 20% of participants in both arms. Duration of infertility was approximately 2.8 years in both arms of the study Oocytes: autologous Embryo transfer: single embryo transfer at day 2 Informed consent: yes Total study duration: May 2010 to Feb 2014 (3 years, 9 months) Funding sources: Sahlgrenska Academy, Sahlgrenska University Hospital, LUA/ALF 70940, Ferring Research Infertility and Gynecology Grant, Hjalmar Svensson Grant, Unisense Fertilitech: Unisense provided the EmbryoScope^{TM} free of charge during the study.
Participants	364 couples with infertility undergoing their first IVF cycle with ICSI. One embryo (in a few cases two embryos, N = 12) of good quality or in some cycles of less good quality (N = 27) was transferred on day 2 and super-numerary good quality embryos were frozen 241 couples were randomised to TLS and 124 were randomised to conventional incubation 1 couple was excluded from the TLS arm as they had been randomised twice Age: (years, mean ± SD, TLS versus conventional incubation): 31.8 ± 4.3 versus 31.8 ± 4.1 P = 0.90 BMI: (kg/m^2, mean ± SD, TLS versus conventional incubation): 24.4 ± 3.9 versus 24.3 ± 4.0; P = 0.70 Ethnicity: not reported Inclusion criteria: • ≤40 years of age; • undergoing their first IVF cycle using ICSI; • at least one oocyte was retrieved. Exclusion criteria: • patients undergoing egg donation.
Interventions	TLS with conventional morphological assessment of still TLS images (intervention) Conventional incubation and assessment(control)
Outcomes	Clinical pregnancy rate per couple randomised Adverse events (miscarriage) per couple randomised
Notes	Live birth and stillbirth data obtained on communication with authors

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was undertaken using: quote: "a web-based randomization programme and all the patients' oocytes were allocated to culture in either a conventional

Park 2015 (*Continued*)

		incubator or in a closed system, in proportion 1:2"
Allocation concealment (selection bias)	Low risk	Quote: "Randomization was carried out by the embryologist after oocyte retrieval". On communication with the authors, it was clarified that the embryologist undertaking the randomisation may have also undertaken the embryo assessment
Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "The patients as well as the treating physician and the person performing the statistical analyses were blinded to which type of procedure was used until the outcome of transfer (pregnant versus not pregnant) was known". Embryologists were not possible to blind, therefore deemed high risk of performance bias
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "The patients as well as the treating physician and the person performing the statistical analyses were blinded to which type of procedure was used until the outcome of transfer (pregnant versus not pregnant) was known. Embryologists were not possible to blind"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Only one woman excluded from analysis in the intervention arm as she was randomised twice. No women excluded from control arm. No drop-outs
Selective reporting (reporting bias)	Low risk	All pre-determined outcomes were reported.
Other bias	Low risk	None detected

Rubio 2014

Methods	Study: completed multi-centre RCT of couples with infertility undergoing ICSI Country: Spain Cause and length of infertility: not reported Oocytes: autologous and donor Embryo transfer: multiple embryo transfer (1.86 per couple, 95% CI 1.8 to 1.9) on day 3 and day 5 Informed consent: not reported Total study duration: February 2012 to July 2013 (17 months) Funding sources: the instrumentation, disposables and utensils used in this study were fully paid for by IVI. IVI is a minor shareholder in UnisenseFertiliTech A/S, but none of the authors have any economic affiliation with UnisenseFertiliTech A/S
Participants	A total of 856 couples with infertility undergoing IVF with autologous and donor oocytes: 444 couples were randomised to TLS and 412 to conventional incubation In all, 13 couples were excluded from the study: 6 in the TLS arm (reasons: 2 had cancelled oocyte donation and 4 had their embryos vitrified) and 7 in the conventional incubation arm (reasons: 1 woman had endometrial bleeding; 2 had cancelled oocyte donation; and 4 couples had their embryos vitrified) Age (years, mean ± SD, TLS versus conventional incubation): 34.7 ± 2.7 versus 34.6 years ± 2.7 years BMI (kg/m^2, mean ± SD, TLS versus conventional incubation): 23.2 ± 3.7 versus 23.04 ± 2.8 Ethnicity: not reported Inclusion criteria: autologous or oocyte donation. Those receiving oocyte donation had one of the following diagnoses: failure to achieve pregnancy after at least 3 cycles of ART, genetic female or chromosomal disorders, or low response to controlled ovarian hyperstimulation Donors were: • aged 18 to 34 years; • BMI 18 to 25 kg/m^2; • had received no endocrine treatment (including gonadotrophins and oral contraception) for the last three months preceding the study and had a normal uterus and ovaries at TV USS (no signs of PCOS). Inclusion criteria for both arms of study: • age 20 to 38 years; • first or second ICSI cycle; • BMI of < 18 and < 25 kg/m^2. Exclusion criteria: • severe male factor (total motile sperm < 1 million); • hydrosalpinx; • presenting uterine diseases after 2D ultrasound evaluation and/or 3D (if in doubt) or hysteroscopy (for acquired or congenital uterine abnormalities); • endocrinopathies (thrombophilia); • recurrent pregnancy losses; • endometriosis; • patients receiving concomitant medications as a treatment for any other condition that might interfere with the results of the study. For autologous oocyte patients: • low-responder patients (fewer than 6 metaphase II per cycle) or those with a FSH

	basal determination > 12 or an AMH concentration of < 1.7 pmol/L (based on our own experience) were also excluded.
Interventions	TLS utilising cell-tracking algorithms (intervention) Conventional incubation and assessment (control)
Outcomes	Adverse events Clinical pregnancy rate per couple Live birth (obtained from Insua 2017 and Insua 2015)
Notes	October 2015: following clarification from authors of comments on this review, it has been made aware to us that the pregnancy data from this study is a combination of biochemical and ongoing pregnancy. Therefore the miscarriage data may also include miscarriages from biochemical pregnancies

