The Istanbul Consensus update: a revised ESHRE/ALPHA consensus on oocyte and embryo static and dynamic morphological assessment

Update 2024

The Working Group on the update of the ESHRE/ALPHA Istanbul Consensus

REVIEW REPORT

November 2024

ALPHA
SCIENTISTS IN REPRODUCTIVE MEDICINE

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The draft of "The Istanbul Consensus update: a revised ESHRE/ALPHA consensus on oocyte and embryo static and dynamic morphological assessment" and an invitation to participate in the stakeholder review were published on the ESHRE website between 31 May and 17 June 2024.

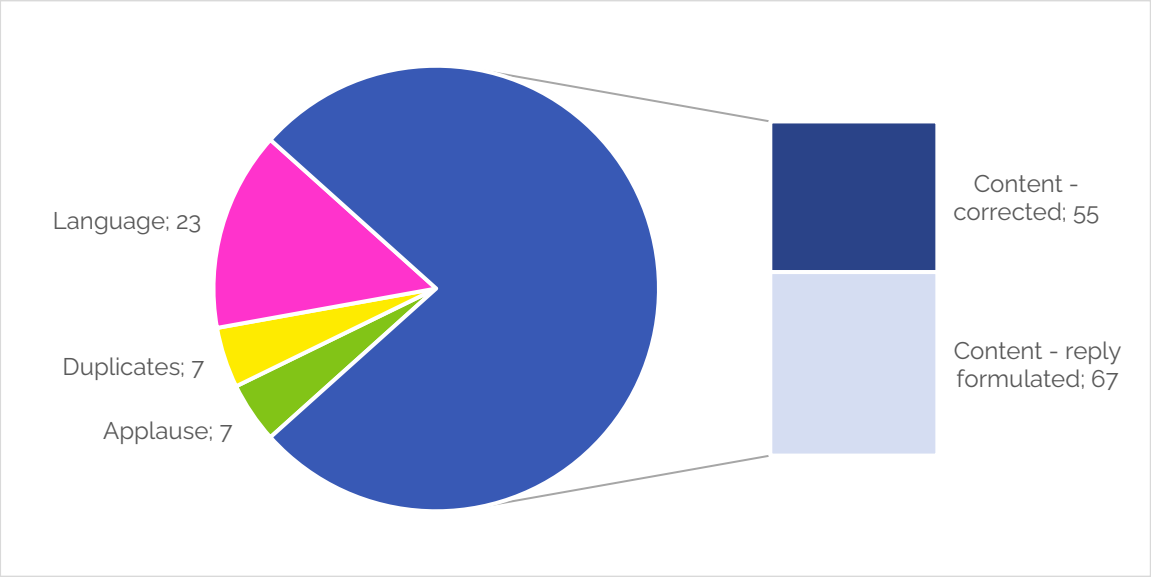
This report summarizes all reviewers, their comments and the reply of the Working Group on the update of the ESHRE/ALPHA Istanbul Consensus and is published on the ESHRE website as an annex to the guideline.

During the stakeholder review, a total of 159 comments were received from 26 reviewers.

The comments were focussed on the content of the consensus statement (n=122), language and style (n=23 comments), were remarks that did not require a reply (n=7) or duplicate comments (n=7). All comments to the language and format were checked and corrected where relevant.

The comments to the content of the paper were assessed by the working group and where relevant, adaptations were made in the consensus paper (n=55; 45.0%). For a number of comments, the working group considered them outside the scope of the consensus paper, or after discussion did not consider it appropriate or relevant to make a change to the text (n= 67; 54.9%). For these comments, a reply was formulated

Overview of comments per type of comment. For the comments to the content of the consensus statement, the graph indicated the proportion where a correction was made to the text



Experts that participated in the stakeholder review

The list of representatives of professional organization, and of individual experts that provided comments to the guideline are summarized below.

Reviewer	Country	Representative of
Munevver Serdarogullari	Cyprus	
Danilo Cimadomo	Italy	
Han Wei	China	
Jean-Léon Maitre	France	
Andrea Abdala	UAE	
Marco Sbarcia	Italy	
Gulcin Ozkara	Turkey	
Daniel Brison	UK	
Daniela Zuccarello	Italy	**Italian society of Human Genetics (SIGU)**
Jessica Eastick	Australia	
Iman Halvaei	Iran	
Liliana Ramos	The Netherlands	
Verena Nordhoff	Germany	
Patricia Fauque	France	
Klaus Wiemer	USA	**Fairtility LTD**
Puga Molina	Germany	
Zhongwei HUANG	Singapore	
Keerti Singh	Barbados	
Mario Sousa	Portugal	
Ashleigh Storr	New Zealand	
Dan Zang	Unknown	
Riddhi Marfatia	Australia	
sayaka Higo	Japan	
Maria carme Pons	Spain	
KARIMA DJERROUDIB	Algeria	
Etienne Van den Abbeel	Belgium	

Reviewer comments and replies

Reviewer	Page/ Line	Comment	Action / Reply
General			
Munevver Serdarogullari	Page 14 - Line 373	In table 2 instead of "/", it can be mentioned as N/R (not reported) or N/A not available	This was corrected in the text.
Munevver Serdarogullari	Page 23 - Line 581	In all text fertilization with "z" in this line it is with "s"	This was corrected in the text.
Danilo Cimadomo		I want to compliment the whole working group for their incredible effort in reviewing the literature on this topic with a sound methodology. This manuscript will be highly relevant to shape the clinical activity as well as Research in the field of clinical embryology worldwide in the coming years.	Thank you for your comment.
Danilo Cimadomo	Embryo priority support tool	Based on the many suggestions listed along the manuscript to define embryo priority for transfer, I think that it might be useful and highly appreciated if Alpha and ESHRE release on their websites an algorithm to support the embryologists to this end. This algorithm might include all the characteristics reported relevant for embryo competence and, through a stepwise sequence of questions about the characteristics of any given embryos, it can support clinical embryologists in their final ranking decision.	The working group think this is worthwhile suggestion. However, it is well beyond the scope of this manuscript and could be considered for future projects.
Daniel Brison		This is an excellent document, and I applaud the organization and hard work which has gone into producing it. The previous iteration was highly influential and I am confident that this one will be similar. Well done all involved. I am not involved in embryo grading and selection now, but have been very active recently in the area of outcomes from ART treatment using UK data, so I have just a few comments in that area.	Thank you for your comment.
Liliana Ramos	Introduction	My compliments to all the authors for such a great piece of work, which is very comprehensive and complete.	Thank you for your comment.
Liliana Ramos		Use of abbreviations I suggest to use a list of abbreviations/definitions as some terms can be less known or used it differently in some countries	A list of abbreviation will be published along with the final version of the document.
Liliana Ramos	Page 2- Line 12	term "sophisticated" is not appropriate in this context, but "enhanced" might be better	The term sophisticated has been removed.
Verena Nordhoff		Overall, I would like to thank the authors for this extremely valuable piece of work. It is very much appreciated!	Thank you for your comment.

