Recommendations for good practice for

The use of Time-Lapse technology

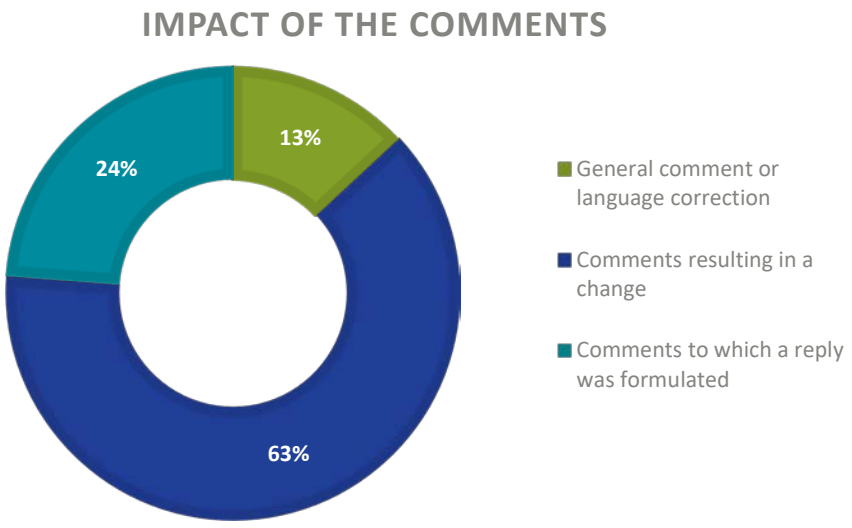
Set-up

The invitation to review was sent to the members of the SIG Embryology (n=3003 email addresses). In addition, the invitation was mailed to the members of the ESHRE Executive Committee and the Committee of National Representatives (n=74). An announcement was also placed on the eshre.eu website.

The stakeholder review started on 17th of June 2019, and was closed after 6 weeks, on the 2nd of August 2019.

Summary

Eleven reviewers, representing ten countries, submitted a total of 168 comments (on average 4 comments per reviewer). All reviewers are listed on page 2.



This report comprises the list of reviewers, and the overview of comments, with a reply from the working group.

List of reviewers

Reviewer	Country	Organisation
Danilo Cimadomo **Laura Rienzi**	Italy	
Kelly Tilleman	Belgium	
Guido Pennings	Belgium	
Zuzana Holubcova	Czech Republic	
Christopher Chen	Singapore	
Gemma Arroyo	Spain	
Sarah Armstrong **Allan Pacey** **Cindy Farquhar**	UK and New Zealand	
Philippe Terriou	France	
Markus Montag *	Germany	Ilabcomm GmbH
Tine Qvistgaard Kajhøj *	Denmark	Vitrolife
Evelyn Cottell *	Germany	Merck

List of comments from the reviewers with reply of the working group

Comments from the industry were also included, however, are indicated with an *

Reviewer	Page	Line	Comment	Reply GDG
General comments				
Markus Montag *	5	Table 1	References are listed alphabetically. In order to trace the first citation of a given Marker listing by year of publication may be better.	Reference format required by Human Reproduction Open
Markus Montag *	7-8	Table 3	References are listed alphabetically. In order to trace the first citation of a given Phenotype listing by year of publication may be better.	Reference format required by Human Reproduction Open
Evelyn Cottell *	2	49	Were the 12 steps followed? This is not indicated	As mentioned in the methods section, it is standard procedure for ESHRE recommendations documents to follow the 12 steps.
Sarah Armstrong Allan Pacey Cindy Farquhar	2	52	The guideline development group was by your own description a meeting of expert professionals on the topic of time-lapse technology. However, you didn't invite any of the clinicians who wrote the Cochrane review on the topic (Prof C Farquhar or Dr S Armstrong), which you cite as being the most up to date review of the literature. In our opinion, it would lend balance to the guideline group to include those with a broad understanding of the quality of the randomised controlled trial evidence. We note from the conflicts of interest form from the guideline development group that almost half of your experts have receive speakers' fees from industry.	The authors of this recommendation paper thank you for your attention to their work. As a preliminary note, we wish to inform you that ESHRE papers are realized according to well-defined internal procedures (https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Guideline-development-process), which were obviously implemented in the case in question. Such procedures were also met to select the members of the working group. Understandably, you, authors of a relevant Cochrane review and esteemed colleagues, were not invited also because the number of experts in the field by far exceed the number of authors that reasonably could be invited to write this

				recommendation paper. In addition, this paper are recommendations for clinical practice, so the focus is (by definition) not only on the clinical evidence. In fact, as well stated, our recommendation paper intends to offer suggestions on how to approach TLT from a practical standpoint, while citing clinical evidence studies only for the sake of completeness. Finally, with regard to your comment on the authors' disclosures, we believe that including experts that have received speakers' fees from industry is acceptable, as long as these are declared and the information transparently available for readers. This is also in accordance with the above mentioned manual. None of the authors share direct or indirect financial interests with companies providing TLT technology and we are confident that the declared speakers' fees did not impact on the integrity of the paper.
Evelyn Cottell *	2	52	Suggest adding: "represented by the authors of this publication". Are the experts of the 2 day meeting the authors, or were other professionals included?	The sentence was amended as suggested by the reviewer.
Evelyn Cottell *	2	53	Suggest "for the meeting" instead of "of the meeting"	The sentence was amended as suggested by the reviewer.
Evelyn Cottell *	2	55	Has a systematic screening and review of the literature been performed according to any guideline eg PRISMA guidelines? If so, please report the search strategy (string and search terms), eligibility criteria, data extraction strategy, PRISMA flow and risk of bias assessment.	A literature search has been performed where relevant, as mentioned in the methods section. However, no systematic screening or review of the literature was performed.

Evelyn Cottell *	2	60	Please specify where review report can be found (i.e. list link or web address, etc.)	The web address was added to the manuscript.
Evelyn Cottell *	2	63	Suggest "When considering TLT for your laboratory: " instead of "Before getting started"	Thank you very much for this suggestion. The working group prefers however to maintain the original text.
Evelyn Cottell *	2	After 77 need a new Table	A "TLT Implementation Guidance Table 2" would be useful, with a more prescriptive and systematic approach recommended, as this is undoubtably the biggest hurdle for labs integrating TLT. A Stepwise approach related to time and experience recommended e.g. (i) Annotate initially, according to labs standard grading system and times; (ii) Determine frequency of TL assessment e.g. daily or on Day 1, 3, 5, 6- according to labs SOPS or according to when most practical/fitting in with workflow (iii) Establish a QC system for annotations among users, with oversight from Super-User (iv) Identify / select additional parameters to be annotated, referring to ESHRE / Ciray table (v) Identify deselection parameters (and define what is meant by "de-selection" e.g. not for transfer but for extended culture to blastocyst stage for cryopreservation. Could go as far as recommending discarding only true 1->3 direct cleavers- care and attention not to discard too early/readily (Noting Lagalla 2013 paper); (vi) Identify selection parameters; (vi) Identify selection algorithms and retrospectively validate 1st, with KID embryos	The working group discussed this suggestion by the reviewer. However, the manuscript is already extensive and exhaustive, so the working group decided not to add another table.
Evelyn Cottell *	3	78	Existing Title is ambiguous. There is overlap in sections 1 and 2- Both cover "Applications and Implications of TLT". Would propose Section 1 Title as "Applications of TLT" 1.1 Enhanced Embryo Assessment system, 1.2 Training / Teaching tool, 1.3 Quality Control system for both Incubator environment and Embryo assessment	The working group discussed this suggestion by the reviewer, however, decided not to implement the suggested changes.
Evelyn Cottell *	3	83	Based on the content of this paragraph, it may be a more appropriate title: Dynamic Embryo assessment based on fertilization milestones	The working group discussed this suggestion by the reviewer, however, decided not to implement the suggested changes.
Evelyn Cottell *	3	100	Keep titles consistent- Embryo assessment "based on" cleavage features Or better, would suggest: Dynamic Embryo assessment during early cleavage stage (This would then include timing parameters and phenotypes)	The working group discussed this suggestion by the reviewer, however, decided not to implement the suggested changes.
Evelyn Cottell *	9	151	Suggest title: TLT as a Training/Teaching Tool	The working group discussed this suggestion by the reviewer, however,

