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Impact of time-lapse imaging incubators with single-step culture medium on cumulative live birth rate in IVF cycles: a retrospective cohort study

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Abstract

Objective To compare the cumulative live birth rate per egg retrieval between time lapse imaging (TLI) incubators and standard culture (SC) incubators both using a single-step culture medium Design Retrospective cohort study Setting A tertiary level fertility-centre Population Women undergoing an IVF cycle between November 2015 and December 2017 Methods Comparison was done between 1219 IVF cycles using TLI and 1039 cycles using SC after accounting for confounding factors such as age and number of oocytes retrieved. Main outcome measure Cumulative live birth rate per egg retrieval Results The live birth rate per egg retrieval following fresh embryo transfer was noted to be higher for TLI cycles (TLI 39.87% vs SC 38.02%, aOR 1.20, 95% CI 1.01 to 1.44). More embryos were available for cryopreservation in the TLI arm (MD 0.08 embryos, 95% CI 0.10 to 0.41). The live birth rate per frozen embryo transfer was not significantly different. The cumulative live birth rate per egg retrieval was significantly higher in the TLI arm (TLI 50.29% vs SC 46.78%, aOR 1.24, 95% CI 1.04 to 1.48) Conclusions With the use of single step medium, there appears to be a greater benefit of TLI through a reduced interruption in embryo culture conditions, resulting in a higher number of embryos available for cryostorage which in turn appears to improve the cumulative live birth rate. Funding No funding was obtained for this study Keywords Time lapse imaging, cumulative live birth rate, single step culture medium, embryo utilization rate

Title page:

Title: Impact of time-lapse imaging incubators with single-step culture medium on cumulative live birth rate in IVF cycles: a retrospective cohort study

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Abstract

Objective

To compare the cumulative live birth rate per egg retrieval between time lapse imaging (TLI) incubators and standard culture (SC) incubators both using a single-step culture medium

Design

Retrospective cohort study

Setting

A tertiary level fertility-centre

Population

Women undergoing an IVF cycle between November 2015 and December 2017

Methods

Comparison was done between 1219 IVF cycles using TLI and 1039 cycles using SC after accounting for confounding factors such as age and number of oocytes retrieved.

Main outcome measure

Cumulative live birth rate per egg retrieval

Results

The live birth rate per egg retrieval following fresh embryo transfer was noted to be higher for TLI cycles (TLI 39.87% vs SC 38.02%, aOR 1.20, 95% CI 1.01 to 1.44). More embryos were available for cryopreservation in the TLI arm (MD 0.08 embryos, 95% CI 0.10 to 0.41). The live birth rate per frozen embryo transfer was not significantly different. The cumulative live birth rate per egg retrieval was significantly higher in the TLI arm (TLI 50.29% vs SC 46.78%, aOR 1.24, 95% CI 1.04 to 1.48)

Conclusions

With the use of single step medium, there appears to be a greater benefit of TLI through a reduced interruption in embryo culture conditions, resulting in a higher number of embryos available for cryostorage which in turn appears to improve the cumulative live birth rate.

Funding

No funding was obtained for this study

Keywords

Time lapse imaging, cumulative live birth rate, single step culture medium, embryo utilization rate

Introduction:

Time lapse imaging (TLI) incubators have revolutionised our understanding of early human embryonic development but their role in clinical laboratory practice remains uncertain. Algorithms have been generated using the information gained from more detailed knowledge of embryo development such as cell division timings (embryo morphokinetics). However, the application of these algorithms to improve embryo selection appears to be hindered by the lack of translatability across laboratories, probably reflective of inter-laboratory variation[1]. Additionally, embryos possess the potential of auto-correction whereby some embryos with abnormal initial morphokinetics go on to form good quality blastocysts. These auto-correcting embryos appear to have a better prognosis than embryos which fail to form good quality blastocysts [2].

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Another school of thought is to use the data derived from TLI incubators as subservient to conventional morphological assessment by using the morphokinetic data as exclusion criteria. This enables differentiation between embryos of similar morphology when choosing the best embryo to transfer [3].