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	Despite adequate random sequence generation, patients were able to request the intervention in some cases and this was granted. See evidence below: Quote: "Patients were allocated to either TMS (study group) or SI (control group) using a computer generated randomisation table which was handled by the embryologist at the laboratory in charge the day before the oocyte retrieval or oocyte donation. The randomisation was not perfectly performed as the patient distribution to the two groups would have been expected to be 50:50 ratio than the reported 51.9:48.1. The main reason for this deviation was limited patient requests for TMS culture"
Allocation concealment (selection bias)	High risk	In some cases allocation was non-random (see above)
Blinding of participants and personnel (performance bias) All outcomes	High risk	Gynaecologist and statistician blinded Patients and embryologist not blinded Quote: "The study is considered double blind because 1) the gynaecologist (evaluating the primary effect) did not know to which group the patients had been assigned, and 2) the statistician evaluating the results only knew the incubators by a binary code, not by type" Communication with author. Quote: "The

		intention was to do triple blinded, but we discovered that some of our patients were informed (because they asked) of the group they were in. Therefore blinding failed in some of our patients. We then decided to describe it as double blind because patients blinding partially failed"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	The gynaecologist evaluating the primary effect was blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	A total of 13 patients excluded from study after randomisation as they suffered adverse events (cancelled oocyte donation, embryos vitrified and endometrial bleeding). Not included in intention to treat, but all excluded patients were accounted for, therefore low risk of attrition bias
Selective reporting (reporting bias)	Low risk	Reported all outcomes declared on www.clinicaltrials.gov On communication with the author: "We are currently collecting data on live birth and stillbirth"
Other bias	Low risk	None detected

Wu 2016

Methods	Study: completed single-centre RCT of couples with infertility undergoing IVF and ICSI Country: USA Cause and length of infertility: 'poor prognosis patients'. Length of infertility not reported Oocytes: autologous oocytes. Embryo transfer: day 3 transfer of embryo. Number not disclosed Informed consent: yes Total study duration: December 2014 to March 2015 (3.5 months) Funding sources: intramural funds from The Center for Human Reproduction and by grants from The Foundation for Reproductive Medicine. Vitrolife, Goteborg, Sweden, contributed a free Embryoscope for the length of the study. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript
Participants	A total of 49 couples with infertility undergoing IVF or ICSI with autologous oocytes: 24 couples were randomised to TLS and 25 to conventional incubation In all, 18 couples were excluded from the study: 8 in the TLS arm (reasons: 6 had no mature oocytes or no fertilisation after ICSI and 2 women had their embryos transferred on day 2) and 10 in the conventional incubation arm (reasons: 5 women had no mature oocytes or no fertilisation after ICSI and 5 women had their embryos transferred on day

Wu 2016 (*Continued*)

	2) Age (years, mean ± SD, TLS versus conventional incubation): 38.8 ± 1.0 versus 40.4 years ± 1.8 years BMI (kg/m^2, mean ± SD, TLS versus conventional incubation): not reported Ethnicity: not reported Inclusion criteria: • couples undergoing autologous IVF (and ICSI) cycles. Exclusion criteria: • not stated.
Interventions	TLS with conventional morphological assessment of still TLS images (intervention) Conventional incubation and assessment (control)
Outcomes	Clinical pregnancy rate per couple randomised
Notes	Contacted authors August 2017 for further information

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Quote: "Computer randomisation to either TLS or standard embryology was the responsibility of a member of the center's Statistics Section (SKD) who was completely dissociated from the patient's IVF cycle"
Allocation concealment (selection bias)	Unclear risk	Randomisation was undertaken by a member of the team not associated with the treatment cycle. Quote: "The designation was then reported to the embryology staff which processed the patient's oocytes/embryos"
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not described. However, given that embryologists would have been impossible to blind, deemed high risk of performance bias
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not possible to blind outcome assessors. The outcomes are objective and are therefore unlikely to be influenced by knowledge of the intervention, therefore we graded this as low risk
Incomplete outcome data (attrition bias) All outcomes	Low risk	Excluded patients were accounted for and were considered by trialists to be valid predecided grounds for exclusion

Wu 2016 (*Continued*)

Selective reporting (reporting bias)	Unclear risk	No access to protocol
Other bias	Low risk	None detected

Yang 2017

Methods	Study: interim results from a single-centre RCT of couples with infertility undergoing IVF and ICSI Country: China Cause and length of infertility: quote: "good prognosis patients". Length of infertility not reported Oocytes: autologous oocytes Embryo transfer: single embryo transfer; day 3 transfer of embryos in intervention group and day 5 transfer in control group Informed consent: Total study duration: October 2015 to October 2016 (12 months) Funding sources: study funded by Ferring
Participants	A total of 334 couples with infertility undergoing IVF or ICSI with autologous oocytes: 167 couples were randomised to TLS utilising cell-tracking algorithms and 167 couple were randomised to conventional incubation and morphology In all, 50 couples were excluded from the study: 23 in the TLS arm (reasons not given) and 27 in the conventional incubation arm (reasons not given) Age (years, mean ± SD, TLS versus conventional incubation): not reported BMI (kg/m^2, mean ± SD, TLS versus conventional incubation): not reported Ethnicity: not reported Inclusion criteria: • couples undergoing autologous IVF (and ICSI) cycles; • </= 36 years; • </= 2 failed IVF attempts using fresh cycles; • >/= 6 normally fertilised embryos (2PN). Exclusion criteria: not described
Interventions	TLS utilising cell-tracking algorithms (intervention) Conventional incubation and assessment (control)
Outcomes	Clinical pregnancy per couple randomised Miscarriage rate per couple randomised (clinical (gestational sac) pregnancy losses)
Notes	Note differing days of embryo transfer (day 3 for intervention group and day 5 for control) Emailed authors 24 August 2017

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**

Random sequence generation (selection bias)	Low risk	Quote: "Randomisation was done 1:1 using random numbers from sealed envelopes". Further communication with authors. Quote: "random numbers were generated by a random number table. Nurses recruited patients and the researchers opened sealed envelopes"
Allocation concealment (selection bias)	Low risk	Communication with authors. Quote: "The researchers, not directly involved in recruiting patients, created opaque numbered envelopes containing the randomization allocation for each participant which were opened in consecutive order after written informed consent was obtained"
Blinding of participants and personnel (performance bias) All outcomes	High risk	Communication with authors. Quote: "The study was not blinded because study participants and clinic staff were aware of which group they were following"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "clinic staff were not blinded". The outcomes are objective and are therefore unlikely to be influenced by knowledge of the intervention, therefore we graded this as low risk
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Attrition mentioned, but reasons not provided
Selective reporting (reporting bias)	High risk	Communication with authors. Quote: "The primary outcome of this study was ongoing pregnancy rate (OPR) as confirmed by the presence of gestational sacs with fetal heartbeat detected by transvaginal ultrasound examination in week 12. For secondary outcomes, we analyzed implantation rates (IR), twin pregnancy rate (monozygotic twins), and ectopic pregnancy (defined as the presence of gestational sacs detected by transvaginal ultrasound examination in week 4). Implantation rate was defined as the presence of gestational sacs detected by transvaginal ultrasound examination in week 4. The other secondary outcomes of this study was early abortion rate defined as when the (implantation-positive) pregnant cycles did not re-

		sult in ongoing pregnancy. We did not include obstetric and neonatal results in current study, but will do a follow-up study to look at these results." Other than pregnancy and miscarriage, no other outcomes were published as this is a conference abstract of interim analysis of full study
Other bias	High risk	Variation in day of transfer between arms of study (day 3 for intervention and day 5 for control)