				decided not to implement the suggested changes.
Evelyn Cottell *	9	162	Suggest tittle: Quality Control of TL Annotations	The working group discussed this suggestion by the reviewer, however, decided not to implement the suggested changes.
Evelyn Cottell *	10	206-207	Suggest titles: 2. TL and culture conditions 2.1 Impact on embryo culture	The working group discussed this suggestion by the reviewer, however, decided not to implement the suggested changes.
Evelyn Cottell *	12	311	Suggest another independent section, instead of a sub-section. Currently as 2.2. 3. TLT and laboratory workflow	The working group discussed this suggestion by the reviewer, however, decided not to implement the suggested changes.
Evelyn Cottell *	13	315	2011 ref?	This was adjusted.
Evelyn Cottell *	13	349	Suggest adding “in an IVF laboratory”	Thank you very much for this suggestion. The working group reckons that “in an IVF laboratory” is implicit.
Introduction				
Evelyn Cottell *	1	12	Change “pronuclear alignment” to “pronuclear presence, size, alignment and dynamics, nucleoli presence and distribution”	The phrase has been changed to "morphology of pronuclei and nucleoli", to include not only alignment but also all other suggested characteristics.
Evelyn Cottell *	1	14	Include ref Otsuki J, Iwasaki T, Tsuji Y, et al. Potential of zygotes to produce live births can be identified by the size of the male and female pronuclei just before their membranes break down. Reprod Med Biol. 2017;16:200-205. https://doi.org/10.1002/rmb2.12032 to support “pronuclear size and dynamics.” Include Fulka et al. Can Nucleoli Be Markers of Developmental Potential in Human Zygotes? Trends Mol Med. 2015 Nov;21(11):663-672. doi: 10.1016/j.molmed.2015.09.005. Epub 2015 Oct 20, to support nucleoli as part of morphological assessment	The references were added. Thank you for your suggestion.

Evelyn Cottell *	1	25	Suggest removing “therefore”. This paragraph introduces a new idea- TLT. Suggest telling more about the history of TL in our field and also introducing the term morphokinetics plus TLT predictive concept as the following paragraph talks about algorithms of selection/deselection. It would still align nicely with section 5 (current state of TLT) – see suggested introductory paragraph below… Time-Lapse Technology (TLT)) was introduced in the ART field many years ago (Payne et al, 1997). However, it was not until 2010 that time-lapse technology shifted from being used to simply observe human embryos while in culture, to being used in a predictive way. Wong et al. described a TL algorithm able to predict blastocyst formation by day 2 of embryo culture. This algorithm was based on cell division timings which included the duration of the first cytokinesis, and durations of the 2 and 3 cell stages (Wong et al., 2010). The year of 2011 marked the official introduction of TLT in the clinical lab, when researchers showed the ability to predict embryo implantation by again using specific cell division timing parameters, introducing the term morphokinetics (Meseguer et al., 2011). References: Payne D, Flaherty SP, Barry MF, Matthews CD. Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. Hum Reprod 1997; 12:532–41. Wong CC, Loewke Kevin E, Bossert Nancy L, Behr Barry, De Jonge Christopher J, Baer Thomas M, et al. Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. Nat Biotechnol 2010; 28:1115–21. Meseguer M, Herrero J, Tejera A, Hilligsoe KM, Ramsing NB, Remohi J. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod 2011;26: 2658–71.	A modified version of the suggested paragraph, and the references, have been added in the Introduction.
Evelyn Cottell *	1	25-28	Suggest starting a new paragraph: “The introduction of TLT in the clinical lab enabled an increased number of observations and the continuous assessment of developing embryos in a dynamic fashion, establishing the concept of Continuous Embryo Monitoring (Mol, B et al 2018). In parallel, TLT introduced the possibility of an uninterrupted culture environment, minimizing embryo handling and the need to expose embryos to conditions outside of the incubator (Meseguer et al; 2012)”.- This conveys more clearly a double advantage Mol, B et al Personalized ovarian stimulation for assisted reproductive technology: study	The proposed changes have been incorporated.

			design considerations to move from hype to added value for patients Fert Steril 2018; 109, (6), 0015-0282. https://doi.org/10.1016/j.fertnstert.2018.04.037	
Zuzana Holubcova	1	28	some embryologists are bothered by the fact that they can´t rotate and orient the embryo to focus on a particular detail	Although this may be a challenge sometimes, this comment is not of direct relevance here.
Evelyn Cottell *	1	29	Change to... stand-alone incubator with " or more" integrated inverted microscopes… As different TLT systems may contain one or more camera systems, this can be one of the major differences between systems	This is a valid point. The change has been included.
Evelyn Cottell *	1	33	Add "embryo" before development	This was adjusted in the manuscript.
Evelyn Cottell *	1	37	Suggest being more precise with refs for integrated software and algorithms. The Ciray ref is fundamentally a paper on standardising guidelines and annotation. e.g. of refs Rubio et al 2014 Fertil Steril -(Embryoscope); Aparicio-Ruiz 2019 Hum Reprod 34(1)i72 (EEVA-Xtend)	Reference by Rubio et al. was added.
Evelyn Cottell *	1	37	In addition, this section only refers to algorithm development- this was the first wave of "development" after implementation of TLT. To make this more contemporary, could you include and ref the development of AI tools and deep learning to assist embryo selection using images only (morphology) in addition to morphokinetics? AI discussed later but good to introduce here also.	This is an introduction section providing a general background on TLT. The use of AI is discussed in a later chapter.
Evelyn Cottell *	1	38	Suggest "This" instead of "The"	This was adjusted in the manuscript.
Evelyn Cottell *	1	40	Suggest "Consider" instead on "choose"	The working group is happy with the choice of wording as it is.
Box: Before getting started with TLT				
Sarah Armstrong Allan Pacey Cindy Farquhar	2	Box	Before getting started with TLT. The second bullet point mentions the financial pros and cons of acquiring a TLT system. We understand that many clinics charge for the use of TLT systems and the ethical stance of passing on the cost to patients is not discussed in this report. This is an important area to consider, given that ESHRE is grounded on scientific research and the current pooled RCT evidence does not reveal an improvement in livebirth or clinical pregnancy from using the technology (Armstrong et al 2019). Therefore, we think another bullet stating "educate clinic staff on the current evidence behind TLT in order to counsel patients alongside offering the technology".	We accept the suggestion to add the bullet point: "educate clinic staff on the current evidence behind TLT in order to counsel patients alongside offering the technology". Thank you for this suggestion.

Evelyn Cottell *	2	Box	Add “ranking” after selection/deselection of embryos	Thank you very much for this suggestion, which was adopted in the text.
Evelyn Cottell *	2	Box	Add “Develop an internal checklist, based on a User requirement Specification for the system, identifying and matching what clinic/lab want in a system e.g. type of gas, humidity, footprint, capacity, type of dish, software, cost, supply chain and manufacturer support etc” (Better to spell out key components)	Thank you very much for this suggestion, which was adopted in the text.
Evelyn Cottell *	2	Box	Suggest adding: “and other” before costs Suggest adding after costs: “ including hardware maintenance and software upgrades (if applicable)	Thank you very much for this suggestion, which was adopted in the text.
Evelyn Cottell *	2	Box	Important to add: “Evaluate technical/customer support available, including accessibility and the level of embryologist support and expertise they will can provide to your team” “Seek appropriate installation and training from the manufacturer/distributor”	Thank you very much for this suggestion, which was adopted in the text.
Evelyn Cottell *	2	Box	Add: Choose appropriate/preferred settings for imaging (e.g. focal plane number, distance between planes, frequency of image taking, light intensity, humidity enabled etc)	Thank you very much for this suggestion, which decided not to include because too specific, although relevant.
Evelyn Cottell *	2	Box	Add: Validate the TL incubator for key parameters (e.g. Temp, C02, alarm triggering/monitoring etc), as you would for any normal incubator.	Thank you very much for this suggestion, which decided not to include because too general, although relevant.
Evelyn Cottell *	2	Box	Identify a Super-user as the TLT referent responsible for the annotation of “initial” morpho-kinetic variables	Thank you very much for this suggestion, which decided not to include because the identification of a "super-user" is one of the diverse approach, but not the one "sine qua non".
1. Why clinics can use TLT (significance of TLT)				
Evelyn Cottell *	3	80-81	Suggest a more neutral approach given the level of current scientific evidence: “This section will review some of the clinical evidence associated with time-lapse parameters and phenotypes being used as tools for embryo assessment and their potential impact on embryo selection/deselection “ Note: Good to introduce the word “phenotype” here as table 3 uses it later but there	The specific sentence was not found in the text. We did however change "phenotype" into "feature".