Klaus Wiemer	AI	Dear Reviewer, The integration of AI with TLT is a new technology that has recently become available to the IVF community. The merger of these two techniques provides a continuous and extensive monitoring system and analysis of embryonic development. AI models evaluate embryos with accuracy, therefore predicting their viability and potential success, thus enhancing selection processes and improving IVF outcomes. This synergy minimizes invasive procedures and human error, ensuring a more stable environment for embryo development and significantly improves IVF outcomes, making treatments more effective and personalized for patients. Therefore, we hope that you will consider and incorporate the suggested comments and additional literature cited to update and improve the comprehensive review that this consensus represents. Thank you, Klaus E. Wiemer and team.	Thank you for your comment.
Puga Molina		Please add links to the corresponding tables and figures in the PDF format, to facilitate the reading. Also links to papers are useful. Thank you for your work.	Thank you for your comment. This has been done in the final version of the manuscript.
Zhongwei HUANG		Consideration of the use of the necessary variables need for static review versus TLT emphasized characteristics to review The entire consensus relied a lot on the latest morphokinetic data from TLT embryos. Using these data how does it translate to IVF laboratories which does not use TLT at all? Unless TLT is used for ALL embryos, are the parameters reviewed in the consensus applied to all embryos i.e. IVF labs using only static single time point light microscopy review versus some IVF labs using ALL TLT versus some IVF labs using both? Suggestion: Is it feasible to have recommendations for – 1. List of mandatory criteria to assess for IVF labs with using only static single time point light microscopy 2. List of entire criteria to assess for IVF labs using ALL TLT 3. Special and priority use of TLT criteria for some IVF labs which cannot use TLT for all cases	The group believes that this manuscript has reasonably addressed the question of TLT vs static observations.
Keerti Singh		Recent references should be included. Some information which in my opinion was important for this document was not included. Kindly note the same in my review comments as well as in comment boxes in the pdf document.	The working group needs further clarification about what recent references the reviewer is referring to. In some chapters the working group decided to refer to the original publications, and the majority of the relevant recent papers were included where relevant.
Ashleigh Storr		This is a wonderfully written summary of the evidence we currently have available. Well done to the working group for putting this together! The summary at the end of each section and the tables are very useful.	Thank you for your comment
Ashleigh Storr	Grading subjectivity	Is there a way to provide additional insights into improving the subjectivity of embryo grading, and consequently, selection? While the manuscript offers valuable discussions on optimising timings, workflow, TLT, and AI, it falls short in suggesting practical steps for laboratories to actively enhance grading consistency, assuming the consensus group perceives this as achievable. Given the current inconsistency among labs in grading and selection practices, as indicated by the survey, and the potential lack of awareness regarding this issue, addressing these concerns could prevent the discard of potentially viable blastocysts.	Training plays a crucial role in reducing inter and intra observer variability in embryo assessment. But assessment of morphology through static observation is inherently subjective and this is very difficult to "improve".

Dan Zang		The Istanbul Consensus update for 2024 draft extensively reviewed literature and analyzed data, utilizing TLT technology to systematically assess the morphological features of embryos at different developmental stages. By consolidating and analyzing data from multiple centers, the study suggested revised evaluation standards and optimal timing. This research offers updated embryo evaluation criteria to IVF laboratories, improving the precision and uniformity of embryo selection and ultimately boosting IVF success rates. The incorporation of TLT technology enhances comprehension of embryonic development processes and provides supplementary assessment parameters.	Thank you for your comment
Dan Zang		Line 49, "perceived potential" in the context of embryo ranking should be elaborated to avoid any ambiguity	The working group replaced 'perceived' with 'estimated'
Dan Zang		Consensus on non-invasive methods for embryo assessment should be added, such as analyzing spent culture media for biomarkers that indicate embryo viability.	All considered experimental. Also, selection based on genetic analysis is not in the scope. This is a practical guideline aimed at a majority of practitioners that depend on morphology and morphokinetics.
Riddhi Marfatia	Embryo Growth	Guidelines towards embryo growth normal and unusual patterns, eg 12 cell embryo on Day 5, on day 7 4BB, or early blastocyst on Day 5 and Day 6, Day 7 expanded blastocyst	This has been covered via selection criteria on different days.
Maria Carme Pons		I like to thank the authors for their outstanding job, for adding information from TLT, and for introducing the idea of embryo ranking.	Thank you for your comment
Karima Djerroudib		Thanks for the working group for this complete and helpful revised consensus	Thank you for your comment
Introduction			
Keerti Singh	Page 2- Line 13-17	Regarding Morphokinetics based on time-lapse technology and continuous monitoring of embryos, please provide recent references after 2020	The working group consider that this is a very general reference for a general statement. They assume that this reference is appropriate and there is no need to add any other references.
Klaus Wiemer	Page 2 - Line 12	Insert highlighted text: In the past decade, the most significant advance in embryo assessment has been the introduction of sophisticated time-lapse microscopy technologies (TLT), and its associated AI algorithms.	The definition of TLT includes also computational and AI tools as illustrated in the ESHRE good practice recommendation paper on TL published in 2019
Zhongwei HUANG	Page 2 - Line 16	Can we change …embryo "viability" to "development" as not dividing cells does not equate to death unless there are obvious morphological changes under the microscope?	This was changed to embryo development in vitro
Keerti Singh	Page 2 - Line 11	Advance to be corrected as 'advancement'	This was corrected in the text
Dan Zang		Emphasizing the importance of standardization and calibration of equipment, terminology, and assessment criteria across laboratories would further highlight the significance of this update.	Added the following to line 37: This document will help re-establish standard terminology and assessment criteria across laboratories.