Abbreviations:
AMA - Advanced Maternal Age
ASRM - American Society for Reproductive Medicine
ART - assisted reproductive technology
BMI - body mass index
ET - embryo transfer
FSH - follicle stimulating hormone
ICSI - intracytoplastic sperm injection
IVF - in vitro fertilisation
PCOS - polycystic ovarian syndrome
RCT - randomised controlled trial
SD - standard deviation
SET - single embryo transfer
TLS - time-lapse system
2D - two dimensional
3D - three dimensional

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Adamson 2016	Not an RCT
Arnesen 2014	Pseudo-randomised
Belles 2014	Randomised oocytes
Cruz 2011	Randomised oocytes
Freour 2014	Letter not containing study data
Huang 2014	Unable to determine the nature of the control arm

(*Continued*)

Ingerslev 2011	Randomised oocytes
Kaser 2014	Systematic review
Kirkegaard 2012	Randomised oocytes
Kirkegaard 2014	Letter not containing study data
Kirkegaard 2015	Systematic review
Loewke 2012	Not an RCT
Lowen 2017	Randomised embryos
Mara 2010	Randomised oocytes
Meseguer 2012	Not an RCT
Nakahara 2010	Randomised oocytes
Polanski 2014	Systematic review
Siristatidis 2015	Pseudo-randomised
Wu 2015	Randomised embryos
Yang 2014	Randomised oocytes

Characteristics of ongoing studies *[ordered by study ID]*

Khan (pers comm)

Trial name or title	TILT - Time-Lapse Imaging Trial
Methods	Multi-centre randomised controlled trial
Participants	Participants undergoing IVF/ICSI treatment and: • the woman is between 18-42 years of age; • receiving the first, second or third IVF/ICSI treatment cycle; • those participants having at least 3 2PN embryos (showing 2 pro-nuclei, which is a sign of normal fertilisation) on day of fertilisation check.
Interventions	Intervention 1: incubation and assessment of embryos using TLI systems (morphokinetic parameters + undisturbed culture + morphological assessment) Intervention 2: incubation of embryos in undisturbed culture and standard embryo assessment (undisturbed culture + morphological assessment)

Khan (pers comm) (*Continued*)

	Control: standard care (morphological assessment alone)
Outcomes	Primary outcome: live birth rate. Secondary outcomes: clinical pregnancy rate, elective single embryo transfer rate, multiple birth rate, miscarriage of clinical pregnancy rate, stillbirth rate and major congenital abnormality rate
Starting date	February 2018
Contact information	Professor Khalid Khan k.s.khan@qmul.ac.uk
Notes	CPMS ID 37510. Undergoing ISRCTN registration

NTR5423

Trial name or title	Embryo SELECtion using TIme-lapse MOnitoring in IVF and ICSI patients
Methods	Multi-centre randomised controlled trial
Participants	Women scheduled for a single embryo transfer (SET) during their first IVF or ICSI cycle at any of the participating IVF centers will be considered for inclusion
Interventions	A) embryo selection based on Eeva results and continuous culture in Geri+ incubator (Geri+Eeva complete) , and B) routine embryo selection based on morphology and continuous culture in Geri+ incubator (Geri culture only), will be compared to C) routine embryo selection based on morphology and interrupted culture in Geri+ incubator (control). Embryos in all three groups will be cultured in the Geri+ time-lapse incubator
Outcomes	Primary outcomes are the ongoing pregnancy rate of the first fresh SET and the cumulative ongoing pregnancy rate including the first fresh SET and all subsequent cryo transfers from the same ovum pick up cycle within one year Secondary outcomes are biochemical pregnancy rate and live birth rate after fresh SET, cumulative live birth rate, miscarriage rate, time to pregnancy, embryo morphology and number of usable embryos (ie embryos used for transfer or cryopreservation), morphokinetic parameters, pregnancy rates in three female age groups, cost-efficiency, outcome of manual time-lapse annotations
Starting date	1 March 2017
Contact information	D.C. Kieslinger. d.kieslinger@vumc.nl
Notes	

DATA AND ANALYSES

Comparison 1. TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Livebirth	2	440	Odds Ratio (M-H, Fixed, 95% CI)	0.73 [0.47, 1.13]
2 Miscarriage	2	440	Odds Ratio (M-H, Fixed, 95% CI)	2.25 [0.84, 6.02]
3 Stillbirth	2	440	Odds Ratio (M-H, Fixed, 95% CI)	1.0 [0.13, 7.49]
4 Clinical pregnancy	3	489	Odds Ratio (M-H, Fixed, 95% CI)	0.88 [0.58, 1.33]

Comparison 2. TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Miscarriage	2	463	Odds Ratio (M-H, Fixed, 95% CI)	1.39 [0.64, 3.01]
2 Clinical pregnancy	2	463	Odds Ratio (M-H, Fixed, 95% CI)	0.97 [0.67, 1.42]

Comparison 3. TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Livebirth	2	1220	Odds Ratio (M-H, Fixed, 95% CI)	1.21 [0.96, 1.54]
2 Miscarriage	3	1351	Odds Ratio (M-H, Fixed, 95% CI)	0.73 [0.49, 1.08]
3 Clinical pregnancy	3	1351	Odds Ratio (M-H, Fixed, 95% CI)	1.17 [0.94, 1.45]

Analysis 1.1. Comparison 1 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1), Outcome 1 Livebirth.

Review: Time-lapse systems for embryo incubation and assessment in assisted reproduction

Comparison: 1 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)

Outcome: 1 Livebirth

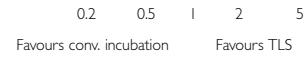
Study or subgroup	TLS n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Kahraman 2013	20/38	19/38		19.6 %	1.11 [0.45, 2.73]
Park 2015	48/240	35/124		80.4 %	0.64 [0.38, 1.05]
Total (95% CI)	**278**	**162**		**100.0 %**	**0.73 [0.47, 1.13]**

Total events: 68 (TLS), 54 (Control)

Heterogeneity: Chi^2 = 1.13, df = 1 (P = 0.29); I^2 =11%

Test for overall effect: Z = 1.41 (P = 0.16)

Test for subgroup differences: Not applicable



Analysis 1.2. Comparison 1 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1), Outcome 2 Miscarriage.