By obviating the need for the embryo to be removed from the incubator for morphological assessment, TLI incubators provide a more stable culture environment. Previous research from our unit indicated that this more stable culture environment might contribute to superior perinatal outcomes with babies born from embryos cultured in TLI incubators having a lower risk of preterm birth and low birth weight [4]. However, there was no difference in cumulative live birth rate. Sequential culture medium had been utilised during the period of that study which required the TLI incubator doors to be opened on the third day of culture for media change. Since November 2015, the culture medium was changed to single step culture medium which allowed for truly uninterrupted culture in TLI incubators until the day of embryo transfer.

Guidance from the British Fertility Society (BFS) [5], European Society for Human Reproduction and Embryology (ESHRE)[6] and the Human Fertilisation and Embryology Authority (HFEA) [7] on the role of TLI incubators in current IVF practice reflects the uncertainty associated with TLI use. Particular criticism has been directed on the absence of cumulative live birth data which would help assess whether the role of TLI incubators is improving embryo selection or whether it improves the culture environment in addition to better embryo selection [6].

The aim of this retrospective study is to compare the cumulative live birth rate between TLI and standard culture (SC) incubators with the use of single-step culture medium.

Materials and Methods:

This study was conducted at a tertiary level infertility unit in the United Kingdom (UK) performing approximately 1500 oocyte retrievals per year. Data extraction was performed from the electronic database of all oocyte retrievals performed from November 2015 to December 2017. This period was chosen because at the start of November 2015 the culture medium was changed from sequential media to a single step culture media and then in January 2018 day three morphology checks were ceased. The interim period therefore had consistent culture conditions for comparison. Neither specific funding nor ethical approval was sought for this study as it was a retrospective analysis of data. There was no patient and public involvement in the design of this study. Only the first cycle in this study period per woman was included for analysis. The primary outcomes analysed were live birth and cumulative live birth per oocyte retrieval. Details of the number of cycles included and the exclusion criteria have been provided in figure 1.

Ovarian stimulation and oocyte retrieval

Ovarian stimulation with gonadotrophins was performed using either a conventional long agonist protocol or antagonist protocol to prevent a premature Luteinising hormone (LH) surge. Follicular monitoring was performed using transvaginal ultrasound with ovulation triggered using either human chorionic gonadotrophin (hCG) or GnRH (gonadotropin releasing hormone) agonist when at least three follicles were 17mm or larger. Oocyte retrieval was performed 35 hours after ovulation trigger under ultrasound guidance. IVF or ICSI was performed between 39 and 41 hours post ovulation trigger depending on the sperm parameters and previous fertilisation results if applicable.

Embryo culture

The patients had the choice of two culture systems in our unit; SC (BT37 bench-top incubators, Planar, UK or Heracell 150 chamber incubators, Thermo Scientific, UK) or TLI at an additional cost (EmbryoScope®, Vitrolife, DK). Where IVF was performed and TLI was required, up to 12 fertilised zygotes were placed in individual wells of an EmbryoScope® Slide at fertilisation check (at 16-19hpi) and placed into the TLI. For

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TLI cases where ICSI was performed, injected oocytes were placed immediately into TLI if there were 12 or fewer mature oocytes injected, or alternatively fertilised zygotes were placed into TLI after fertilisation check where there were more than 12 mature oocytes injected. Where there were more than 12 zygotes for a patient the additional zygotes were placed into SC. Cycles where both TLI and SC incubators were used were excluded from the analysis.

A low oxygen (5%) culture environment with 6% carbon dioxide at a temperature of 37^0C was used in both SC and TLI culture systems. Quinn's Advantage Fertilisation Medium (Cooper Surgical, Denmark) was used from oocyte retrieval to fertilisation check for IVF cases and until denudation for ICSI cases. This was followed by Sage 1-Step (Cooper Surgical, Denmark), with both types of culture medium having an oil overlay (Origio Liquid Paraffin, Cooper Surgical, Denmark). In both TLI and SC incubators, single embryo culture was performed in micro-drops. Embryos in SC incubators were removed from culture on day 3 for morphology assessment, whereas the TLI embryos had undisturbed culture until the day of embryo transfer. TLI embryos were annotated throughout culture as has been described previously[4].