			was no mention in the texts. Also, morphokinetics include timing parameters but not the phenotype(s) which are included in this and other paragraphs.	
Evelyn Cottell *	3	88	If this paragraph is discussing how TLT can improve embryo selection towards a healthy live birth, then the data included here needs to support this level of discussion further than how it is currently referenced.	The first sentence was changed.
Evelyn Cottell *	3	92	tPNf, PN breakdown or PN fading or disappearance? The term fading is used later in the paragraph, then disappearance later in table 1. It is good to keep it consistent.	This was adjusted in the manuscript.
Evelyn Cottell *	3	95	Please spell out tPB2 before using abbreviation	A comment was added that the abbreviations are explained in Table 2.
Gemma Arroyo	3	95	In relation to Extrusion of the second polar body, Aguilar et al., 2014 (Reprod Biomed Online. 2014 Apr;28(4):475-84. doi: 10.1016/j.rbmo.2013.11.014.) found that "The timings at which second polar body extrusion (3.3-10.6 h), pronuclear fading (22.2-25.9 h) and length of S-phase (5.7-13.8 h) occurred were linked successfully to embryo implantation. The other parameters were apparently not related, as determined by image acquisition and time-lapse analysis".	A sentence was added to the manuscript with the reference.
Evelyn Cottell *	3	99	Missing important publication on PN sizes just before nuclear membrane breakdown: Otsuki J, Iwasaki T, Tsuji Y, et al. Potential of zygotes to produce live births can be identified by the size of the male and female pronuclei just before their membranes break down. Reprod Med Biol. 2017;16:200-205. https://doi.org/10.1002/rmb2.12032	PN size is not a commonly monitored parameter. The working group therefore decided not to include the reference
Markus Montag *	3	100 ff	Clearly state that selection parameters used should be based on transfer day. This applies also for the model section, page 18, Topic 3.3…	The working group appreciates the detailed comment of the reviewer on specific references, however, the main focus of the manuscript covers technical and methodological aspects, rather than clinical aspects of TLT.
Evelyn Cottell *	3	101	Suggest adding: "(atypical phenotypes)" after Discrete cleavage anomalies.	This section focuses on cleavage features not on morphology.
Gemma Arroyo	3	101	Some authors found association between predictive parameters of blastocyst formation, such as direct cleavage to 3- cell stage (Cruz M, Garrido N, Herrero J, Pérez-Cano I, Muñoz M, Meseguer M. Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality. Reprod BioMed Online. 2012;25(4):371–81). Moreover, direct cleavage embryos had a lower implantation rate than other embryos studied with a normal cleavage pattern, as other authors had published before (Rubio I, Kuhlmann R, Agerholm I, Kirk J, Herrero J, Escribá MJ, et al. Limited implantation success of direct-cleaved human zygotes: a time-lapse study. Fertil Steril. 2012;98(6):1458–63).	The sentence was modified to include the information.

Evelyn Cottell *	3	103	Include additional reference (first publication describing abnormal cleavage data beyond first cleavage): Athayde Wirka, et al., 2014 Fertil Steril	The references were taken out, due to a previous comment, and the reader is referred to Table 1.
Evelyn Cottell *	3	104	Add text " " …blastocyst development can be predicted with high sensitivity "(94%) and specificity (93%). The strength of the described algorithm was actually based on its high specificity".	The text was adjusted as suggested by the reviewer.
Evelyn Cottell *	3	101-108	Could you be more specific about how these abnormal divisions impact implantation potential? All the references support this finding. It is a very clinically relevant finding as such abnormal divisions negatively impact embryo implantation. In the beginning of this section it seems that more data relevant to selecting embryos with improved potential of "making a healthy live birth" will be discussed. It would be good to include some more information otherwise it seems quite shallow. If this is not the goal here, then a suggestion is to change the introduction of this section.	The beginning of the section was changed so this comment is no longer relevant.
Evelyn Cottell *	3	106-107	Better to described both ways: …the time interval between the end of the first mitosis and the initiation of the second (duration of 2 cell) and the time interval between the second and third mitoses (duration of the 3-cell stage)	The text was adjusted as suggested by the reviewer.
Evelyn Cottell *	3	109-111	Better to place paragraph at beginning of section 1.1, after line 82? It is out of place here. Re-order Tables 1&2	The 2 sections are placed in a chronological order (fertilization marker and subsequently, cleavage). The working group decided not to make the proposed change.
Evelyn Cottell *	3	112	"Two to five cell cleavage timing" .. (t5..)- this is not correct definition of t5	The reviewer is correct, this was corrected in the manuscript.
Evelyn Cottell *	3	112-113	Incorrect….should read …The time to 5 cell and durations of the 2-cell stage and the 3-cell stage (t5, cc2, s2,) were shown to be most predictive parameters for embryo viability and implantation (Meseguer, et al., 2011).Note: It is interesting and important to show that two key publications found the time interval between the 2-cell and the 3-cell stage relevant to their predictive algorithms. (Wong 2010 and Meseguer 2011)	Table 2 was referenced for the definition of the abbreviation and the reference was added as suggested by the reviewer.
Evelyn Cottell *	3	118	Missing key publication by Fishel et al 2018 correlating time to start of blastulation and duration (tSB) and duration (dB{tB – tSB}) with Live Birth. This is a more valuable endpoint to Ploidy and should be referenced. 10.1016/j.rbmo.2018.05.016. Also Fishel 2017 RBMO paper	The sentence was amended, and the reference was included as suggested by the reviewer.

Gemma Arroyo	3	118	However, Rienzi (Rienzi L, Capalbo A, Stoppa M, Romano S, Maggiulli R, Albricci L, et al. No evidence of association between blastocyst aneuploidy and morphokinetic assessment in a selected population of poorprognosis patients: a longitudinal cohort study. Reprod BioMed Online. 2015;30(1):57–66) did not find any association between morphokinetic variables and the presence of aneuploidies in the embryo.	A sentence was added to the manuscript with the reference.
Evelyn Cottell *	4	122	Very important to support the variety of studies/markers/phenotypes described under Table 1 and 3: Suggest adding a paragraph discussing that the various atypical phenotypes and timing parameters published by various groups show different impacts on clinical outcomes - which contributes to mixed messages and growing scepticism regarding TL scientific evidence. This can be, at least partially, attributed to the lack on consensus on definitions and one time when milestones/phenotypes are being evaluated. For instance, abnormal cleavage and direct cleavage seem to have different clinical implications depending on when it is detected (milder later than on the first and second embryo divisions) (cite Meseguer, Athayde Wirka and Desai). Reverse cleavage and blastomere multinucleation also seem to show different impact on implantation potential based on when it is detected (cite Desai, Liu). Therefore, future TLT studies should aim to minimize the mismatch of markers'/phenotype definition when comparing previous seminal work and avoiding "grouping" data when definitions are not appropriately used. When discussing and presenting new, confirmatory or non-confirmatory data, researchers should take this into consideration. Previous attempt to standardize the language and the definitions involving TLT research has been published early in 2014 (cite Kaser and Racowsky) This new paragraph could be nicely placed after this paragraph below and both paragraphs could be the last ones before table 1: Guidelines were proposed on the nomenclature and annotation of the events observed during embryo development followed with a TL system (Ciray, et al., 2014). The variable and the description of the events are summarized in Table 2.	This issue has already been covered in a more general manner elsewhere in the manuscript.
Markus Montag *	5	Table 1	Should include as phenotype "Blastomere movement" Ezoe et al., Reprod Biomed Online. 2019 May;38(5):659-668. doi: 10.1016/j.rbmo.2018.12.014. Epub 2018 Dec 22	This was included in Table 1.
Tine Qvistgaard Kajhøj *	5	Table 1	For table 1: suggest inclusion of the following papers: Multinucleation: Ergin et al (2014): Fertil Steril. 2014 Oct;102(4):1029-1033.e1. Direct cleavage: Zhan et al., PLoS One. 2016 Dec 1;11(12):e0166398. doi: 10.1371/journal.pone.0166398. eCollection 2016.	The references are added with one exception: Blastocyst expansion cannot be included in Table, since this is not an abnormality.