Review: Time-lapse systems for embryo incubation and assessment in assisted reproduction

Comparison: 1 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)

Outcome: 2 Miscarriage

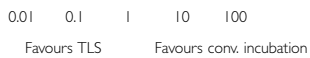
Study or subgroup	TLS n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Kahraman 2013	4/38	4/38		59.1 %	1.00 [0.23, 4.33]
Park 2015	15/240	2/124		40.9 %	4.07 [0.91, 18.08]
Total (95% CI)	**278**	**162**		**100.0 %**	**2.25 [0.84, 6.02]**

Total events: 19 (TLS), 6 (Control)

Heterogeneity: Chi^2 = 1.78, df = 1 (P = 0.18); I^2 =44%

Test for overall effect: Z = 1.62 (P = 0.11)

Test for subgroup differences: Not applicable



Analysis 1.3. Comparison 1 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1), Outcome 3 Stillbirth.

Review: Time-lapse systems for embryo incubation and assessment in assisted reproduction

Comparison: 1 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)

Outcome: 3 Stillbirth

Study or subgroup	TLS n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Kahraman 2013	2/38	2/38		100.0 %	1.00 [0.13, 7.49]
Park 2015	0/240	0/124			Not estimable
Total (95% CI)	**278**	**162**		**100.0 %**	**1.00 [0.13, 7.49]**

Total events: 2 (TLS), 2 (Control)
Heterogeneity: not applicable
Test for overall effect: Z = 0.0 (P = 1.0)
Test for subgroup differences: Not applicable

Analysis 1.4. Comparison 1 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1), Outcome 4 Clinical pregnancy.

Review: Time-lapse systems for embryo incubation and assessment in assisted reproduction

Comparison: 1 TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)

Outcome: 4 Clinical pregnancy

Study or subgroup	TLS n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Kahraman 2013	26/38	26/38		17.6 %	1.00 [0.38, 2.63]
Park 2015	63/240	37/124		76.9 %	0.84 [0.52, 1.35]
Wu 2016	3/24	3/25		5.5 %	1.05 [0.19, 5.78]
Total (95% CI)	**302**	**187**		**100.0 %**	**0.88 [0.58, 1.33]**
Total events: 92 (TLS), 66 (Control)					
Heterogeneity: Chi^2 = 0.15, df = 2 (P = 0.93); I^2 =0.0%					
Test for overall effect: Z = 0.62 (P = 0.54)					
Test for subgroup differences: Not applicable					

Analysis 2.1. Comparison 2 TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2), Outcome 1 Miscarriage.

Review: Time-lapse systems for embryo incubation and assessment in assisted reproduction

Comparison: 2 TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2)

Outcome: 1 Miscarriage

Study or subgroup	TLS n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Goodman 2016	12/150	10/150		83.1 %	1.22 [0.51, 2.91]
Kaser 2017 (1)	5/54	1/27		10.9 %	2.65 [0.29, 23.92]
Kaser 2017 (2)	1/56	0/26		6.0 %	1.43 [0.06, 36.35]
Total (95% CI)	**260**	**203**		**100.0 %**	**1.39 [0.64, 3.01]**

Total events: 18 (TLS), 11 (Control)

Heterogeneity: $Chi^2 = 0.42$, df = 2 (P = 0.81); $I^2 = 0.0\%$

Test for overall effect: Z = 0.83 (P = 0.41)

Test for subgroup differences: Not applicable

(1) Day 5 ET. Control group split between two intervention arms

(2) Day 3 ET. Control group split between two intervention arms

Analysis 2.2. Comparison 2 TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2), Outcome 2 Clinical pregnancy.

Review: Time-lapse systems for embryo incubation and assessment in assisted reproduction

Comparison: 2 TLS utilising embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2)

Outcome: 2 Clinical pregnancy

Study or subgroup	TLS n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Goodman 2016	88/150	83/150		62.0 %	1.15 [0.73, 1.81]
Kaser 2017 (1)	23/56	13/26		18.9 %	0.70 [0.27, 1.78]
Kaser 2017 (2)	21/54	13/27		19.1 %	0.69 [0.27, 1.74]
Total (95% CI)	**260**	**203**		**100.0 %**	**0.97 [0.67, 1.42]**

Total events: 132 (TLS), 109 (Control)

Heterogeneity: Chi^2 = 1.52, df = 2 (P = 0.47); I^2 =0.0%

Test for overall effect: Z = 0.14 (P = 0.89)

Test for subgroup differences: Not applicable

(1) Day 3 ET. Control group split between two intervention arms

(2) Day 5 ET. Control group split between two intervention arms

Analysis 3.1. Comparison 3 TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3), Outcome 1 Livebirth.

Review: Time-lapse systems for embryo incubation and assessment in assisted reproduction

Comparison: 3 TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3)

Outcome: 1 Livebirth

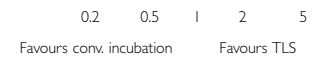
Study or subgroup	TLS n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Park 2015	48/240	35/124		30.0 %	0.64 [0.38, 1.05]
Rubio 2014	216/444	162/412		70.0 %	1.46 [1.11, 1.92]
Total (95% CI)	**684**	**536**		**100.0 %**	**1.21 [0.96, 1.54]**

Total events: 264 (TLS), 197 (Control)

Heterogeneity: Chi^2 = 8.16, df = 1 (P = 0.004); I^2 =88%

Test for overall effect: Z = 1.60 (P = 0.11)

Test for subgroup differences: Not applicable



Analysis 3.2. Comparison 3 TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3), Outcome 2 Miscarriage.