Etienne van den Abbeel		The gold standard for defining a successful treatment cycle outcome is the birth of ONE healthy child. Therefore single embryo transfer should be performed not only in the fresh cycle but also in the cryopreserved cycles avoiding at all cost multiple pregnancies. It is my belief that blastocyst transfer/extended culture is the best way to go . Therefore, It should be maybe stated in the document presented by the authors that ranking/grading human embryos should rather be seen as a de-selection tool especially when extended culture and blastocyst culture is performed , better discriminating between viable an non viable blastocysts. In this specific comments section I will indicate a couple of examples demonstrating some potential risks for "error" in selecting/de-selection the viable /non viable embryo.	The question of SET is relevant but has been addressed in the ESHRE guideline 2024. This manuscript is aimed to rank embryos rather than selecting and de-select embryos. This terminology has been specifically defined throughout the manuscript.
Chapter 1			
Marco Sbarcia		Have the committee taken in consideration in development time the sex of embryo? Several observations reported differences in time development of male versus female embryos.	A new section on the development time according to the sex of the embryo has been included in the text.
Gulcin Ozkara	Page 5- Line 111-113	"Culture medium type and oxygen tension have also been studied in relation to the timing of preimplantation developmental events, including a large analysis of over 10,000 embryos (Dietrich et al., 2020). This study compared two culture media and reported differences in the timing of compaction initiation but not the start of blastulation." The given reference is not true. In the study of Dietrich et al., 2020, ovarian stimulation protocols were compared in terms of embryo morphokinetics and the number of embryos are not as much as 10.000.	This statement has been removed from the text.
Iman Halvaei	Page 4- Line 98	T2 should be defined first in its appearance	This was corrected in the text.
Iman Halvaei	Page 4 to 6	Study limitations should be noted	The following statement was added to the text: "Although the studies are heterogeneous and drawing strong conclusions is difficult, TLT studies can help inform and optimise static assessment timing windows in the IVF laboratory."
Klaus Wiemer	Page 5 - Line 125-130	Insert: An AI platform was able to determine that morphokinetic timing events from tPNf to tEB for embryos that were diagnosed as euploids were less variable than aneuploid counterparts. In the present study aneuploid embryos had longer time intervals within their morphokinetic parameters than euploid embryos (reference below). Source: (S Sharma, A Doshi, C Bhatia, A Zepeda, A Brualla, C Hickman, P-741 Morphokinetic Goldilocks: assessing the morphokinetic range to identify embryos with the optimal chance of being euploid, Human Reproduction, Volume 38, Issue Supplement_1, June 2023, dead093.1060, https://doi.org/10.1093/humrep/dead093.1060)	The working group has included a meta-analysis (Bamford et al., 2023) about AI and morphokinetics and consider that this is sufficient as reference given the topic of this manuscript.
Puga Molina	Page 4 -	Line 96 line: change "that only the time to 2-cell (t2)" and delete from 98 Clarify that t2 is time to 2-cell in this line instead of line 98	This was corrected in the text

Zhongwei HUANG		Paternal and maternal ageing impacts on both gametes and resultant fertilization and embryological outcomes. It would be important to discuss in brief on the impact of parental ages on the gametes and how ageing impacts on the quality of the gametes, fertilization and embryological development. If the impact is significant especially in men and women with low number of gametes, the assessment of these gametes and subsequent embryos will be more vital to ensure that the couples do not undergo a transfer with high chance of failure or miscarriages. Importantly, it may guide the embryological team to monitor the development closer or prioritize the use of the TLT for these cases to determine the reproductive outcomes. Would the age effects be altered or improved with culture conditions/media and prioritize the use of TLT for monitoring of their developments to optimize outcomes in the poor prognosis patients – few gametes available for ART?	This manuscript is not intended to discuss the benefit of using TLT for different populations and the working group believes that this is outside of the scope of the paper.
Keerti Singh	page 3 - Line 57	Suggest include the word 'cleavage' after continues with cleavage a series of…	This was added in the text
Keerti Singh	Page 5 - Line 114-121	The role of oxidative stress can be included. References can be included on studies done with spent culture medium of individually cultured embryos. Are there any prospective RCTs done?	The WG gave some examples of intrinsic and extrinsic factors that can impact the embryos, although they believe that it is not the focus of the paper.
Keerti Singh	Page 5 - Line 128	Suggest – including information with references on unique negative phenomenon observed with time lapse systems "reverse cleavage"	This was detailed in the cleavage stage assessment chapter.
Keerti Singh	page 6 - Line 148	Advantage of more stable incubation conditions with TLT, without the need of opening the incubator doors	The group considered that the TLT benefits are outside of the scope of the paper. Thus, only brief statements about the impact of TLT on embryo development can be found in different sections of the manuscript (introduction and Chapter 1).
Keerti Singh	Page 7 - after line 149	Suggest including - Information on application of AI in ART and use of deep learning models may improve effectiveness of time-lapse systems. AI also offers capability for automation and standardization of embryo selection	This was added in the recommendations for future research. All ESHRE/ALPHA papers are updated typically every 4 years in case of the publication of new relevant evidence
Mario Sousa	Page 3 Line 59	The correct biological terms should be better explained. Fertilization refers to sperm-oocyte fusion (syngamy); Zygote refers to a normal fertilized oocyte, displaying 2PN and 2PB; the 2PN need to become juxtaposed (there is no karyogamy in humans); the nuclear envelopes of the juxtapose PN break into multiple fragments (PN break-down stage), which, morphologically, is observed as a progressive fading of the surface of the 2PN; the mixture of gamete chromosomes should not be called syngamy.	The word syngamy has been removed.
Ashleigh Storr	Page 5 - Line 142	It might be beneficial to mention some the evidence for (e.g. https://doi.org/10.1093/humrep/deac233) and against (e.g. 10.1016/S0140-6736(23)00168-X) uninterrupted culture of embryos. It's not just the algorithms that have been introduced with TLT, but also the incubators themselves which could have an impact on embryo development?	The benefit of TLT is already described in the introduction and the last chapter of the manuscript. Please see the introduction section.