Embryo transfer and follow up

Morphological assessment and grading of embryos was performed according to UK guidelines [8]. Embryo transfer was performed on either day 3 or day 5 depending on patient and embryo criteria consistent with NICE guidelines [9]). Patients who had two or more good quality embryos on day 3 (at least 6 cells, grade 3 or 4 for both evenness and fragmentation) were offered extended culture to day 5. The number of embryos transferred was based on patient age, embryo quality, the number of previous transfers (if any), and considering the unit's multiple births minimisation protocol[10]. Morphology grading alone was used to select embryo(s) to transfer in SC cycles. In TLI cycles, morphological grading was the primary selection tool and in cases where there was more than one morphologically good quality blastocyst, avoidance criteria and the KIDScore D5 score (V2.0 algorithm, Vitrolife, Denmark) were used to exclude embryos with lower implantation potential. The avoidance criteria included unevenness at the two-cell stage, multinucleation at four-cell, direct first cleavage and irregular or reverse cleavage as described in detail previously [4] . Any surplus good quality blastocysts on day 5 or 6 were cryopreserved by vitrification (Cryotop(r) and KT801 vitrification media, Kitazato, Japan). Embryo utilisation rate was defined as the number of embryos transferred or cryopreserved per 100 zygotes.

Pregnancy was established using a urinary pregnancy test 18 days following oocyte retrieval which was followed by a transvaginal ultrasound scan at 7 weeks' gestation. The International Glossary on Infertility and Fertility Care and the Core Outcome Measure for Infertility Trials (COMMIT) were used to define the outcomes assessed[11,12]. A clinical pregnancy was confirmed when cardiac activity was visualised on ultrasound. A multiple pregnancy was diagnosed where there was more than one foetal pole with cardiac activity on ultrasound. Pregnancies which did not reach the stage of a clinical pregnancy were defined as biochemical pregnancy loss and loss of a clinical pregnancy prior to 22 completed weeks was classified as a miscarriage. The birth of at least one viable infant after 22 weeks' gestation was counted as a live birth.

Frozen embryo transfer

The electronic database was checked manually to ascertain the outcomes of frozen embryo transfer (FET) cycles of women who did not have a live birth after fresh embryo transfer but had surplus blastocysts cryopreserved and women who had embryos electively cryopreserved after oocyte retrieval (freeze-all embryos). FET cycles were either artificial or natural and embryos were warmed rapidly following standard protocols (VT802 warming media, Kitazato, Japan). In artificial cycles the endometrium was primed with oestrogen following a long agonist down-regulation protocol with progesterone supplementation when the endometrium measured >7mm on ultrasound scan. In natural cycles, blastocyst transfer was performed six days after an early morning urine LH surge had been detected. If a live birth had occurred after frozen embryo transfer, this was documented in order to calculate the cumulative live birth rate. All live births reported until July

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2020 were included as a reasonable time-frame for assessing the short term cumulative live birth rate given that the study period encompassed November 2015 to December 2017.

Statistical analysis:

The number of oocyte retrievals was taken as the unit of denominator for comparison between TLI and SC incubators. As only the first cycle per woman was included in this study, the per-oocyte retrieval data is identical to per-woman data. Continuous variables were described by mean and standard deviation and compared through mean difference (MD) with 95% confidence intervals (CI) and p values obtained through unpaired t-tests. Categorical variables were described using percentages and comparison performed with Chi-Square test and reporting utilised odds ratio (OR) with 95% CI and p values. For the primary outcomes of live birth and cumulative live birth, adjusting for confounding variables (age and number of oocytes retrieved) was performed using binomial logistic regression and adjusted OR reported. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0.

Results:

From November 2015 to December 2017, 2539 women underwent an IVF cycle with their own eggs. Of these cycles, 121 were abandoned before egg collection or had no eggs collected. On three occasions there was failure to obtain sperm. There were 78 cycles with complete failure to fertilise and two with failed cleavage. Mature oocyte cryopreservation was performed for 17 patients and there were 8 cycles where no eggs were suitable to inject following denudation for ICSI. There were 21 cases of freeze all embryo cycles and 11 where no embryo transfer was performed due to abnormal development. Three surrogacy cycles were excluded as were two cycles which were lost to follow up leading to no outcome information being available. We also excluded 15 cycles where both TLI and SC incubators were used. This resulted in a total of 2258 cycles included in this retrospective analysis, of which 1219 were in the TLI arm and 1039 in the SC arm (figure 1).