Review: Time-lapse systems for embryo incubation and assessment in assisted reproduction

Comparison: 3 TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3)

Outcome: 2 Miscarriage

Study or subgroup	TLS n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Kovacs 2013	3/80	2/81		3.3 %	1.54 [0.25, 9.47]
Rubio 2014	45/444	59/412		95.0 %	0.67 [0.45, 1.02]
Yang 2017 (1)	2/167	1/167		1.7 %	2.01 [0.18, 22.41]
Total (95% CI)	**691**	**660**		**100.0 %**	**0.73 [0.49, 1.08]**

Total events: 50 (TLS), 62 (Control)

Heterogeneity: Chi^2 = 1.46, df = 2 (P = 0.48); I^2 =0.0%

Test for overall effect: Z = 1.59 (P = 0.11)

Test for subgroup differences: Not applicable

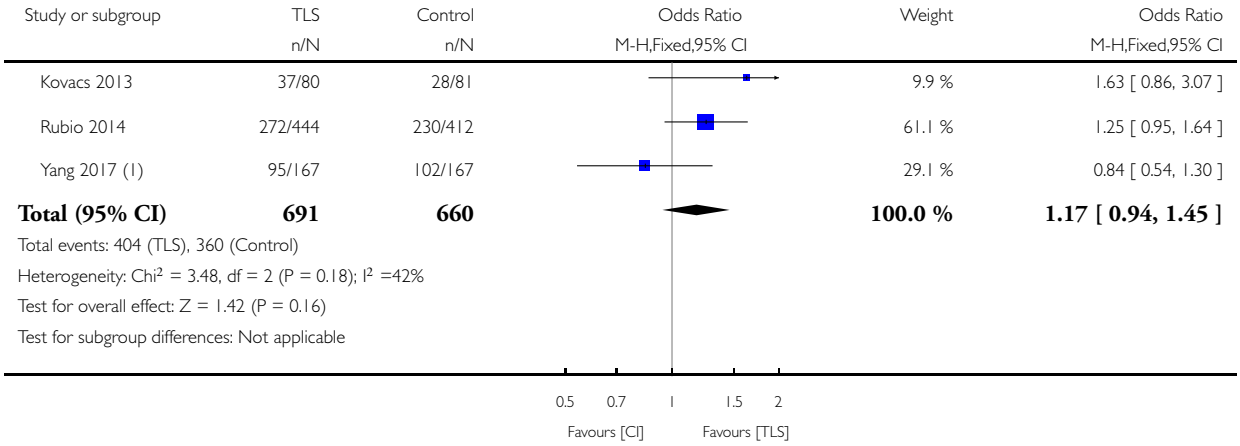
(1) Miscarriage rates calculated from clinical pregnancy and ongoing pregnancy rates reporting in paper

Analysis 3.3. Comparison 3 TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3), Outcome 3 Clinical pregnancy.

Review: Time-lapse systems for embryo incubation and assessment in assisted reproduction

Comparison: 3 TLS utilising embryo selection software versus conventional incubation and assessment (trial design 3)

Outcome: 3 Clinical pregnancy



(1) Day 3 embryo transfer (ET) in TLS arm and Day 5 ET in control arm

APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility specialised register search strategy

PROCITE platform
Searched 2 August 2017
Keywords CONTAINS “time lapse monitoring” or “time lapse” or “embryoscope” or Title CONTAINS “time lapse monitoring” or “time lapse” or “embryoscope” (38 hits)

Appendix 2. CENTRAL CRSO search strategy

Web platform
Searched 2 August 2017
#1 MESH DESCRIPTOR Fertilization in Vitro EXPLODE ALL TREES (1847)

#2 MESH DESCRIPTOR Sperm Injections, Intracytoplasmic EXPLODE ALL TREES (477)

#3 MESH DESCRIPTOR Embryo Transfer EXPLODE ALL TREES (959)

#4 (in vitro fertili?ation):TI,AB,KY (2077)

#5 (ivf or icsi):TI,AB,KY (3838)

#6 (intracytoplasmic sperm injection*):TI,AB,KY (1223)

#7 embryo*:TI,AB,KY (4487)

#8 blastocyst*:TI,AB,KY (672)

#9 MESH DESCRIPTOR Ectogenesis EXPLODE ALL TREES (9)

#10 MESH DESCRIPTOR Embryonic Development EXPLODE ALL TREES (517)

#11 MESH DESCRIPTOR Reproductive Techniques, Assisted EXPLODE ALL TREES (2823)

#12 (assisted reproduct*):TI,AB,KY (773)

#13 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 (7591)

#14 Eeva*:TI,AB,KY (13)

#15 (Primo Vision*):TI,AB,KY (8)

#16Embryoviewer*:TI,AB,KY (2)

#17 Embryoscope*:TI,AB,KY (28)

#18 timelapse*:TI,AB,KY (3)

#19 (time lapse*):TI,AB,KY (184)

#20 (sequential embryo*):TI,AB,KY (4)

#21 #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 (200)

#22 #13 AND #21 (103)

Appendix 3. MEDLINE search strategy

OVID platform
Searched from 1946 to 2 August 2017
1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (37573)

2 in vitro fertili?ation.tw. (20414)

3 ivf-et.tw. (2124)

4 icsi.tw. (7103)

5 intracytoplasmic sperm injection$.tw. (6181)

6 ivf.tw. (20613)

7 (embryo or embryos).tw. (167517)

8 blastocyst$.tw. (19707)

9 exp ectogenesis/ or exp embryonic development/ (52957)

10 exp Reproductive Techniques, Assisted/ (62314)

11 assisted reproduct$.tw. (12269)

12 or/1-11 (252182)

13 time lapse.tw. (9857)

14 timelapse.tw. (118)

15 Embryoscope$.tw. (47)

16 Embryoviewer.tw. (0)

17 Eeva$.tw. (54)

18 Primo Vision$.tw. (5)

19 (sequential embryo$ adj2 scor$).tw. (2)

20 (sequential embryo$ adj2 assess$).tw. (2)

21 or/13-20 (10021)

22 randomized controlled trial.pt. (470503)

23 controlled clinical trial.pt. (94472)

24 randomized.ab. (413060)

25 randomised.ab. (81022)

26 placebo.tw. (197078)

27 clinical trials as topic.sh. (187632)

28 randomly.ab. (286156)

29 trial.ti. (185498)

30 (crossover or cross-over or cross over).tw. (76371)

31 or/22-30 (1210915)

32 exp animals/ not humans.sh. (4445831)

33 31 not 32 (1116903)

34 12 and 21 and 33 (44)

Appendix 4. EMBASE search strategy

OVID platform
Searched from 1980 to 2 August 2017
1 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/ (57045)

2 in vitro fertili?ation.tw. (25997)

3 ivf-et.tw. (2883)

4 icsi.tw. (13507)

5 intracytoplasmic sperm injection$.tw. (8172)

6 ivf.tw. (33791)

7 (embryo or embryos).tw. (179999)

8 blastocyst$.tw. (24876)