Karima Djerroudib	Page 6 - Line 151	In addition of observational timing, especially at transfer/vitrification day (day 2-3-5), we can consider that there is a second timing "decisional timing". in several laboratory, Decisional timing is not always the same of observational one.Example of two blastocysts at day 5, which is the better? 1-Expanded blastocyst at 116h => expanded blastocyst at 120h 2-Full blastocyst at 116h => hatched blastocyst at 120h (time of vit/transfer)	This has been clarified in the text. Please refer to table 1 of the manuscript.
Iman Halvaei	Page 5 - Line 107	The effects of ovarian stimulation of embryo development are noted briefly. I recommend more discussion in this regard	The working group believes that this impacting factor has been sufficiently described in the chapter, taking into consideration the main topic of the paper and the length of the manuscript.
Chapter 2			
Munevver Serdarogullari	Page 11- Line279	RFs has been used as a abbreviation but in other parts it has been used as"refractile bodies". It can be all the same in the whole document	This was corrected in the text.
Munevver Serdarogullari	Page 17 - Line 436	Nucleolar precursor bodies: has ":" other do not have, it can be removed.	This was corrected in the text.
Iman Halvaei	Page 9 - Line 233-243	Clinical outcomes of large PB should be noted	A recent reference has been added: Liu Y, et al., 2024. Live birth derived from a markedly large polar body oocyte: a rare case report. Zygote. 2024 Apr;32(2):170-174. doi: 10.1017/S0967199424000054.
Iman Halvaei		Please refer to a last meta-analysis in each section regarding oocyte abnormality	The authors cited the original studies in the text. The reviewer did not specify which meta-analysis is being referred to. A possible recent meta-analysis: Nikiforov D, et al., 2022. Human Oocyte Morphology and Outcomes of Infertility Treatment: a Systematic Review. However, the working group believes that the citation of original paper is sufficient.
Liliana Ramos	Page 8 - Line 178	Which type of oocytes (except for giant oocytes or too small oocytes) should never be used for IVF/ICSI? If these the only ones to be discarded at all times? I suggest to mention this in the text and not only in the table.	In the various paragraphs on the different morphological characteristics of oocytes, it is explained whether their clinical use is recommended or not. Stating this information again in the introductory cap might be redundant.
Liliana Ramos	Page 8 - Line 200	A very compact COC might be related to the early time of triggering (when smaller follicles < 15 mm are triggered, these present immaturity without been abnormal)	The working group considered that there are no clear data to confirm this assumption.
Liliana Ramos	Page 10 - Line 263	A vacuole is not a SER vesicle	The text has been modified.
Liliana Ramos	Page 11- Line 290	What is the "a" in SER-a ? define or explain what this means	sER-a refers to "aggregates of smooth ER". This abbreviation was defined in the introduction of Chapter 2.

Liliana Ramos	Page 12- Line 327	What is COS (define this before of use table with abbreviations)	This was corrected in the text
Klaus Wiemer	Page 13 - Line 364	Insert: Since various morphology assessment points have low or no clinical correlation. Considering Artificial intelligence models for oocyte quality assessment and prediction of blastulation is an option that needs further study	The potential of AI models is cited later on page 49: . "Undoubtedly, the next decade will bring a more substantial incorporation of AI in the ART laboratory, offering solutions to the perpetually challenging problem of viable gamete and embryo selection"
Zhongwei HUANG	Page 11 - Line 261	The authors can include a statement what oocyte sizes should one considered unsuitable for use? This is not included and readers have to infer	The dimensions are cited in the text "Without consideration of the ZP thickness, small (<100 µm diameter) and large oocytes (≥125 µm diameter) have been reported to have very low developmental potential (Bassil et al., 2021). Giant oocytes (e.g. >180 µm diameter) should be excluded from clinical use due to their possible tetraploid origin"
Keerti Singh	Page 8 - Line 170	Suggest including - Information on application of AI in ART and use of deep learning models may improve effectiveness of time-lapse systems. AI also offers capability for automation and standardization of embryo selection	This information is provided at page 49.
Keerti Singh	Page 8 - Line 195-196	Please provide recent references. Suggest including negative impact due to oxidative stress (OS). High levels of OS produced by blood clots may affect DNA integrity of both oocyte and sperm during conventional IVF. Some labs do a mechanical cutting of cumulus corona mass	Regrettably, there are no recent references available on this specific topic.
Keerti Singh	page 9 - Line 217-218	It has been reported that oocytes with thin zona pellucida may increase fertilization rate. Please include with references	Some data on ZP thickness and polarized light macroscopy have been added to the text.
Keerti Singh	Page 10 - Line 250	Suggest including references in support of the statement. Anagnostopoulou C et al (2022) stated irregular shaped oocytes are not correlated with cryo-survival, aneuploidy, fertilization, high quality embryos, implantation and pregnancy rates". The sentence can be modified as there are conflicting reports regarding using irregularly shaped oocytes.	The reference has been added
Keerti Singh	Page 12 - After line 317	Suggestion – include granularity and meiotic spindle disorders appear during early stages of development compared to SER and vacuole aggregations that appears at the end of oocyte maturation.	The WG considered that the origin of the oocyte abnormalities is outside the scope of this paper.
Keerti Singh	Page 13 - Line 349	Under heading oocyte morphology and morphokinetics with information on oocytes with centrally located granular cytoplasm (CLGC) show declined cryosurvival poor quality blastocysts also, BUB1 & BRCA1 expression and a very high aneuploidy rate	A reference has been added