Table 1 outlines the baseline characteristics of the two groups for comparison. As reported previously [4], there was a bias to older patients in the TLI group where the mean age was 35.15 years as compared with 33.47 years in SC (MD, 1.68 years, 95% CI 1.31 to 2.05). Despite being older, the women in the TLI arm had more oocytes retrieved, with a mean of 12.15 oocytes vs 10.87 oocytes for SC (MD 1.28, 95% CI 0.74 to 1.82). Age and number of oocytes were therefore adjusted for when analysing the outcomes. The proportion of cycles that had a transfer performed was similar between the two groups, as was the mean number of embryos transferred. However the proportion of cycles with embryos available for cryopreservation (TLI 49.38 % vs SC 43.79%, OR 1.25, 95% CI 1.06 to 1.48, p = 0.008) as well as the mean number of embryos cryopreserved (TLI 1.37 +- 2.03 vs SC 1.10 +- 1.68, MD 0.08, 95% CI 0.10 to 0.41, p = 0.001) was higher in the TLI group. The embryo utilisation rate was also higher in the TLI group, but this did not reach statistical significance (TLI 37.08% vs SC 36.29%, OR 1.03, 95% CI 0.97 to 1.11, p = 0.323)

Table 2 outlines the clinical outcomes in the two groups. The clinical pregnancy rate per cycle was significantly higher in the TLI group (TLI 46.84% vs SC 41.87%, OR 1.22, 95% CI 1.04 to 1.45, p = 0.017). There was no significant difference in the multiple pregnancy rate (TLI 6.48% vs 4.83%, OR 1.37, 95% CI 0.79 to 2.37, p = 0.267). There was a significantly higher live birth rate per cycle in the TLI arm (TLI 39.87% vs SC 38.02%, OR 1.08, 95% CI 0.91 to 1.28, p = 0.039). After adjusting for the baseline characteristics of age and number of oocytes which had been noted to be significantly different between the TLI and SC groups, the live birth rate was still significantly higher in the TLI group (aOR 1.20, 95% CI 1.01 to 1.44, p = 0.040).

As noted above, a higher proportion of cycles in the TLI arm had embryos available for cryostorage which in turn translated to a non-significant increase in the proportion of women who had an embryo available in cryostorage following an unsuccessful fresh embryo transfer (TLI 41.80% vs SC 36.69%, OR 1.24, 95% CI 0.99 to 1.55, p = 0.058). This is turn led to a non-significant trend towards a higher number of frozen

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embryo transfers being performed in the TLI arm (TLI 1.25 +- 0.90 vs SC 1.22 +- 0.90, OR 0.03, 95% CI -0.12 to 0.19, p = 0.707). There was no significant difference in the live birth rate per frozen embryo transfer (TLI 30.96% vs SC 30.39%, OR 1.03, 95% CI 0.74 to 1.42, p = 0.377).

The cumulative live birth rate per cycle after taking into account both fresh and frozen embryo transfers from the study period was on initial analysis not significantly different between TLI and SC groups (TLI 50.29% vs SC 46.78%, OR 1.15, 95% CI 0.97 to 1.36, p = 0.125). However, upon adjusting for age and number of oocytes, the cumulative live birth rate was significantly higher in the TLI group (aOR 1.24, 95% CI 1.04 to 1.48, p = 0.016).

Discussion:

Main findings:

The cumulative live birth rate is significantly higher with TLI incubators when compared with SC incubators. This appears to be driven by two factors. Firstly, there was an improvement in live birth rate per fresh embryo transfer in the TLI arm with no significant change in the number of embryos transferred or the multiple pregnancy rate. Secondly, there was a significant increase in the number of embryos available for cryopreservation in the TLI arm which translates into a trend towards a higher proportion of those with unsuccessful fresh embryo transfers having access to cryopreserved embryos in the TLI arm.