			Cell exclusions: Lagalla et al., Reprod Biomed Online. 2017 Feb;34(2):137-146. doi: 10.1016/j.rbmo.2016.11.008. Epub 2016 Nov 24. Cottichio et al., Human Reproduction Update, Volume 25, Issue 4, July-August 2019, Pages 422–438 Blastocyst expansion: Huang et al., Reprod Biomed Online. 2019 Jul;39(1):27-39. doi: 10.1016/j.rbmo.2019.01.010. Epub 2019 Jan 23.	
Evelyn Cottell *	5	Table 1	There is a need for clear definitions in this table 1 It is critical that the various atypical phenotypes are defined as clearly as possible and in relation to specific cell stage of embryo. A contribution to inconsistencies with TL data is due to abnormal phenomena being pooled together and with different interpretations of the a given phenomena e.g. Rubio's 2012 Direct cleavage (DC2-3= cc2 = t3–t2 < 5 hours) defines this differently to Athayde Wirka's 2014 abnormal cleavage, or cell cycle stage not specified with the abnormality. These differences should be highlighted in the guideline document and the various definitions for each paper presented in Table 1. As a separate comment, recommendations should be made for future studies/ papers to standardise definitions, sub-categorise and clearly define Irregularly Cleaved Embryos. One such proposal by Lagalla et al 2017 could be considered: 1–3 direct cleavage; 1–3 rapid cleavage; 2–5 cleavage; Reverse cleavages; Prolonged S2 (t4-t3). Summaries of Lagalla 2017 and Zhan 2016 papers are worthy of inclusion, as they address stage specific events and associated incidences of atypical phenotypes and importance of clear definitions. Reverse cleavage: Athayde Wirka is mistakenly a reference for this phenotype. Additional references for Reverse cleavage: Liu et al, 2014; Desai et al, 2014; Goodman et al, 2016; Barrie et al, 2017 Multinucleation: Ergin et al, 2014; Desai et al, 2014; Goodman et al, 2016; Balakier et al, 2016; Hashimoto et al, 2016	Suggested references and the comment in the text are added and the clarification is made to distinguish direct from fast cleavage.
Evelyn Cottell *	5	Table 1	Add Otsuki et al Reprod Med Biol. 2017;16:200-205. paper on male and female nuclei size to Table, under PN section	This reference was added to Table 1.
Evelyn Cottell *	5	Table 1	Add key paper by Zhan et al, (Zaninovic group) 2016 Direct Unequal Cleavages: Embryo Developmental Competence, Genetic Constitution and Clinical Outcome to Table 1, under Direct cleavage. N.B. Stage specific DUCS, strong correlation of DUCS with epididymal / testicular sperm and Multinucleation. PLOS ONE | DOI:10.1371/journal.pone.0166398	This reference was added to Table 1.

Evelyn Cottell *	5	Table 1	Add key paper by Lagalla et al to Table 1, under new section Irregularly Cleaved Embryos, as described above. "Irregularly cleaved embryos should be cultured to blastocyst stage as they have the potential to become euploid. These embryos are observed to exclude cells from compaction. These cells could be analyzed to investigate a possible aneuploidy rescue mechanism. It is also recommended that their collection during biopsy procedures is avoided to prevent misdiagnosis".	This reference was added to Table 1.
Zuzana Holubcova	6	Table 2	It would be useful to unify nomenclature regarding morpho-kinetic parameters. tPB2: 2nd PB detached from oolema – more specifically – "abscission is completed". tPNf: some people use this abbreviation for PN formation instead of fading which brings about a lot of confusion. The mitosis starts with the break-down of the nuclear membrane (the moment when the sharp edge of interphase nuclear membrane turns blurry). With 5 min resolution, it is observable that interval from membrane break-down to the total disappearance of pronuclei (clearance of the area) could be up to 30 minutes. It should to be specified which moment the evaluators annotated. tn: sometimes the moment when cytokinesis start is scored instead of completion of cell division. tSC, tM, tSB, tB tE … these parameters are very subjective, could it be better defined? For instance tSB – does it meant the first time point when the sign of cavitation can be observed or some the time point when the cavity reaches some critical volume? tE – unclear to me, the increase of diameter would have to plotted first to define the starting moment… I am missing abbreviation for the duration of the first mitosis (the time interval tPNf (or tPNBD) to t2) and duration of the first cytokinesis (t2-start of cleavage event) ECC1: first cell cycle: please define t2-tPNa or t2- tPB2 ? cc2: t3-t2 OR t4-t2 and ECC2: t4-t2 – confusing. Shouldn´t cc2 be restricted to t3-t2 only? cc3 – a= t5-t4, b= t6-t4, c= t7-t4, d= t8-t4 again very very confusing, is it subcategories? wouldn´t it be better using cc3 (3rd cycle) = t5-t3? – the faster cell usually divides first… In embryos producing live births, there is only short s2 and s3. To my opinion, the length of the cell cycle should be the time interval between the first division of the previous and the following cycle. IMPORTANT – a relative timing vs. diverse "time zero" - ICSI, D1 (18h?), tPB2, tPNBD..	Some of the commented nomenclature appear in previously published guidelines; Changes were made when relevant

			I would add a warning that this inconsistency has to be taken into account when comparing data form different studies…..	
Philippe Terriou	6	Table 2	Dynamic events / cc2 cc2 Blastomere cell cycle: Duration of the second cell cycle (a=t3-t2, b=t4-t2)** footnote: **: note that morphokinetic automated annotation does not make the difference between true t3 (apparition of a third cell at the beginning of the third cycle) and false t3 (apparition of third cell during a direct division from 1 cell to 3 cells) and that cc2 will then be wrong.	This was considered outside the scope of table 2.
Tine Qvistgaard Kajhøj *	6	Table 2	Definition of tSB should be "Initiation of blastulation (first frame in which the blastocoel is visible)" (table says "blastocyst").	The reviewer is correct, this was corrected in the manuscript.
Evelyn Cottell *	6	Table 2	Adapted table 2 "Nomenclature of morphokinetics" from consensus paper 2011?- Should it not be Ciray 2014 paper? Important to define t0 in Table (time of IVF insemination or mid time of ICSI) and also discuss / debate the use of this start point, as this has likely contributed to discrepancies in literature regarding TL data. As the time of insemination can vary widely from clinic to clinic, this start point is likely not to be the best one when trying to standardise morphokinetic assessment (Kaser and Racowsky (2014) discuss this issue). Also, time of fertilisation is likely to be dependent on time of hCG administration. When assessing morphokinetics of embryo development, it is worth considering using a different start point- PN faded as reported by Fishel / Campbell group or even the first cytokinesis (as suggested by Kaser and Racowsky, 2014. Under "Dynamic events" Missing time of syngamy (Psyn) before "Not mentioned" : defined as the time from PN disappearance (when PN can no longer be seen) to the first cytokinesis (when the furrows of division are visualized) reference: Athayde Wirka et al., 2014	The changes were done, and the reference was added to the manuscript as suggested by the reviewer
Evelyn Cottell *	6	Table 2	Table 2 Replace "dynamic events" by Dynamic Intervals"	As Blastocoele compaction is not an interval, the name was changed to 'Dynamic events and time intervals'
Evelyn Cottell *	7	Table 3	Table 3 Add PN size and dynamics Otsuki et al 2017; tn? Add Wong, Conaghan refs to duration of 2 cell and 3 cell. Add Fishel 2018 ref to t(SB) and add tB-tSB interval, with supporting ref to RBMonline and Live Birth as Prediction/endpoint	References were added as suggested by the reviewer.
Danilo Cimadomo Laura Rienzi	7	Table 3	Both the studies reporting an association (Table 3) and not reporting an association with IVF outcomes (absent from this version) should be shown in a Figure or Table, like Pennetta et al did in their review based on the association between time lapse parameters and embryo ploidy. Similarly, also in this manuscript the association	Studies not reporting an association are already included in the table. In the text, there is now a comment to underline the fact that the comparison

			between embryo ploidy and time lapse parameters deserves a Figure or a Table. Moreover, the absolute number, study design, patient population and statistical analyses conducted should be clearly stated in the Table/Figure for each mentioned study. Please clearly underline also the absence of reproducibility across different studies in the identification of the Time Lapse variables putatively associated with IVF clinical outcomes.	between the studies in difficult due to variable methodologies. Adding another table is not feasible, and outside the scope of this recommendations document.
Gemma Arroyo	7-8	Table 3	Include the study published by Carrasco et al 2017, J Assist Reprod Genet 34:983-990. The authors have reported significant differences for t4, t7 and S3 between embryos that implanted and those that did not. This is an study that included 439 KID cycles .	The table was amended to include this reference
Markus Montag *	7-8	Table 3	It lists the biological and clinical significance on outcome. The problem I see here is, that the outcome is defined very different in the different publications that are cited. especially important for implantation (GS / FHB / Week of gestation). Further, there is a huge difference for a given MARKER if it is used for D3 or D5 transfers. So the definitions used in the different publications should be given, also in view of the excellent Cochrane session at ESHRE 2019 that clearly pointed to this problem.	The aim of this table is to give a general overview of existing data not to go to much into details.
Markus Montag *	7-8	Table 3	Include "Ratio of cell cycle durations": Cetinkaya et al., J Assist Reprod Genet. 2015 Jan;32(1):27-35. doi: 10.1007/s10815-014-0341-x. Epub 2014 Nov 5	The working group discussed this and decided not to include this parameter in the table.
Evelyn Cottell *	8	133	A section focusing on later embryo development is needed to discuss some of the related work already published. It would be sequential to the previous one ("Dynamic Embryo assessment during early cleavage stage") and it would focus on morula (Cottichio et al., 2019) and blastocyst stage (many others as described under the tables). This could include timing parameters and phenotypes.	The working group discussed this suggestion, however, decided not to add another section to the manuscript.
Danilo Cimadomo Laura Rienzi	8	135	"Screening of embryo ploidy status" is a misleading terminology. Please refer to it as "Testing" as suggested by The International Glossary on Infertility and Fertility Care, 2017 (Zegers-Hochschild et al, FS & HR, 2017).	The reviewer is correct, this was corrected in the manuscript.
Evelyn Cottell *	8	134-37	Ref needed to support statement. Sentences are too general and need to be qualified.	We agree with the reviewer, this was adjusted in the manuscript.
Danilo Cimadomo Laura Rienzi	8	137-139	The following statement should be revised by including also evidences in favor of PGT-A "However, PGT-A is not permitted in some countries, and there remains doubt regarding its cost-effectiveness and clinical relevance (Griffin and Ogur, 2018)". Please better refer to committee opinions (e.g. the American position of the Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology published in Fertil Steril 2018; the Canadian position of the Society of Obstetricians and Gynecologists of Canada published in the J Obstet Gynaecol Can 2015; the view of several experts in the field published in Molecular	We agree with the reviewer, this was adjusted in the manuscript. The suggested references were added to the manuscript.