Karima Djerroudib	Page 13 - Line 364	Suggestion to add the proportion of immature oocyte (GV+MI) in a cohort to not only predict outcome of the MII oocyte in the same cohort but to postponed ICSI for couple of hours §Regarding page 8/line 182-183-184 §Regarding Atlas of embryology "In particular, some oocytes can be immature (at the stage of early telophase I) when observed with polarized light microscopy, despite the presence of PBI in the PVS. At this stage, in fact, there is continuity between the ooplasm of the oocyte and the forming PBI and the MS is interposed between the two separating cells (Figs 19–22). This condition normally has a duration of 75–90 min." § Regarding this article "Egg maturity assessment prior to ICSI prevents premature fertilization of late-maturing oocytes". Holubcová Z, Kyjovská D, Martonová M, Páralová D, Klenková T, Otevřel P, Štěpánová R, Kloudová S, Hampl A. J Assist Reprod Genet. 2019 Mar;36(3):445-452. doi: 10.1007/s10815-018-1393-0. Epub 2019 Jan 12. PMID: 30635815; PMCID: PMC6439061. §Regarding this publication "High oocyte immaturity rates affect embryo morphokinetics: lessons of time-lapse imaging system Amanda Setti, Daniela Braga, Patricia Guilherme, Assumpto, Iaconelli Jr.Edson Borges Jr. Published:June 14, 2022DOI:https://doi.org/10.1016/j.rbmo.2022.06.005"	Some suggestions have been added in the text. Please see Oocyte assessment - Immaturity section.
Karima Djerroudib	Page 9 - Line 220-227	Take in consideration that periviteline space can be induced by osmolarity of media or some media which have caracteristic to have precipitation after couple of hours incubation at 37°c	The WG considered that the origin of the oocyte abnormalities is outside the scope of this paper.
Sayaka Higo		"Follow-up of babies born from oocytes with atypical phenotypes and rescue IVM demands attention" I agree about this comment, but would prefer to add prenatal follow up.	Added "prenatal follow-up" to the sentence.
Mario Sousa	Page 8 - Line 172-174	Add: Bull-eye and granular vesicles as additional cytoplasmic dimorphisms(1)	The text has been modified accordingly.
Mario Sousa	Page 9 - Line 219	Replace (showing different ZP phenotypes are therefore considered suitable for clinical use) by: Due to their lower developmental potential, oocytes showing different ZP phenotypes should be considered for clinical use only in poor prognosis cases	The group considered there are no sufficient evidence for this statement.
Mario Sousa	Page 9- Line 229	Replace (Oocytes showing different PVS phenotypes are therefore considered suitable for clinical use) by: Due to their lower developmental potential, oocytes showing different PVS phenotypes should be considered for clinical use only in poor prognosis cases.	The group considered there are no sufficient evidence for this statement.
Mario Sousa	Page 10 - Line 239	Replace (Oocytes showing fragmented or large PB are therefore considered suitable for clinical use) by: Oocytes showing fragmented PB are therefore considered suitable for clinical use. I would prefer to add in L242: However, a disproportionately large polar body, although very rare, could be associated with abnormal meiotic spindle placement and deserves more attention. Therefore, as disproportionately large polar bodies have been associated with abnormal oocyte meiosis, these oocytes should not be used for treatments.	It is reported in the final recommendations section.
Mario Sousa	Page 10 - Line 243-249	There are extremely abnormal oocyte shapes, such as cucumber shaped (2). I would prefer to add in L250: Irregularly shaped oocytes are considered suitable for clinical use. However, disproportionately oocytes should not be used for treatments, unless in poor prognosis cases.	A similar statement can be found in the final recommendations
Mario Sousa	Page 10 - Line 255-261	Add after the last sentence: However, as Giant oocytes have been associated with abnormal oocyte meiosis, these oocytes should not be used for treatments, unless in poor prognosis cases.	The group believes that giant oocyte should never be used.

Mario Sousa	Page 10/P11-Line 262-278	At the end (L277) replace (Oocytes showing vacuoles are therefore considered for clinical use) by: Due to their lower developmental potential, multivacuolated oocytes or with a large vacuole, should be considered for clinical use only in poor prognosis cases.	A similar statement can be found in the final recommendations
Mario Sousa	Page 11-Line 279-285	At the end (L288) replace by: Due to their lower developmental potential, oocytes showing RB larger than 5 µm (3), should be considered for clinical use only in poor prognosis cases	The group considered there are no sufficient evidence for this statement.
Mario Sousa	Page 11-Line 289-306	At the end (L306) replace by: Due to their lower developmental potential and imprint risks, oocytes showing aSERT larger than 14 µm, should be considered for clinical use only in poor prognosis cases. Additionally, fully informed consent and strict follow-up of fetal development are mandatory (4, 5).	A similar statement can be found in the final recommendations
Mario Sousa	Page 11/P12-Line 307-317	At the end (L317) replace by: Due to their lower developmental potential, oocytes showing marked central granularity, should be considered for clinical use only in poor prognosis cases	The group considered there are no sufficient evidence for this statement.
Mario Sousa	Page 12-Line 326	Before the subchapter "Immaturity" it should be added text on the inclusion Bull-eye, and on the inclusion granular vesicles (1)	The text has been modified accordingly
Mario Sousa	Page 13-Line 364	Why not to add a subchapter on oocyte spindle observation? And on ZP birefringence evaluation?	Information about polarized light microscopy has been added
Mario Sousa	Page 8 - Line 198	Do not use the term conventional IVF. There are IVF (in-vitro insemination) and ICSI (in vitro microinjection)	The working group believes that conventional IVF is a good term since IVF alone includes also ICSI
Dan Zang	Line 262	The section could be strengthened by including more recent references, as some of the cited studies are relatively old (e.g., Veeck, 1999; Van Blerkom, 1990). Incorporating more current research would ensure that the information provided is up-to-date and reflects the latest understanding in the field.	The WG thank the reviewer for the suggestion; however, studies on oocyte morphology are quite outdated, and more recent references are not available.
Dan Zang	line 289	The passage presents a comprehensive overview of the current research and debates surrounding smooth endoplasmic reticulum clusters (SER-a) in oocytes and their potential implications for oocyte quality and reproductive outcomes. One potential area for improvement could be the inclusion of more details or explanations regarding the potential mechanisms or reasons behind the contrasting findings. While the passage effectively summarizes the research, it does not delve deeply into the possible explanations for the discrepancies or the factors that might influence the impact of SER-a on reproductive outcomes.	The group believes that the origin of the oocyte abnormalities is outside the scope of this manuscript.
Mario Sousa	Page 13-Line 366-368	Add granular vesicles and the Bull-eye inclusions	The text has been modified accordingly
Etienne van den Abbeel		SER: The consensus document states that the clinical use of SER-a positive oocytes may be considered , I would add that however consent document would be appropriate	The group considered that it is out of the scope of the document.
Chapter 3			
Munevver Serdarogullari	Page 20 - Line 533	Timing of observation: has ":" other have only "." ":"can be changed to "."	This was corrected in the text