9 exp ectogenesis/ (144)

10 exp embryo development/ (141940)

11 exp infertility therapy/ (83945)

12 assisted reproduct$.tw. (18431)

13 or/1-12 (334023)

14 time lapse$.tw. (13030)

15 timelapse.tw. (460)

16 Embryoscope$.tw. (454)

17 Eeva$.tw. (146)

18 Primo Vision$.tw. (34)

19 (sequential adj2 embryo$ scor$).tw. (3)

20 (sequential adj2 embryo$ assess$).tw. (4)

21 or/14-20 (13515)

22 Clinical Trial/ (935393)

23 Randomized Controlled Trial/ (462287)

24 exp randomization/ (75009)

25 Single Blind Procedure/ (28727)

26 Double Blind Procedure/ (138658)

27 Crossover Procedure/ (52614)

28 Placebo/ (297274)

29 Randomi?ed controlled trial$.tw. (164270)

30 Rct.tw. (25112)

31 random allocation.tw. (1667)

32 randomly allocated.tw. (27945)

33 allocated randomly.tw. (2255)

34 (allocated adj2 random).tw. (781)

35 Single blind$.tw. (19533)

36 Double blind$.tw. (173847)

37 ((treble or triple) adj blind$).tw. (701)

38 placebo$.tw. (253177)

39 prospective study/ (393425)

40 or/22-39 (1776265)

41 case study/ (48863)

42 case report.tw. (334596)

43 abstract report/ or letter/ (1005182)

44 or/41-43 (1380597)

45 40 not 44 (1730865)

46 13 and 21 and 45 (175)

Appendix 5. CINAHL search strategy

EBSCO platform
Searched from 1961 to 2 August 2017

#	Query	Results
S17	S12 AND S16	29
S16	S13 OR S14 OR S15	669
S15	TX Eeva*	234
S14	TX Embryoscope*	0
S13	TX time lapse	386
S12	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11	13,400
S11	TX ectogenesis	10
S10	TX assisted reproduct*	1,882
S9	(MM "Reproduction Techniques+")	4,715
S8	(MM "Fetal Development")	2,447
S7	TX blastocyst*	860
S6	TX(embryo or embryos)	4,992
S5	TX intracytoplasmic sperm injection*	369
S4	TX IVF or TX ICSI	2,052
S3	(MM "Fertilization in Vitro")	1,718
S2	TX vitro fertilization	3,731
S1	TX vitro fertilisation	3,731

FEEDBACK

Feedback on review protocol

Summary

Summary of a letter sent to David Tovey, Editor in Chief at the Cochrane Editorial Unit, London on 2nd February 2015:
1. The title of the review does not reflect the protocol and would better read "Time-lapse systems for embryo monitoring/assessment in assisted reproduction".
2. To the best of our knowledge several studies are ongoing in the field of time-lapse, while only one RCT has been published and this has as primary endpoint clinical pregnancy rate. There is not a single peer-reviewed published RCT that currently would fulfil the primary outcome measure of this intended time-lapse review.
3. Totally, two intervention studies of this time-lapse review emphasize the concept of cell-tracking algorithms. The cell-tracking algorithm per definition is an inherent and patented feature of a commercial product and hence only applied to a small fraction of patients in published time-lapse studies. Focusing on this could bias the neutrality of an evaluation. Hence we propose that the concept of cell-tracking algorithms should be re-phrased including morphokinetic evaluation models, clearly stating the aim of the model (e.g. blastocyst prediction or implantation prediction based on day 2/3/4/5 parameters), and how the model was applied in relation to the actual transfer day.
4. We would like to ensure that studies comparing "standard morphology" provide an exact definition of the standard evaluation. We particularly note several studies, which refer to a single day 3 observation as a "standard" evaluation when comparing to effectiveness against an automated cell-tracking algorithm.
5. The protocol mentions light exposure as a potential negative aspect of time-lapse imaging. In view of this we like to bring to the attention to the authors that there is a recent publication on this topic. This study has investigated the light exposure in a time-lapse system and concludes, that the overall exposure even in a 5 day culture period is much lower compared to standard observation as currently practiced. Similar findings were reported earlier.

Reply

Summary of the reply sent by the review authors to Professors Pribenzky and Montag on 20th February 2015:
1. The title of the review includes the words 'embryo incubation' because it is currently not possible to detect whether the potential advantage of time-lapse lies in its capacity for embryo monitoring/assessment or as a method of achieving a stable culture/incubation environment. For that reason we would defend keeping the title as it is.
2. Your second point surrounds the use of live-birth as a primary outcome despite the paucity of trials assessing this outcome. The Cochrane Menstrual Disorders and Subfertility Group provide guidelines on appropriate outcomes in reviews that are pertinent to both patients and clinicians. In the case of fertility interventions, live-birth is accepted as a suitable primary outcome. We currently have one study that reports this outcome, and in the future, more eligible studies assessing this outcome will be added to the review making this an important primary outcome.
3. Your third point raises the question on authors' neutrality when describing 'cell-tracking algorithms', which is considered by you to be an inherent and patented feature of a commercial product. We consider the phrase 'cell-tracking algorithms' to have no connection to any particular commercial product and adequately explains a process to the reader. The review does not name the manufacturer of time-lapse technology used in each study. Sadly information from published studies does not provide detailed information on the aims of the cell-tracking algorithm model, making it impossible to comment on what basis the information was applied. We will look to include this information in future updates of this review.
4. Your fourth point similarly outlines the importance of describing 'standard morphology' in each study. We agree that where this information is available, it should be described as part of the characteristics of each study.
5. Finally, you question the sentence in the protocol surrounding the potential negative aspect of light exposure on embryos associated with time-lapse systems given the recent findings of two studies. However, this review merely aims to establish both the potential benefits and harms of time-lapse, and offers up possible suggestions as to areas of potential harm. Potential harm is being assessed through adverse events; in this case miscarriage rate.
The authors thanked Professors Pribenzky and Montag for taking the time to carefully consider the protocol.

Contributors

Associate Professor Csaba Pribenszky, St Istvan Univ. Faculty of Vet. Sci., Budapest, Hungary
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Associate Professor Csaba Pribenszky and Prof Markus Montag certified on their feedback submission form that they have 'no affiliations with, or involvement in any organization or entity with a financial interest in the subject matter of my feedback'.