			Human Reprod in 2016 by Sermon et al; the debate published by Fertil Steril in 2018, whose first author is Rosenwarks). Moreover, although it is hard (if not impossible) conducting a cost-effectiveness analysis universally-valid worldwide in different socio-economic backgrounds and settings, we are aware of at least three studies published to date and based on the Austalian, Italian and American scenarios (Lee et al, Aust N Z J Obstet Gynaecol, 2019; Somigliana et al, Fertil Steril, 2019; Neal et al, Fertil Steril, 2018). These papers should be mentioned.	
Evelyn Cottell *	8	150	Suggest title: Dynamic Embryo Assessment and ploidy status Very important to add a paragraph discussing the possibility that a combination of ploidy status with morphokinetic analysis may help to select embryos with highest potential to implant/ form Live birth. (Or the potential to identify the most suitable blastocysts for biopsy, using TLT data has not been addressed). Ref Rocafort et al. JARG. 2018 Sep;35(9):1573-1583. "Automated TLI combined with PGS is a useful prognostic tool to identify euploid embryos with the highest potential for implantation and pregnancy. Furthermore, these results provide evidence that a healthy pregnancy does not only depend upon normal chromosomal status". Cytoplasmic health (possibly reflected by morphokinetics) vs nuclear health is an important consideration and one potential reference which discusses this aspect is Meldrum, 2016 Fertil and Steril Vol. 105, No. 3, March 0015-0282	We agree with the reviewer, this was adjusted in the manuscript.
Evelyn Cottell *	9	152	Suggest rephrasing to …." tool for teaching embryology and standardizing assessment" Standardising assessment is an important requirement to teaching	Thank you very much for this suggestion, which was adopted in the text.
Evelyn Cottell *	9	153	Add "morphology assessment and" before morphokinetics and using "dynamic events" instead of morphokinetics (to be inclusive of TLT-related phenotypes) – see below: "….incubator to record their morphology assessment and dynamic events,.."	Thank you very much for this suggestion, which was adopted in the text.
Evelyn Cottell *	9	155	Suggest adding "standard morphology assessments" after example, and adding "normal and" before different cleavage patterns - see below: "…examples of standard morphology assessment and examples of normal and different cleavage patterns can easily…"	Thank you very much for this suggestion, which was adopted in the text.
Evelyn Cottell *	9	164	Ref needed for intra and inter-op variability with traditional evaluation -could use Sundvall	Thank you very much for this suggestion, which was adopted in the text.
Evelyn Cottell *	9	181-182	Storr et al., 2017 is related to traditional Day 5 morphology grading. This is not the correct reference. Perhaps it should be their 2015 publication on morphokinetics?	Thank you very much for this suggestion, which was adopted in the text.

Zuzana Holubcova	9	184	apart from time resolution also the Z resolution (adjustable in some TLT devices) effects the level of observable detail (e.g. PNB scoring)	Thank you for your comment. A relevant amendment was made.
Evelyn Cottell *	10	After 201	Very important. Add a subsection to discuss: TLT as a Quality Control Tool. TLT systems may offer important Quality Control tools (not just for assessment). Tools that could be used to monitor and audit the incubator environment i.e. Temp. & CO2 levels, recovery rates, door opening events, alarms etc have not been discussed and should be included as another important application of TLT systems	Thank you very much for your suggestion. Due to size constraints we cannot add a new section. However, a relevant reference (Wolf et al., 2013) was added elsewhere
Markus Montag *	10	202	This deserves an own heading 1.4 "Other benefits" and should mention safety (less handling of dishes) and include a reference to TL as early warning for wromng goings. Reference: Wolff et al., Hum Reprod. 2013 Jul;28(7):1776-82. doi: 10.1093/humrep/det102. Epub 2013 Apr 16.	Thank you very much for this suggestion. The suggested reference was included
2. Implications of TLT				
Zuzana Holubcova	10-13		I am missing comments about the importance of humidity	The literature on humidity is scarce and since the effect of humid incubators is related to osmolality. This parameter now is discussed there (as suggested by another reviewer).
Christopher Chen			culture dishes; the small media volumes may affect osmolality and the avoidance of bubbles formation that affect viewing embryos.	This is correct and is already discussed in the text. Air bubbles is general information, not to discuss in the context of culture conditions.
Gemma Arroyo	10	207	In "Implications of TLT" it would be necessary to include a subsection called "Influence on embryo morphokinetics". One of the parameters that can influence the morphokinetics of the embryo is the stimulation protocol. Although it is named in table 5, we think that is interesting to introduce "Patient-related factors" as a specific subsection.	The working group thinks it is sufficient that the stimulation protocol is mentioned in table 5, instead of as a separate paragraph.
Markus Montag *	11	252	Reduced oxygen was used much longer than only in the last 10 years. This statement should be changed as many have used this in routine much longer.	This phrase has been deleted.
Evelyn Cottell *	11	252	"low 02 conditions …have been replicated in the last 10 years" needs to be revised. No ref here and not correct (Many labs introduced low 02 20 years ago). More accurate to say "importance of low O2 conditions has gained recognition and been more widely applied, in the last 10 years could add CATT et al Human Reproduction, Vol. 15, (Suppl. 2), pp. 199-206, 2000	This phrase has been deleted.
Evelyn Cottell *	11	257	Key paper-Bontekoe S et al 2012 Cochrane review on low O2 should be referenced and discussed here	This publication has been added to this section.

Evelyn Cottell *	11	257	"Such benefits of lower oxygen levels will almost certainly apply to time-lapse incubators". Need to make a connection first that most TLSs recommend low 02 use and therefore the benefits of lower oxygen levels will almost certainly apply to these incubators. And suggest a comment that for many labs now facing replacement of old box incubators operating with high O2 levels, a TLS offers an opportunity to introduce both low 02 incubation with the other benefits/applications of Time-lapse technology	A comment was added that suppliers of TLT systems recommend the use of lower oxygen levels.
Evelyn Cottell *	12	281-4	Messaging from Kelley 2016 paper not clear	The message from the Kelley and Gardner paper (2016) has been rephrased.
Tine Qvistgaard Kajhøj *	12	287	"ECM" should be "ICM"	Thank you, this was indeed a typing error.
Evelyn Cottell *	12	287	ECM? Inner Cell Mass not Embryonic Cell Mass	Thank you, this was indeed a typing error.
Gemma Arroyo	12	288	The sentence "It is important to stress that with current time-lapse systems in principle ideal group culture is not possible due to the design of the culture dishes" is not correct. In fact in the next sentence there is a comment on the contrary sense.	On the balance of published evidence in animal models, the working group remains content with the view points in the manuscript.
Evelyn Cottell *	12	295	"However, a similar effect can be achieved with simply increasing the volume of individual droplets so that they have contact to each other". Sentence would be against manufacturer recommendations for dish preparation	A disclaimer has been added after this sentence to indicate that this may not be in compliance with some of the manufacturer recommendations for dish preparation.
Markus Montag *	12-14	296ff / 356f	Statements in lines 356-362 are somehow in contradiction to earlier statement on importance of distance between wells (lines 296-298)	There is no contradiction here, 356-362 is the manufacturers information, lines 296-298 is the opinion of the working group.
Markus Montag *	12	299	Add to the para on humidity that the starting osmolality of the media used is of uttermost importance.	We agree with the reviewer, this was adjusted in the manuscript.
Evelyn Cottell *	12	299	Swain paper was on mouse embryos. Important to note mouse or human sources when discussing studies	This information has been added to the manuscript.
Evelyn Cottell *	12	After 306	Propose adding a paragraph on importance of Humidity on culture conditions and potential effects on TLTS, as this would tie in nicely with Swain work on Osmolality. Refs Fawzy et al . Fertil Steril® 2017; 108, (2) 0015-0282, also Morbeck papers ESHRE 2018	It was tried to elaborate on that topic. Fawzy et al. (2017) is included in the manuscript. Morbeck is a conference abstract, which is not an appropriate evidence-format.