Patricia Fauque	Page 16 - Line 403	The sentence lines 403-404 is the same as in lines 387-389	in line 387-389: we present the survey results regarding the timing of assessment of zygote stage at 17h ± 1 hpi; However, in line 403-404, we present the survey results regarding the scoring of the zygote based on the Istanbul consensus recommendations.
Patricia Fauque	Page 17 - Line 429	In our study (Barberet et al., 2019) we not reported a significant difference in the PN size between male and female PN.	The citation is correct because the text does not mention "significant difference", but rather a trend towards a larger male PN that is consistent with other studies
Patricia Fauque	Page 17 - Line 432	In Barberet et al., we assessed the influence of the size assessed at 17hpi +/-1hour on live birth rate and we did not find any significant difference. Therefore, this statement is tempered by our results.	In their study, Barberet describe the assessment of male and female area, but it is not clear whether they assessed these two parameters separately or in combination (difference between the male and female areas)
Patricia Fauque	Page 17 - Line 434	I propose to add "and results from TLI showed that the PN size could not be an independent parameter associated with outcome."	The working group added the text in italic: "that resulted in live births (Otsuki et al., 2017, Otsuki et al., 2019). In addition, results from TLT are not conclusive on the value of PN size as an independent parameter associated with outcome. Collectively, ..."
Patricia Fauque	Page 19 - Line 501	Is it possible to distinguish 1PN results obtained from cIVF to those from ICSI? The significance and therefore results could be not the same. In addition the term "in significant proportion" is too vague form me, is it possible to add some data?	The text in italic has been added: "Consistent with this, several studies reported that 1PN blastocysts screened by PGT-A were diploid/euploid in significant proportions (40-50% of tested samples), in some cases similar to those of 2PN controls (Bradley et al., 2017, Capalbo et al., 2017, Destouni et al., 2018, Xie et al., 2018, Zhao et al., 2022). In addition, while such studies involved ICSI as part of the PGT-A procedure", live births from 1PN zygotes have also been obtained in standard IVF cases (Li et al., 2020). Documented use ..."
Patricia Fauque	Page 19- Line 504	Again, this part needs to be more precise. These 1PN are they with the second extruded PB? Are they with a larger size ? and Are they obtained from cIVF or ICSI? This statement/conclusion that 1PN have the same embryonic developmental competences seems too hazardous for me. How many embryo transfers were performed ? and as said, this 1PN were selected by an extended embryo culture, so what was the proportion of these 1PN which were able to develop to blastocyst?	The reviewer is reminded that the cited text only offers background information. The recommendations on 1PN use are based on principles of caution.

Patricia Fauque	Page 20-Line 529	It would be important to give data because probably the difference is not significant due to the small sample size, is it possible to provide % in each group of zygote?	Again, this should be considered background information. What really matters is the relevant recommendation on the use of 2.1PN, which is based on principles of caution similar to the previous case
Patricia Fauque	Page 20-Line 551	The discussion on 0PN with 1GP or 2GP extruded is absent herein. Could it be possible to add that?	The text in italic has been added: "... a significant proportion of 2PN zygotes undergo PNBD at earlier times than the fertilisation check interval recommended by the original Istanbul Consensus (2011). In such cases, the presence of the second PB should accompany 2PN fertilization and therefore used as a scoring criterion. While these zygotes may be categorized as 0PN, if cultured, they may produce normal laboratory and clinical outcomes"
Patricia Fauque	Page 21-Line 557	I think this statement is too peremptory, see above my comment	The working group does not agree with the peremptory character of this statement, also because the following text states: "Collectively, this evidence supports cautious clinical use of 1PN zygotes, combining blastocyst culture and -if available- PGT-A technology appropriate for biparental diploidy assessment"
Patricia Fauque	Page 21 - Line 558	It is in contrast to the previous sentences which are in favor of "no caution"	The previous statements are not recommendations, but citations of relevant studies
Klaus Wiemer	Page 17 - Line 435	Insert: Lee et al., (2024) recently demonstrated that zygotes possessing uneven PN (>20% in size) resulted in slower developing embryos as assessed by AI. However, resulting AI score, blastocyst score, and blastocyst conversion rates were not impacted by this morphological characteristic. This data suggests that although uneven PN size may result in slow growing embryos, those embryos that achieve the blastocyst stage, are not impacted by this observation. (reference). Source: UNEVEN PRONUCLEATES (PN) ARE ASSOCIATED WITH SLOWER EMBRYO DEVELOPMENT Authors: Lee SW, Neblett M, Zepeda A, Herman, Hickman C, Babayev S. (Accepted abstract, PCRS 2024) Link to the abstract: P66_Lee_Siwon.pdf (memberclicks.net)	Unfortunately, only full peer-reviewed papers can be cited, not abstracts.
Klaus Wiemer	Page 18 - Line 407	Insert: Moderately sized embryos were found to have higher LB outcomes. An Optimal embryo surface area and diameter were determined by the highest live birth rate and the suboptimal range was determine by a decreased Live birth rate. At tSB, optimal surface area was 11771-13577 um2, while optimal embryo diameter was 120-129µm. LB rate for optimal embryo surface area was higher than for suboptimal [Optimal 80% (20/25) vs Suboptimal 55% (40/72), p<0.001]. Source: Not too big, not too small: Euploid blastocysts that lead to Live birth have an optimal surface area, diameter and optimal pace of development. CRGH Clinic, Alexa Zepeda, Pedro Pini, Klaus Wiemer, Cristina Hickman. (Accepted abstract for ESHRE 2024)	Unfortunately, only full peer-reviewed papers can be cited, not abstracts.