Feedback on review

Summary

1. Cochrane guidelines state that in case of fertility interventions, live birth has to be the primary outcome. In the view of this we have looked carefully at the data that were given by Cochrane on live birth from the one study for which the authors provided live birth data. As these data were not available in the original publication - because it was not a primary outcome - we contacted the authors ourselves to access the data, The study by Kahraman et al. reported to Cochrane for the time-lapse system (TLS) group 18 term live births, 2 miscarriages after 5 months of pregnancy and for the conventional incubation (CI) group 17 term live births, 1 preterm still birth and 1 induced abortion.
This leaves the fact that Cochrane reported two pregnancies more for each group than the authors of the study did. The authors reported in their response letter to Cochrane that 5 patients were excluded in the TLS group and 7 in the CI group. The reason for exclusion was, that these patients did not meet the inclusion criteria and received two embryos for transfer (which was not mentioned in the response letter to Cochrane) due to bad embryo development, freeze-all after OHSS or no selection on day 5. From these excluded patients, 2 patients in the TLS group and 3 patients in the CI group became pregnant, resulting in 2 live births in each arm (one in a fresh day 3 transfer and one in a freeze-thaw transfer for the TLS group; one in a fresh day 5 transfer and one in a freeze-thaw transfer for the CI group).
From this, we assume that the 2 live births from patients excluded from the study were added in each arm to give the final 20 live births for the TLS group and 19 for the CI group. But we clearly consider that this is not an adequate evaluation and presentation of the data, unless these details are made available to the readers. It is also highly questionable, if using outcome details from patients that were excluded or dropped out is in general a proper way to evaluate the effectiveness of a new treatment technology. Since this is unpublished and thus information that has not been verified we are interested in knowing what Cochrane guidelines are for such decisions and what the decision making process and control mechanisms are to guarantee correctness of such modifications?

2. We agree that it is a challenge to distinguish in some published studies if a cell-tracking algorithm has been used or not. ..Whether a cell-tracking algorithm has been used or not, it is always the embryologist (or physician) who makes the final choice for transfer, because every algorithm may have flaws and embryos may not develop as nicely as expected, despite an algorithm was applied.
For the studies that were used for the Cochrane review, one study applied only standard morphology on day 5 for the decision on which embryo to transfer (Kahraman et al., 2013). However, the study by Kovacs clearly uses an algorithm that combines scores based on kinetics and morphology, all scored on the time-lapse sequences: these scores add up for each embryo, where the one with the highest is selected for transfer. The same holds true for the study by Rubio et al. (2014). One of the authors of this letter to Cochrane (CP) was involved in and is a co-author to the study by Kovacs et al.; therefore this statement is a fact and not fiction.
Consequently the subgrouping done in the current Cochrane review is wrong and the evaluation shown in Figure 6 of the review not correct.
3. In the Cochrane review the number of positive ß-hCG pregnancies from the Rubio paper (so called "biochemical pregnancies") and the clinical pregnancies from the Kahraman & Kovacs paper are used to evaluate the Clinical Pregnancy Rate. As Rubio et al. defined "pregnancies" as those having a positive ß-hCG serum level; the Cochrane report includes a mix of different definitions. Biochemical pregnancy rate is not the same as clinical pregnancy rate unless it is confirmed by the authors that the number is the same. Therefore, stating a mix of biochemical pregnancies and clinical pregnancies as THE CPR in the Cochrane review without further explanation to the readers is unacceptable and wrong.
The number of pregnant patients is related to the number of patients included in the study (Intention to treat; TLS / CI: 444/412 for Rubio; 30/32 for Kovacs; 38/38 for Kahraman). Although this may be standard according to Cochrane guidelines, it is a bit odd that patients that dropped out or were excluded are still considered. Taking these into account the real number of patients treated and analysed would be different (TLS / CI: 438/405 for Rubio; 24/25 for Kovacs; 33/31 for Kahraman).

The dropouts mentioned by Kahraman (TLS: 5, CI: 7) were re-added by Cochrane to the total numbers, however; also the clinical outcome was re-added. The problem is that patients with unusual bad embryo development on D3 and D4, as well as frozen-thawed transfer cycles, received 2 embryos for transfer and not a single embryo as initially planned for the study. Therefore it is a point for discussion to include the outcome of these (excluded) cycles in the evaluation.
4. In the fifth point of your reply you refer to miscarriage rates.
The different studies included in the report used different time-points to define ongoing pregnancy:
- Rubio et al. define ongoing pregnancy as presence of fetal heart beat in week 12 of pregnancy
- Kahraman et al. consider ongoing pregnancy as being beyond week 5 after pick up (positive gestational sac)
- for the study by Kovacs et al. no definition has been given by the authors.
A lack of uniform definition and different interpretation of terms such as miscarriage, clinical pregnancy or ongoing pregnancy makes assessment of miscarriage difficult.
Considering the different end points defined by the studies included in this report, we think it is not possible to make a clear assessment of miscarriage rate. Also, there is no information for the readers that the respective papers use different definitions.
The Early pregnancy loss in the Kahraman paper is a mix of pregnancy losses such as ectopic pregnancy or a presence of a gestational sac without fetus or fetal heartbeat.
Since Rubio et al define ongoing pregnancy as presence of fetal heart in week 12 of pregnancy; it is not possible to distinguish between biochemical pregnancy loss and clinical pregnancy loss before week 12.
We think that either more information should be provided to the readers allowing for a correct interpretation of the results or that this calculation should be excluded due to heterogeneity of the data from the different studies.
We understand that for the primary end point the number of patients treated is used for comparison of results. However, what strikes us, is the fact that in the Cochrane review the number of the "miscarriages" is put in relation to the number of intention to treat - instead of relating these to real clinical pregnancies (which is difficult as discussed on the previous topic on definition of clinical pregnancy).
Based on this there are considerable flaws in the calculation presented in the Cochrane review for the miscarriage rate! In clinical embryology miscarriage rates are always seen in relation to the pregnancies achieved and considered as an important indicator for embryo viability beyond implantation.