Strengths and limitations:

Strengths:

To our knowledge, this is the second study comparing cumulative live birth rate per oocyte retrieval between TLI and SC incubators and the first to show a significant difference. A criticism of TLI incubators in the ESHRE guideline was the paucity of cumulative live birth reporting in scientific literature which would help to assess the relative importance of the 'undisturbed culture' versus 'embryo selection'. The data presented here aims to provide a better perspective on this underexplored aspect of TLI incubators. Additional strengths of the study include the single-centre nature which ensures that the laboratory conditions were uniform throughout the study period and the large sample size which provided statistical power. Furthermore, the baseline denominator was per oocyte retrieval which reduces selection bias due to failed embryo transfer.

Limitations:

A retrospective study does carry its limitations, primarily in terms of confounding factors. We attempted to adjust for this by an analysis for baseline characteristics and adjustment for factors such as age and number of oocytes retrieved but there is still a potential for unknown confounding factors that we have not adjusted for. As the findings are from a single unit, generalisability of these findings will be limited but the findings of this study may form the rational basis for approval for a well-powered randomised controlled trial. Findings from other centres are urgently needed to either confirm or refute these findings to determine whether change in laboratory practice to incorporate TLI would help in other fertility clinics.

Interpretation (in light of other evidence):

Comparison with previous literature:

The first studies comparing TLI and SC incubators were exploratory, had a small sample size and primarily focussed on the safety and comparability of TLI to SC incubators. The latest Cochrane review[13] noted an absence of RCTs examining cumulative live birth rate and the ESHRE recommendations on good practice

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of TLI incubators [6] was able to identify only one study (previously published from our unit) on cumulative live birth rate in the available literature. In this previous study, we did note an improvement in live birth per fresh embryo transfer but failed to note a significant difference in cumulative live birth per oocyte retrieval[4]. However, in the period of the previous study, sequential culture media was used and so the opening of TLI incubator doors was required on day 3 for a change of the culture medium.

Biological plausibility of the findings:

The availability of morphokinetic data from TLI incubators led to the identification of 'poor prognostic' features which were developed into factors for exclusion. The suggested exclusion factors included unevenness at the two-cell stage, multinucleation at four-cell, direct first cleavage and irregular or reverse cleavage [3]. Apart from true direct cleavage from one to three cells for which there appears to be reasonable evidence for a poor prognosis, the rest of the exclusion criteria do not appear to have a consistent prognostic effect between studies [14,15]. Additionally, embryos may be capable of auto-correction, and an embryo which displays features of the exclusion criteria, but nonetheless develops into a top-quality blastocyst appears to have a better prognosis than embryos which arrest during their development [2]. An alternate approach has been to not rely on exclusion criteria, but generate algorithms based on embryo morphokinetics to select the best embryo. Such algorithms have been generated through statistical analysis in combination with observation and expert opinion of embryologists and more recently using artificial intelligence for data analytics. Whilst these algorithms have shown promise in initial studies, they do not appear to be translatable across different embryology laboratories, which is a major limitation to widespread adoption [1]. There is ongoing research on using artificial intelligence and deep learning to improve algorithms, with early attempts being made into introducing such algorithms into clinical practice [16,17].

The Cochrane review noted that use of algorithms did not appear to confer an additional benefit to TLI with conventional morphological assessment (OR for ongoing pregnancy rate 0.61, 95% CI 0.32 to 1.20, 1 RCT, N = 163) [13].

An increase in cumulative live birth rate with TLI with this group suggests that the undisturbed culture may contribute to this uplift. We have hypothesised in our previous paper on perinatal outcomes that the undisturbed culture in TLI may play a role in the impact of TLI systems. The choice of culture media may play a role, but successful embryo development also relies on a stable embryo culture environment.

Laboratory factors can impact on media efficacy and embryo development resulting in significantly different outcomes [18]. A change in temperature can destabilise the cellular cytoskeleton (including the meiotic spindle) and might also affect embryo metabolism. Differences in embryo aneuploidy rates amongst egg donor programmes between different clinics [19] serves to illustrate the crucial factor of laboratory conditions (including maintaining a stable temperature) on the development of a competent embryo. A recent review listed several patient, clinical and laboratory factors which might affect the risk of embryo aneuploidy indicating that there is room for further research to improve embryo competence in IVF clinics[20].