Evelyn Cottell *	12	308	Please add “does not APPEAR TO affect osmolality. Conclusive summary statements cannot be made on the basis of 2 papers.	The reviewer is correct, this was corrected in the manuscript.
Evelyn Cottell *	12	After 310	Partly discussed in 242-244 but suggest a separate section: Under TLT and embryo culture: Impact of a less disturbed culture system Elaborate on this section to discuss a less disturbed culture environment,, potential benefits and existing literature. (Zhang, RBMonline 2010; McEvoy Hum Reprod 2016 ESHRE abstract. Minimizing embryo handling, environmental changes/fluctuation associated with taking embryos out of incubator for assessment, minimizing risk, stress (potentially)? It is probably one of the main agreed benefits that TLT offers.	Due to restrictions in chapter length it is not possible to extend all hypotheses. If this indeed needs further elaboration this should be done in the introduction since it is a rather general statement. Zhang et al. (2010) was not included on purpose due to the study design. The authors compared a poor approach (6 door openings) with a less poor one (4 door openings). No (or fewer) door openings would have been the proper control group. McEvoy et al was an abstract at ESHRE. Our strategy was to include original papers only (prospective in design if possible).
Evelyn Cottell *	12	After 314	One important implication of TLS that could be covered under section 2.1, after the O2 tension, is the more stable gas and temperature environments and faster recovery rates of TLTS compared to box / benchtop incubators. It is noted under Safety in 3.2, but it is more than just a safety consideration-it has implications of an added benefit. This to be included under section mentioned above: Impact of a less disturbed culture system	It was a conjoint decision of the working group not to include this information here in detail, only in the safety section.
Evelyn Cottell *	13	317	Suggest adding a new sentence before “importantly” This additional flexibility can potentially improve efficiency as it allows better planning and timing for specific tasks (i.e. fertilization check, embryo biopsy, etc.)	A change was made based on the comment; This flexibility can improve efficiency as it allows for better planning and timing of specific tasks (i.e. fertilisation check, embryo biopsy) and use of equipment (such as inverted microscopes).
Evelyn Cottell *	13	319	more information is available for “RANKING THEIR ORDER” and choosing which embryo/embryos to transfer/biopsy /cryopreserve	Change made based on the suggestion: more information is available with TLT for ranking and selecting embryos

Evelyn Cottell *	13	319	TLT systems also remove the common pressure to access "high ín demand" ICSI inverted microscopes, often used for embryo assessments in addition to micromanipulation techniques	A change was made based on the comment; This flexibility can improve efficiency as it allows for better planning and timing of specific tasks (i.e. fertilisation check, embryo biopsy) and use of equipment (such as inverted microscopes).
Evelyn Cottell *	13	319	Regarding ICSI cases, TLT systems ease the pressure on embryologists to sometimes observe the fertilization status very early in the following morning. It also provides security that key fertilisation events are not being missed for those cases that go into syngamy earlier than usual. Eg Campbell, A /Fischel paper showing a % number of ICSI PNs missed when only looking at 16+2hrs post insemination	This is covered by "providing the flexibility of reviewing developmental history at any appropriate time", but an addition has been made; This flexibility can improve efficiency as it allows for better planning and timing of specific tasks (i.e. fertilisation check, embryo biopsy) and use of equipment (such as inverted microscopes).
Evelyn Cottell *	13	320	Videos instead of film sequences?	This was adjusted in the manuscript.
Evelyn Cottell *	13	After 324	"It may be wise to proactively develop strategies to manage any effect on laboratory productivity", "including facilitation of sufficient time and resources to introduce and train embryology staff, so that TL systems can be optimized". suggest adding last	A clarification was made: It may be wise to proactively develop strategies to ensure the availability of sufficient resources during the introduction and training of staff and to manage any effect on laboratory productivity.
Zuzana Holubcova	13	330	In our experience, the subjectivity can be at least partially mitigated when consensus is sought amongst different observers instead of limiting the number of evaluators as seen in some publications	The second opinion refers to a general second opinion that could likewise be needed during traditional embryo grading. Subjectivity is discussed in chapter 1.3 (Quality control)
Evelyn Cottell *	13	331	..will be able to implement more "CONFIDENTLY" and incisively, a deselection and ranking..	This was adjusted in the manuscript.
Christopher Chen	13	341	Staff training - Authors may want to mention importance of proper preparation of TLT	Proper preparation is indeed important. The working group is not sure what the reviewer is indicating.
Evelyn Cottell *	13	332-3	Change "good quality" to "good and fair" and "for any remaining embryos" to "for any remaining poor quality embryos"	This was adjusted in the manuscript.

Evelyn Cottell *	13	After 333	Another potential impact on workflow/lab dynamic could be related to improved communication between lab and physicians/nurses (cross-functional) when reporting results related to embryo development/assessment.	Good point, addition made: It is important to inform non-laboratory staff of the new routines concerning assessment and culture. TLT can also here be used to increase understanding of embryo development but also as an important aid in making embryo assessments more descriptive, hence facilitating cross-functional communication.
Evelyn Cottell *	13	334	Policy: This whole paragraph is confusing, what are authors trying to say? Message is not clear here.	A clarification was made: When implementing a TLT approach, it is essential that clinics perform a detailed analysis to develop a tailored policy for its use to be implemented in case the availability of TLT systems is limited.
3. How to introduce TLT				
Zuzana Holubcova	14	362	TL dishes differ in diameter in the bottom of well – conic shape can create undesirable shadow during imaging (some features can´t be reliably evaluated). Another factor to consider when purchasing TLT devise is gas supply available at the clinic – premix bomb vs. gas mixing at site	Thank you for your suggestion. We added the type of gas needed for each TLT in the table 4.
Gemma Arroyo	14	369	Include the reference of Carrasco et al, 2017 after the last statement. In this paper the authors argue the algorithm used must be designed in accordance with the relevant parameters specific to each centre and propose an strategy to implement a morphokinetic model for embryo selection in the laboratory based on 1- measure morphokinetic parameters, 2-identify relevant parameters, create own algorithm and 4-incorporate the algorithm for clinical use in the corresponding SOP.	Thank you for your comment, but in the algorithm is discussed in detail in section 3.3 "Morphokinetic algorithms for embryo selection"
Evelyn Cottell *	14	363	..factors „influencing“ a decision.. rather than “factors suggesting a decision”	Thank you for your suggestion. We have modified the sentence.
Evelyn Cottell *	14	367	The algorithm described by Conaghan is no longer available and has been updated (Aparicio-Ruiz 2019 Hum Reprod 34(1)i72 Also, Table 4, Geri is F and G, correct Geri doesn’t offer single culture.	Thank you for your comment. We have deleted group culture.
Zuzana Holubcova	15-16	Table 4	why are different TLT systems codes and not named? Any particular reason?	The devices were blinded to ensure that the paper would be objective and