5. We do agree that more studies are important for all aspects mentioned in the Cochrane review, but we do not agree with the presentation and evaluation of the data as they are presented right now.
We would therefore like to ask the authors of this Cochrane review:
- to withdraw the current review
- to state the reason for withdrawal
- to reassess the data, provide more information for the readers allowing correct interpretation and, where necessary, redo the calculations
- to include experts with deeper knowledge in clinical embryology and time-lapse imaging for a revised version of the Cochrane review

Reply

1. We thank you for your feedback and confirm that the data from Kahraman et al. reported in the Cochrane review includes two additional live births per group and the denominator includes the patients who were excluded post randomisation. The reason for these additions are that we have applied the intention to treat principle, which we stated we would utilise in our protocol. This is in line with Menstrual Disorders and Subfertility Cochrane group (MDSG) guidelines and the CONSORT statement. The intention to treat principle is a standard, uncontroversial, and well recognized protocol. Item 16 of the CONSORT statement states for example "Intention-to-treat analysis is generally favoured because it avoids bias associated with non-random loss of participants". The MDSG guideline states under unit of analysis issues that "the primary analysis should be per woman randomized and that data will be analysed on an intention to treat basis and attempts will be made to obtain missing data from original trialists." We believe that the reasons for drop-outs were clearly described in the characteristics of study table and risk of bias tables in addition to describing the unpublished nature of the data.
In this case, the numbers of drop-outs and additional pregnancies is similar in both arms of the study therefore we can confirm that the inclusion of the additional data would not have affected the overall results. Whilst we value your thoughts we are of the view that this is the most methodologically correct approach and therefore it will remain unchanged in this review.
2. Thank you for the additional information and clarification of the design and therefore classification of the Kovacs study. We consider that it is difficult to assess whether the algorithms have been used to make clinical decisions or not. This additional information can be incorporated at the next scheduled update and we will add footnotes indicating this in the meantime. It does not change the overall analysis or the results for either of the subgroups.

3. We agree that mixing biochemical and clinical pregnancy rate is not the ideal study design. In the case of the Rubio study, raw data on ongoing pregnancy rate was not provided to us despite a number of requests to the study authors. Therefore the best available data was utilized. We acknowledge that we should have made this clear in the characteristics of study table, and we will add a footnote to this effect. In the updated review, we will contact the Rubio study authors again to request data on ongoing clinical pregnancies.
You briefly touch on the challenge of various studies using single or dual embryo transfer, as well as fresh and frozen-thawed transfer cycles and different days of transfer. You highlight this in conjunction with the intention to treat principle which includes outcomes from patients with a variety of these procedures. In our protocol we outlined that we would include studies that utilize any of these variations in treatment, as occurs in real life. We have detailed the number of embryos transferred, including details on planned day of transfer in the characteristics of studies table for each included study.
4. We acknowledge the heterogeneity in the definition of miscarriage between the included studies. Unfortunately this is an unresolved academic issue in the field of fertility research, where there is a lack of uniform definition, not only amongst journals, but also between countries. As you have highlighted, often papers do not provide a definition. In our protocol we described that miscarriage and stillbirth would be expressed per woman randomized. If we were to report per pregnancy, there is a risk of unbalancing the groups and adding bias to the analysis.
5. The review will be updated with new data when it becomes available. We do not consider that the points raised justify withdrawing the review. We would like to assure you that as part of the publication process this review has been through a rigorous peer review process. This included peer review from embryologists within the field.
The authors thanked Professors Pribenzky and Montag for taking the time to carefully consider the review.

Contributors

Associate Professor Csaba Pribenszky, St Istvan Univ. Faculty of Vet. Sci., Budapest, Hungary
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Associate Professor Csaba Pribenszky and Prof Markus Montag were asked to disclose their conflicts of interest. They stated they have 'no affiliations with, or involvement in any organization or entity with a financial interest in the subject matter of my feedback'.

WHAT'S NEW

Last assessed as up-to-date: 2 August 2017.

Date	Event	Description
2 August 2018	Amended	Correction of a typo in Characteristics of studies

HISTORY

Protocol first published: Issue 9, 2014

Review first published: Issue 2, 2015

Date	Event	Description
23 January 2018	New search has been performed	Five new studies were added for this update (Goodman 2016; Kaser 2017; Park 2015; Wu 2016; Yang 2017). Kovacs 2013 had new data published in 2017.
23 January 2018	New citation required but conclusions have not changed	The addition of five new studies has not led to a change in the conclusions of this review. The comparisons have been restructured
5 October 2015	Feedback has been incorporated	Feedback on the review was received in April 2015. The feedback and the review authors' response has been summarised in the "Feedback" section. Footnotes were added to Summary of Findings Tables 1 and 3 and to the Characteristics of study table for Rubio 2014.
5 March 2015	Feedback has been incorporated	Feedback applicable to the review protocol was received in February 2015. The feedback and the review authors' response has been summarised in the "Feedback" section. No changes were made to the review

CONTRIBUTIONS OF AUTHORS

SA developed the protocol and wrote the first draft of the review. PB, VJ, AP and CF commented on and made changes to the review. SA and PB screened the search titles and extracted data from full text articles. SA and PB contacted authors for further information. VJ gave her methodological and content opinion on the full review.

Ms Nicola Arroll and Dr Lynsey Cree were both authors in the first iteration of this review, but have not participated in this update of the review.

DECLARATIONS OF INTEREST

Dr Priya Bhide is a co-investigator for the TILT trial, an RCT of TLS versus undisturbed culture versus conventional incubation and assessment, which has recently obtained ethics approval. TILT is funded by the Barts Charity. Dr Sarah Armstrong is hoping to recruit for the trial.

There are no other conflicts of interest for any of the authors.

SOURCES OF SUPPORT

Internal sources

- The University of Auckland, New Zealand.

Clinical Fellow 2013-4

External sources

- None, Other.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The title has been altered to reflect both the assessment and culture capability of TLS.

The wording of the 'types of interventions' in the methods has been altered to clarify the comparisons we made. We have sought to divide studies into three comparisons depending on the nature of the intervention and the control, in order to truly assess if there is a clinical benefit to TLS, and where the benefit of TLS might lie.

1) TLS with conventional morphological assessment of still TLS images versus conventional incubation and assessment (trial design 1)

2) TLS utilizing embryo selection software versus TLS with conventional morphological assessment of still TLS images (trial design 2)

3) TLS utilizing embryo selection software versus conventional incubation and assessment (trial design 3)

The outcome 'adverse events' has been re-worded to include miscarriage and stillbirth.

We have removed 'alternative imputation strategies' from Sensitivity analysis

INDEX TERMS

Medical Subject Headings (MeSH)

*Embryo Culture Techniques; *Reproductive Techniques, Assisted; Abortion, Spontaneous [epidemiology]; Embryonic Development [*physiology]; Live Birth [epidemiology]; Pregnancy Rate; Randomized Controlled Trials as Topic; Stillbirth [epidemiology]; Time-Lapse Imaging [*methods]

MeSH check words

Female; Humans; Pregnancy