Conclusion:

Use of TLI incubators appears to increase the cumulative live birth rates in IVF providing additional reassuring data about the safety and effectiveness of TLI incubators for clinical practice in IVF. The fact that this effect on cumulative live birth rate has been seen with single-step culture medium and not with sequential culture medium (explored in our previous study) adds additional strength to our hypothesis that TLI incubators do not affect embryo selection alone but improve embryo quality through the uninterrupted culture conditions. This forces a rethink of the interrelationships between the type of incubator systems and type of the culture medium used in the clinical laboratory. Research studies such as meta-analyses exploring the impact of incubator systems should consider the type of culture medium used. Further research is being

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undertaken at our unit to assess the impact of not opening the incubator doors on day 3 in SC incubators to assess whether this adaptation might confer some of the benefits of TLI systems to SC incubators.

Authors' roles

MM developed the idea for the project. The study was designed by MM, SF and EF with inputs from KT and AHB. MM, SF and EF performed the data extraction and MM performed the data analysis and takes full responsibility for the integrity of the data. MM, EF and SF drafted the manuscript with inputs and critical discussion from KT and AHB. The final version has been approved by all authors

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Disclosure of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1 Barrie A, Homburg R, McDowell G, Brown J, Kingsland C, Troup S. Examining the efficacy of six published time-lapse imaging embryo selection algorithms to predict implantation to demonstrate the need for the development of specific, in-house morphokinetic selection algorithms. *Fertil Steril* 2017; **107** : 613–621.

2 Lagalla C, Tarozzi N, Sciajno R, Wells D, Santo MD, Nadalini M*et al.* Embryos with morphokinetic abnormalities may develop into euploid blastocysts. *Reproductive BioMedicine Online* 2017;**34** : 137–146.

3 Meseguer M, Herrero J, Tejera A, Hilligsoe KM, Ramsing NB, Remohi J. The use of morphokinetics as a predictor of embryo implantation.*Human Reproduction* 2011; **26** : 2658–2671.

4 Mascarenhas M, Fox SJ, Thompson K, Balen AH. Cumulative live birth rates and perinatal outcomes with the use of time-lapse imaging incubators for embryo culture: a retrospective cohort study of 1882 ART cycles. *BJOG: An International Journal of Obstetrics & Gynaecology* 2019; **126** : 280–286.

5 Bolton VN, Leary C, Harbottle S, Cutting R, Harper JC. How should we choose the 'best' embryo? A commentary on behalf of the British Fertility Society and the Association of Clinical Embryologists.*Hum Fertil (Camb)* 2015; **18** : 156–164.

6 ESHRE Working group on Time-lapse technology, Apter S, Ebner T, Freour T, Guns Y, Kovacic B *et al.* Good practice recommendations for the use of time-lapse technology+. *Hum Reprod Open* 2020;**2020** : hoaa008.

7 Time-lapse imaging | Human Fertilisation and Embryology Authority. https://www.hfea.gov.uk/treatments/treatment-add-ons/time-lapse-imaging/ (accessed 30 Dec2020).

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8 Association of Clinical Embryologists – Guidelines on Good Practice in Clinical Embryology Laboratories 2012. *Human Fertility* 2012;**15** : 174–189.

9 National Institute for Health and Care Excellence. (2013). Fertility problems: assessment and treatment (NICE Quality Standard No. 156). Retrieved from https://www.nice.org.uk/guidance/cg156. .

10 Cutting R, Morroll D, Roberts SA, Pickering S, Rutherford A, BFS and ACE. Elective single embryo transfer: guidelines for practice British Fertility Society and Association of Clinical Embryologists. *Hum Fertil (Camb)* 2008; **11** : 131–146.

11 Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R *et al.* The International Glossary on Infertility and Fertility Care, 2017. *Hum Reprod* 2017; **32** : 1786–1801.

12 Duffy JMN, AlAhwany H, Bhattacharya S, Collura B, Curtis C, Evers JLH*et al.* Developing a core outcome set for future infertility research: an international consensus development study+++. *Human Reproduction* 2020; **35** : 2725–2734.

13 Armstrong S, Bhide P, Jordan V, Pacey A, Farquhar C. Time-lapse systems for embryo incubation and assessment in assisted reproduction.*Cochrane Database Syst Rev* 2018; **5** : CD011320.