Klaus Wiemer	Page 20 - Line 554-565	Insert: Of note, new technologies such as AI can detect the number of Pronuclei with significant agreement with experienced embryologists (references). This new technology may assist in documentation of pronuclear formation in those cases that exhibit rapid tPNf. Source: A Florek, R Odia, S Theodorou, M Duran, W Saab, V Seshadri, P Serhal, C Hickman, A Brualla Mora, R Derrick, M Gaunt, P-258 Impact of Direct Unequal Cleavage (DUC) on embryo development, blastocyst formation and ploidy - artificial intelligence (AI) analysis, Human Reproduction, Volume 37, Issue Supplement_1, July 2022, deac107.248, https://doi.org/10.1093/humrep/deac107.248 J Teruel Lopez, C Miret Lucio, M Lozano Zamora, M Escribá Suarez, M Benavent Martínez, J Crespo Simó, I Erlich, M Tran, N Bergelson, P-269 A validation study for artificial intelligence (AI) compared with manual annotation, using donor eggs reveals that AI accurately predicts blastulation, Human Reproduction, Volume 37, Issue Supplement_1, July 2022, deac107.258, https://doi.org/10.1093/humrep/deac107.258	Unfortunately, only full peer-reviewed papers can be cited, not abstracts. In addition, this abstract is more pertinent to automation, not the significance of PN number.
Mario Sousa	Page 18- Line 453-467	The oocyte cortex is reach in SER small, medium and large vesicles, in small aggregates of SER tubules (aSERT), in isolated SER tubules and in mitochondria. SER elements and mitochondria are rich in calcium deposits. After fertilization, aSERT disaggregate, and SER vesicles and mitochondria are largely displaced to around PN. This was shown to face the calcium oscillation vector, from the periphery to the center before PN formation, and then from the center towards the periphery since the existence of PN and then embryo nuclei (6)	Thank you for reporting such details. Unfortunately, although relevant, they cannot be included, for the sake of conciseness.
Ashleigh Storr	PN number	Adding more detail to the information on 1PN oocytes could be beneficial, particularly considering some older evidence suggesting a potentially higher rate of aneuploidy with ICSI-derived 1PNs compared to those from IVF. Not all laboratories have the resources to conduct PGT in such cases. Given these considerations, based on the available information, would the consensus group still recommend culturing 1PN ICSI zygotes until the blastocyst stage?	In the absence of PGT-A, laboratory are not obliged to use 1PN zygotes (from iVF or ICSI). But if they opt for use, such zygotes should be exposed to extended culture as haploid embryos are predicted to be far less competent to develop to the blastocyst stage.
Dan Zang	line 484	One potential area for improvement could be the inclusion of more detailed information on the specific criteria or methodologies used to select the more developmentally competent 1PN zygotes or embryos, as this could further inform the interpretation of the reported clinical outcomes.	Please see lines 492-493: "It is plausible that a larger size of the single PN reflects a higher, possibly diploid, DNA content".
Sayaka Higo	1PN	I strongly agree about the "possibility of 1PN embryo use, not simply dispose", and prefer "discuss and consider by PGT-A", but I still wonder how clinical staff can suggest to patient for proceed PGT-A. For patients who doesn't have any factors (AMA, RIF..) but no suitable embryos are available for transfer originated from 2PN, could we add this criteria for PGT-A?	It is for laboratory/clinical teams to decide how to integrate the recommendations into their workflow. Common sense would suggest applying chromosome screening not to all 1PN cases, but only to those derived from cycles with a specific and independent indication of PGT-A.
Mario Sousa	Page 19- Line 484-508-	I believe that the results presented in (7) and (8) should be added and discussed. In (8) it is well explained all the possible mechanisms. Due to their lower diploidy status, these oocytes should not be considered for clinical use. If used, PGT is advised.	It is not clear which text the reviewer refers to.
Chapter 4			
Munevver Serdarogullari	Page 37 - Line 920	AI has been mentioned only with abbreviation during all document it would be good to add as (artificial intelligence)	This was corrected in the text
Patricia Fauque	Page 21 - Line 725	Perhaps add that it was found for multinucleation at 2-cell stage?	This has been added to the text

Patricia Fauque	Page 21 - Line 731	Only one study cited	The sentence was modified and 3 references have been added.
Patricia Fauque	Page 21 - Line 737	Add the reference : Desch et al 2017	This has been added to the text
Patricia Fauque		I'm not sure to understand: the embryos were selected with direct uneven or irregular chaotic division and then developed up to blastocyst, so they inevitably had this abnormality. We could say: "However, for those that reached the blastocyst stage, the aneuploidy was not increased as compared to those without these early abnormalities?	It has been clarified in the text
Patricia Fauque	Page 27 - Line 743	At what stage (i.e. 2-cell or 4-cell stage)?	This has been added to the text
Klaus Wiemer	Page 24 - Line 622-628	Insert: Through the use of TLT and AI, several direct unequal cleavage patterns (DUCs) have been described. These patterns of cleavage can have different effects on subsequent embryo development and can have negative impact on resulting blastocyst formation (reference). These authors further elucidated that the type of DUCs into 6 different patterns: Minor Chaotic, DUC1, Indirect DUCs, Major Chaotic DUCs, DUC2 and fragmented DUC1. Source: (C Miret Lucio, M Lozano, A Brualla, A Zepeda, C Hickman, N Bergelson, M Escribá, M Benavent, A García, J Crespo, J Teruel, P-300 Not all DUCs are the same: Impact of DUC type on the blastulation, utilization and ploidy, Human Reproduction, Volume 38, Issue Supplement_1, June 2023, dead093.658, https://doi.org/10.1093/humrep/dead093.658)	This reference is an abstract with rather preliminary results. The full publication is not available and cannot be included in the manuscript.
Klaus Wiemer	Page 25 - Line 658	Insert: AI models can accurately predict blastulation and embryo utilization on day 2 (30hpi) as well as on day 3. These models are based upon the ability of TLT and AI to detect and determine optimal cleavage timing and events associated with cleavage (reference). Source: Lucio, C. M., Lozano, M., Mora, A. B., Sakov, A., & Hickman, C. (2022). CAN CHLOE EQTM, AN AI-BASED EMBRYOLOGIST ASSISTANT TOOL, AUTOMATICALLY PREDICT WHETHER AN EMBRYO WILL BLASTULATE, BE UTILISED AND/OR IMPLANT? Fertility and Sterility, 118(4), e116. https://doi.org/10.1016/j.fertnstert.2022.08.344	This reference is an abstract. The full publication is not available and cannot be included in the manuscript.
Klaus Wiemer	Page 27 - Line 745	Insert: More recently, the application of AI for cleavage assessment has determined that direct uneven cleavage results in blastocysts with lower euploid rates compared to embryos that do not exhibit these characteristics. In addition, direct uneven cleavage also impacted quality of ICM and embryos having severe fragmentation (reference). Source: Hickman, C., Zaninovic, N., Malmsten, J., Zhan, Q., Mora, A. B., Har-Vardi, I., & Ben-Meir, A. (2022). TURNING THE BLACK BOX INTO a GLASS BOX: USE OF TRANSPARENT ARTIFICIAL INTELLIGENCE TO UNDERSTAND BIOLOGICAL MARKERS USEFUL FOR EMBRYO SELECTION. Fertility and Sterility, 118(4), e5–e6. https://doi.org/10.1016/j.fertnstert.2022.08.032	This reference is an abstract. The full publication is not available and cannot be included in the manuscript.