				could not be interpreted as ESHRE giving preference to a certain system.
Philippe Terriou	15-16	Table 4	Table 4. After line “Dry or humid culture system”, add line: “pH monitoring” (pH monitoring is available in system E)	Thank you for your suggestion. We added a line with pH monitoring in the table 4.
Christopher Chen	15-16	Table 4	Culture environment – temperature, CO2 and 02 were mentioned. Author may want to mention dry and humid TLT incubator systems.	Dry or humid culture system is mentioned in the second part of the table.
Markus Montag *	15-16	Table 4	Other important/practical information include: possibility to integrate with EMR systems	Thank you for your suggestion. We added this information in the table 4.
Markus Montag *	15-16	Table 4	Define what is meant by “Remote Control”, as this can be very different solutions / options.	Thank you for your comment. We clarified it, as suggested.
Tine Qvistgaard Kajhøj *	15-16	Table 4	Specifications (illumination) is not correct for system B (Primo Vision); the wavelength used is 590nm (amber). See: https://www.vitrolife.com/products/time-lapse-systems/primo-vision-time-lapse-system/	Thank you for your correction. We changed the wavelength to 590nm.
Tine Qvistgaard Kajhøj *	15-16	Table 4	Specifications (time of light exposure) for system D (EmbryoScope): note that total value (seconds /day /embryo) depends on settings for both number of focal planes and interval of image acquisition.	Thank you for your comment, which is valid, however, not relevant to the table.
Tine Qvistgaard Kajhøj *	15-16	Table 4	Cost (general) for system C (EmbryoScope+): labels are provided without extra charge as part of the service package.	Thank you for your comment. We have deleted the labels as an extra cost.
Tine Qvistgaard Kajhøj *	15-16	Table 4	Impact of compartment failure: it should be noted that for systems C and D (EmbryoScope+ and EmbryoScope) there is a separation between incubation and computer meaning that computer system failure will not affect incubation system.	Thank you for your comment. We have inserted this information.
Evelyn Cottell *	15-16	Table 4	For “Impact of compartment failure”, it only indicates the sequence of failure of temperature. Failure of camera systems may also lead to different outcomes within different TLT systems, and should be incorporated into table	Thank you for your suggestion. We added this information in the table 4.
Evelyn Cottell *	15-16	Table 4	All currently available systems are described as System A to G: please add a footnote which system from which company is referred to, and at a link to the relevant website/user manual for each System. As the different systems continuously change, it is also important to clarify the exact date when this information was obtained.	The devices were blinded to ensure that the paper would be objective and could not be interpreted as ESHRE giving preference to a certain system. We added the date, as suggested.
Evelyn Cottell *	17	406	Add “stability” after “Culture Environment”	This was added in the manuscript.

Markus Montag *	18	416ff	Should include the paper by Petersen et al., Hum Reprod. 2016 Oct;31(10):2231-44. Doi: 10.1093/humrep/dew188. Epub 2016 Sep 8	This reference was included in the manuscript, as suggested by the reviewer.
Evelyn Cottell *	18	418	Correction: The first publication of an algorithm predicting development to the blastocyst stage was Wong 2010, 28 (10) NatBiotech which was subsequently validated clinically by Conaghan et al 2013 Fertil Steril, 100 (2) 0015-0282. Wongs publication was closely followed in 2011 by Meseguer et al, with validation, adaption and improvements (Basile etc …)	This was adjusted in the manuscript.
Evelyn Cottell *	18	421	..tendency towards better clinical outcomes was concluded when "an algorithm" was used (Pribenszky, et al., 2017)- Change to "algorithms and cleavage anomalies were used". (5 RCTs were assessed, all using variations of/ additions to Meseguer 2011 algorithm)	This was adjusted in the manuscript.
Evelyn Cottell *	18	421	"although concerns were raised on the reproducibility of the results (Barrie, et al., 2017, Freour, et al., 2015, Kirkegaard, et al., 2015, 422 Neyer, et al., 2015)". Sentence requires editing as the concerns raised in these papers relate to reproducibility of Meseguer 2011 algorithm I believe and not the reproducibility of the 2017 Pribenszky paper	This was adjusted in the manuscript.
Danilo Cimadomo Laura Rienzi	18	424-425	"Each and every lab introducing TLT should do their proper validation, as algorithms could be influenced by several confounding factors (see Table 5)". This suggestion is misleading, since this might not be feasible in every clinic. Well-designed, well-controlled and powered studies are needed to build or validate an algorithm that should then also undergo a peer-reviewed publication process. To date, not even reference laboratories worldwide were able to consistently validate an algorithm on independent datasets. Therefore, a statement such this might generate false expectations and flawed data, particularly if the algorithms are not produced based on appropriate sample size or post-hoc power analyses certifying the value of each variable introduced (that must be also corrected for putative confounders). In general, we doubt that a predictive model of whichever IVF outcome could be solely identified within each laboratory (where the in vitro culture condition do also change with time); conversely, it should be rather established by reference clinics and then proven reproducible and consistent across different datasets from other laboratories. In our opinion, an ESHRE recommendation paper should not suggest such a workflow, which does not follow the premises of an evidence-based medicine.	In a way we can agree with the reviewer, however, a validation is nonetheless needed. We amended the sentence: Each and every lab introducing TLT should, if possible, do their proper validation based on appropriate sample size or post-hoc power analyses certifying the value of each variable introduced and corrected for putative confounders, as algorithms could be influenced by several confounding factors (see Table 5)
Zuzana Holubcova	18	425	it should be emphasized that comparison of preimplantation development under different conditions should be done on siblings oocytes/embryos, sadly, people often present comparison of (non)matched IVF cycles. When evaluating the data, it is	This was amended in the manuscript (also with the comment of another reviewer).

			important to check whether the distribution of data is homogenous. In a small dataset containing extreme values, the median is more representative than average (+- SD) .	
Gemma Arroyo	18	425	Include the reference of Carrasco et al, 2017 after the last statement.	This reference was included in the manuscript, as suggested by the reviewer.
Markus Montag *	19	Table 5	Several important confoundimng factors are not mentioned: Oxygen tension / PÜloidy status / Culture media / Handling protocols / Gas source / Sperm factor (Baart et al.)	These factors were not mentioned in this table to avoid repetition. Oxygen tension and culture media are covered in section 2.1, embryo ploidy status is covered in section 1.1, sperm factor is included in table 5. Unfortunately, the reviewer provided to little information to be able to find the correct publication by Baart et al.
Evelyn Cottell *	19	Table 5	Fertilisation technique /influence on algorithms - new paper published online in JARG by Inoue, Taketo, July 2019 (https://doi.org/10.1007/s10815-019-01521-x) worth including, looking at differences in hatching patterns between ICSI and IVF (oral presentation at ESHRE), confirming Kirkegaard 2013	This reference was included in the manuscript, as suggested by the reviewer.
4. Evidence of a clinical benefit of TLT				
Evelyn Cottell *	19	429-430	These sentences should be rephrased or removed altogether. TLT is a Technology allowing a more continuous monitoring of embryos together with an enhanced stability of culture environment. Using an algorithm versus standard morphological assessment to choose an embryo for transfer is the "intervention" that should be validated with user-defined laboratory and clinical endpoints. Once any TL incubator has been validated operationally (like any other incubator) it can be implemented into routine clinical practise?! Any subsequent intervention e.g. a selection algorithm/software, should indeed be validated, scientifically and clinically. Prior to this section, the manuscript has presented many applications and implications of TLT, which can improve routine clinical practise in the IVF lab- e.g. standardizing assessments; counselling patients who show poor developmental patterns not previously detected with conventional microscopy; improving workflow; allowing remote assessment. This would be a more appropriate introduction to section 4- "Evidence of a Clinical benefit", to precede sentence "However, a clear increase of IVF success rates with the use of TLT remains to be firmly proven". ESHRE guidelines should be factual and fair and avoid referring to rhetoric like "TLT should be implemented in routine clinical practice only	The manuscript recognises the benefits of time-lapse technology, however, the working group does acknowledges that the evidence of a clinical benefit is not there yet.

			after stringent testing that demonstrates a proven benefit for patients" There are clear clinical results now by many authors, to show reduced implantation rates with embryos showing abnormal cleavage patterns.	
Sarah Armstrong Allan Pacey Cindy Farquhar	20	438	The report devotes a paragraph to the systematic review by Pribenszky et al 2017, and is described as revealing a significantly higher ongoing pregnancy and live birth rate and a lower early pregnancy loss when using TLT compared to conventional embryo incubation and assessment. As Cochrane authors we have a number of concerns about this particular review, which we published in RBM online (reference below). The main concerns are that authors of the review combined trials with differing intervention and control arms, and omitted certain eligible trials that were included in our Cochrane review. The review incorrectly included a prospective cohort study as a randomised study, and the data were not analysed on an intention to treat basis. A concern over the equipoise of the authors is also raised given they are employed by Vitrolife. Armstrong S, Bhide P, Jordan V, Pacey A, Farquhar C. Time lapse systems for ART. Reproductive Biomedicine Online 2017. Doi: https://doi.org/10.1016/j.rbmo.2017.12.012	Thank you for your comment. We do understand your reasoning. Nevertheless, we must be impartial and cite papers that sometime present contrasting results. To address your comment, we have added a phrase "Conversely, one meta-analysis, with a different methodological approach, has suggested a beneficial effect of TLT.....". In addition, all meta-analyses are new described in the same paragraph.
Evelyn Cottell *	20	438-442	An explanation is needed as to why the conclusions of the Pribenzky MAL 2017 are different from the Chen 2017, Armstrong 2018 and Armstrong 2019 reviews. It is not enough to just present the results of the MALs.	Differences mainly due to different methodological approach. This is now mentioned.
Evelyn Cottell *	20	443	Suggest changing "a significant cause" to "a significant confounder". There are many other confounders to TLT clinical efficacy controversy, which should also be listed and summarised here e.g. (i) Different study designs e.g. Day 2, 3, 5, 6 TF's (Park study only Day 2 TF's- so the full benefit of extended undisturbed culture was not assessed); (ii) different endpoints; (iii) wide array of morpho-kinetic times and intervals assessed; (iv) different interpretations/definitions of a specific feature e.g. direct cleavage; (v) inter and intra-operator variations in when a key developmental stage is annotated and inter and intra-operator variations in std morphological assessment; (vi) general grouping together of an abnormal phenotype, but not specifying at what cell stage it was observed e.g. multi-nucleation (vii) Table 5's Patient related confounders (viii) Table 5's Gamete, embryo and Lab related factors (ix) the different types of TLT systems used in different studies	The paragraph has been modified to provide this information.
Markus Montag *	20	449	The Mascarenhas paper shows a positive effect for TL after adjustment for age (patients in one system were older) – such info is important!	The suggested sentence has been included in the manuscript.
Zuzana Holubcova	20	457	(de)selectin vs. ranking of embryos. Some embryos with developmental ussues can still produce live births. Until the strong evidence about morphokinetic parameters is provided, strict deselection should be avoided.	Thank you for your comment. The sentence you refer to states that "...it is reasonable to assume that, compared with static observations,