14 Desai N, Goldberg JM, Austin C, Falcone T. Are cleavage anomalies, multinucleation, or specific cell cycle kinetics observed with time-lapse imaging predictive of embryo developmental capacity or ploidy? *Fertil Steril* 2018; **109** : 665–674.

15 Zhan Q, Ye Z, Clarke R, Rosenwaks Z, Zaninovic N. Direct Unequal Cleavages: Embryo Developmental Competence, Genetic Constitution and Clinical Outcome. *PLoS One* 2016; **11** . doi:10.1371/journal.pone.0166398.

16 Babayev E, Feinberg EC. Embryo through the lens: from time-lapse cinematography to artificial intelligence. *Fertility and Sterility* 2020; **113** : 342–343.

17 Tran D, Cooke S, Illingworth PJ, Gardner DK. Deep learning as a predictive tool for fetal heart pregnancy following time-lapse incubation and blastocyst transfer. *Hum Reprod* 2019; **34** : 1011–1018.

18 Swain JE, Carrell D, Cobo A, Meseguer M, Rubio C, Smith GD. Optimizing the culture environment and embryo manipulation to help maintain embryo developmental potential. *Fertil Steril* 2016;**105** : 571–587.

19 Munne S, Alikani M, Ribustello L, Colls P, Martinez-Ortiz PA, McCulloh DH *et al.* Euploidy rates in donor egg cycles significantly differ between fertility centers. *Hum Reprod* 2017;**32** : 743–749.

20 Rodrigo L, Clemente-Ciscar M, Campos-Galindo I, Peinado V, Simon C, Rubio C. Characteristics of the IVF Cycle that Contribute to the Incidence of Mosaicism. *Genes* 2020; **11** : 1151.

Figure legends

Figure 1: Flowchart depicting cycles included within this study

TLI (Time lapse incubators); SC (standard culture incubators)

Table legends

Table 1: Baseline characteristics

Table 2: Outcomes

Table 1: Baseline characteristics

	TLI (n=1219)	SC (n=1039)	Mean difference/Odds ratio (95% con
Age, mean (SD)	35.15 (4.27)	33.47 (4.75)	1.68 (1.31 to 2.05), <0.001
Number of oocytes retrieved, mean (SD)	12.15 (6.64)	10.87 (6.37)	1.28 (0.74 to 1.82), <0.001
Fresh embryo transfer performed, n (%)	1186 (97.29)	1011 (97.30)	1.03 (0.61 to 1.72), 0.921

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	TLI (n=1219)	SC (n=1039)	Mean difference/Odds ratio (95% con
Cycles with embryos cryopreserved, n (%)	602 (49.38)	455 (43.79)	1.25 (1.06 to 1.48), 0.008
Embryos transferred, mean (SD)	1.20 (0.48)	1.17 (0.44)	0.02 (-0.01 to 0.08), 0.059
Embryos cryopreserved, mean (SD)	1.37 (2.03)	1.10 (1.68)	0.08 (0.10 to 0.41), 0.001
Embryo utilisation rate, % (n/100 2PNs)	37.08 (3141/8472)	36.29 (2357/6495)	1.03 (0.97 to 1.11), 0.323

*Embryo utilisation rate = number of embryos transferred or cryopreserved per 100 two-pronuclear embryos

Table 2: Outcomes

	TLI (n=121
Clinical pregnancy, n (%)	571 (46.84)
Multiple pregnancy, n (%)	37 (6.48)
Live birth, n (%)	490 (39.87)
Proportion of cycles with cryopreserved embryos available following unsuccessful fresh embryo transfer, % (n)	41.80 (293/
Number of frozen embryo transfers, mean (SD) ˜	1.25 (0.90)
Live birth per frozen embryo transfer, % (n)	30.96 (122/
Cumulative live birth per oocyte retrieval, n (%)	612 (50.29)

Adjusted Odds ratio - adjusted for age, number of oocytes retrieved

˜ Amongst women with cryopreserved embryos following unsuccessful fresh embryo transfer

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`figure 1.pdf` available at https://authorea.com/users/412615/articles/521248-impact-of-time-lapse-imaging-incubators-with-single-step-culture-medium-on-cumulative-live-birth-rate-in-ivf-cycles-a-retrospective-cohort-study