				continuous embryo monitoring in an undisturbed environment will offer more information into embryo development…". The issue of selection and deselection is not addressed here.
Evelyn Cottell *	20	461	"RCTS with adequate design and sufficient power" are for sure needed; but would replace "adequate" with "well considered" design. Calling for RCTS with Live birth outcomes is extremely challenging. Ongoing clinical pregnancy as a very good surrogate endpoint to Live birth rate should also be considered. Also, the value of Real world data should be included	This was changed to "...more well-designed and sufficiently powered RCTs,..."
5. Current state of TLT				
Tine Qvistgaard Kajhøj *	20	464-465	TLT became commercially available for human IVF in 2009 (both EmbryoScope and Primo Vision)	The reviewer is correct, this was corrected in the manuscript.
Evelyn Cottell *	21	After 485	Insightful survey presented at ESHRE 2018, O-228- worthy of discussing here. Differences in time-lapse practice: is a consensus on standards needed? Cristina Hickman UK survey on clinics using a common TL system and how its use varies widely between IVF clinics, particularly with regards to patient and cross-department involvement, communication, embryo selection and how process-efficiencies are optimized.	The suggested survey is a conference abstract, which is not the type of evidence to be used in recommendations documents.
6. Current and future research perspectives				
Markus Montag *	21	486ff	It is stated that TL is in its infancy. This is hard to believe given the number of abstracts at ESHRE and other conferences and the huge number of peer-reviewed publications.	The sentence is slightly modified: "In comparison with rapid technical development of TLT and combination with other technologies in basic research of cell biology, the TLT in clinical embryology remains in its infancy…"
Evelyn Cottell *	21	505	Replace "embryos" with "blastocysts", to indicate clearly that this AI study looked at full imaging all way to blast	This was adjusted in the manuscript.
Evelyn Cottell *	21	506	TLT and AI: Consider including the work of Khosravi et al., npj Digital Medicine (2019)2:21 ; https://doi.org/10.1038/s41746-019-0096-y	The reference suggested by the reviewer was added to the manuscript.
Markus Montag *	22	519	How can a review from 2013 be indicated to sum-up papers published after 2013? Better phrasing: New observations have been revealed with TL (Chen (2013)) and summarize findings from publications from following years. Publications/parameters in this section should be moved up to/included in Table 3.	Rephrased: The observation of such crucial developmental events in real time has revealed a number of new

				parameters that have been introduced into embryology (listed in Table 3).
Zuzana Holubcova	22	522	I am missing a few important references here: 1) Holubcova et al 2015 – 1st fluorescence live imaging of human oocyte maturation (instead of Zeielinska 2015 which was a follow-up study), Hashimoto et al 2016 – fluorescence live imaging of human embryos; 2) Strnad et al 2016 – "in toto imaging" – fluoresce light sheet imaging of developing mouse embryos; 3) Chavez at al 2012 – a combination of the time lapse + CGH of individual blastomeres + immunofluorescence in human embryos; 4) Daughtry et al - a combination of time-lapse + single-cell sequencing of individual blastomeres + immunofluorescence in primate embryos	All references suggested by the reviewer were added to the manuscript.
Evelyn Cottell *	22	546	"To date, despite significant research effort, no single reliable biomarker of embryo quality has been identified" what is the point of this sentence? Can this sentence be changed to say that "we are continuously searching for improved biomarkers of embryo viability, to reflect the complexity of pre-implantation development?"	The suggested rephrasing was partially adapted in the document.
7. How to share TLT data with patients				
Kelly Tilleman	22	550	This section is far from supported by scientific evidence and therefore it is in contrast with the rest of the document. Although I realize that some guidance on the counselling of patients does add value to the recommendation, the fact that this comprises of 6 paragraphs is a bit over the top. The example of the short explanation is truly redundant. In my opinion clinics who use TLT are quite experienced in counselling of patients. Maybe this section can be reduced or at least re-written based on scientific evidence as the rest of the content.	Thank you for your comment. Yes, to provide a report or not to the patients from clinics with TLT is lacking scientific evidence. However, does not mean that the clinic has a procedure in place, and it is one of the questions raised by the users and the patients. Additionally, it is far away from the reality that the clinics who use TLT are quite experienced, on the contrary, there are many clinics (personal experience visiting more than 200 clinics all around the globe) that have TLT and have no idea how to take profit of them. This section is the shortest of the paper and we have included the only existing reference related with the report.

Markus Montag *	22-23	550 ff	I miss published data to support this. But there are published data – see below: Blomquis et al: http://www.alliedacademies.org/articles/patients-experience-of-viewing-timelapse-sequences-a-prospective-surveystudy-6312.html	Thank you for the suggestion, this reference was included in the manuscript.
Evelyn Cottell *	23	592	Replace “proper” indication by “most suitable”? or “an indication that might benefit most” from TLT	This was adjusted in the manuscript.
Evelyn Cottell *	23	593	“lacks a convincing evidence base to prove any clinical efficacy” . However, it may provide otherwise unknown information on embryo quality and development and may help to counsel couples in decisions making regarding further treatment, donor egg use, adoption etc.	This was added to the manuscript.
Evelyn Cottell *	22-23	551-593	This section is well written but perhaps a little long and requires some editing. Also, it makes no reference to provision of video to patients, which is common practice in many centers and avoids all these “paper report issues” . There is increasing evidence that patients feel more engaged with the treatment process and that the IVF is more transparent when videos are provided. Ref Bui, D et al Hum Reprod 2018; 33 (suppl 1)	We have included two references that address your concerns about video provision.
Sarah Armstrong Allan Pacey Cindy Farquhar	23	595	If this statement is meant for patients, then it is far too clinical in its language and difficult to decipher.	This section is not intended for patients, but rather for fertility practitioners.
Evelyn Cottell *	24	602-603	“May, in the future provide a valid adjunct to select/deselect embryos ”. I think it is doing so to some degree at the moment. Would change to …”in the future improve its power as an adjunctive test to select embryos with the highest implantation potential/deselect embryos with lowest implantation potential”	This was added to the manuscript.
8. Summary/conclusions				
Guido Pennings	24	618	I do not understand how the authors arrive at their conclusions. They very clearly state that there is no evidence of any clinical benefit, yet they conclude that the technique is here to stay, is very promising and should be mastered by all embryologists. Such strong conclusions in support of a technique that at present is little more than another add-on should have a much stronger foundation. If these are to be ESHRE guidelines the conclusions should be much more prudent.	Thank you for your comment, in clinical IVF (and not only), a technology does not need to have a clinical benefit (yet) to stay. The downstream implications of TLT that justify its permanent adoption are clearly described in the manuscript, including (but not limited to) detection of aberrant developmental phenomena incompatible with implantation and/or viability (e.g. late formation of a 3rd PN

				or direct cleavage of a 2PN into three blastomeres), time and staff management, teaching and training, quality control and research.
Evelyn Cottell *	24	619	Suggest “monitoring” instead of “observation”: Continuous embryo monitoring has allowed…”	Thank you very much for this suggestion, which was adopted in the text.
Evelyn Cottell *	24	623	Ref?	In a summary section we prefer not to include references
Evelyn Cottell *	24	605-632	626: “make patients aware” Excellent Summary and conclusion	Thank you very much for this suggestion, which was adopted in the text.