Klaus Wiemer	Page 29 - Line 811	Insert: Recently, the combination of TLT and AI algorithms have successfully determined ranges for cellular development that correspond with blastocyst development and outcomes. Studies involving AI have successfully predicted cleavage patterns and other anomalies that can impact blastocyst development that are often missed using traditional culture without TLT (references). Source: Yelke, H. K., Ozkara, G., Kahraman, S., Yildiz, S., Hickman, C., & Mora, A. B. (2022). STRONG AGREEMENT BETWEEN MANUAL AND ARTIFICIAL INTELLIGENCE (AI) SUPPORTS AUTOMATED ANNOTATIONS OF TIME-LAPSE CULTURED EMBRYOS AT a SINGLE FERTILITY CLINIC. Fertility and Sterility, 118(4), e155. https://doi.org/10.1016/j.fertnstert.2022.08.452 A Barrie, R Smith, C Hickman, I Erlich, A Campbell, P-287 An assessment of agreement between automated embryo annotation, through artificial intelligence, and manual embryo annotation, Human Reproduction, Volume 37, Issue Supplement_1, July 2022, deac107.276, https://doi.org/10.1093/humrep/deac107.276 J A Castilla, N Almunia, A Brualla, R Jiménez, A M Villaquirán, I Har-vardi, A Ben-Meir, E Gomez, O-067 Artificial intelligence system detects "goldilocks" morphokinetic zone for embryos transferred or frozen in time-lapse videos, Human Reproduction, Volume 37, Issue Supplement_1, July 2022, deac104.081, https://doi.org/10.1093/humrep/deac104.081	This reference is an abstract. The full publication is not availbale and cannot be included in the manuscript.
Maria Carme Pons	Page 25 - Line 649-652	It is stated that "fast growing embryos on day 3 (>8 cells) have a higher rate of aneuploidy and an increased incidence of abnormal cleavage patterns and are less likely to make blastocysts than 8-cell embryos," including our paper (Pons et al., 2019) as a reference to the statement.However, our findings do not fully support this affirmation.Our study distinguished two groups of fast-cleaving embryos that differ in blastocyst formation and ploidy. The 9- to 11-cell embryos had lower competence compared to 8-cell embryos, and the >11-cell embryos were just as likely to develop to the blastocyst stage and be euploid as 8-cell embryos. I'd suggest adding a new sentence including these data to illustrate that this point is controversial since not all papers find a negative impact of the fast-cleavage on further embryo development. Some papers with similar results are Shapiro et al., 2000, and Luna et al., 2008.	This point has been discussed by the group, but not included since it became quite complicated. A short sentence was however added in the text.
Maria Carme Pons	Page 25 - Line 654-656	Suggestion: Add some publications supporting the claim (There is clear evidence that slow-growing embryos always perform worse)	Some references were added.
Etienne van den Abbeel		Fragmentation on day2/3: In the consensus paper fragmentation is described in a pure mathematical way (= as a percentage of the volume of the embryo). It is very well known that fragmentation has some spatial and temporal characteristics so defining fragmentation solely as a percentage of the volume might lead to problems. For example a 4-cell embryo where one blastomere is fragmented results in a grading of 3-cell embryo with 25% fragmentation, 3 equal blastomeres and not cell size stage-specific, while the 3 equal blastomeres of the size of a 4-cell blastomere do represent the correct cell size.	This has been inserted under the section "Fragmentation"
Chapter 5			
Munevver Serdarogullari	Page 32- Line 821	In the morula stage section, there are two separate terms, morula and morulae. When referred to as a stage, this section mentions the morulae stage. Would it be better to use same term not to cause confusion during reading.	The term "morulae" is the plural form of "morula" and is used when referring to multiple such structures.
Patricia Fauque	Page 31 - Line 744	Typo : Day 4	This was corrected in the text

Etienne van den Abbeel		Morula: The human embryo is said to have become a morula at approximately 8 to 16-cell stage. A morula cn be compacting/ compacted with many cells ,with few cells, complete or incomplete. The authors refer to the Ciray et al , 2014 terminology where the term morula refers to "end of the compaction process" which is to my belief a somewhat misleading definition	Indeed there is some inconsistency in literature regarding the term „morula". The group adapted the text accordingly.
Jean-Léon Maitre	Compaction and blastomere exclusion	We have recently published a study on human embryo compaction (Firmin et al, Nature, 2024, doi.org/10.1038/s41586-024-07351-x), which reports on the role of contractile forces during compaction. We report that embryos that fail compaction have a low surface tension, which is indicative of weak cell contractility rather than defective cell-cell adhesion. Also, cell exclusion at the time of compaction is also associated with low surface tension, which points at cell contractility being too weak. We think it may be relevant to mention in the sections relative to compaction but, more importantly, we think that the section about cell exclusion and defects in cell adhesion and polarity should be amended. The study from Alikani et al 2005 on E-cadherin (CDH1) localization is very interesting. However, it lacks quantifications and is more of a case study of a few embryos. We think it should be grouped together with the next sentence referring to the study from Zhu et al 2021, which is, we agree, rather speculative. Instead, we would recommend adding a statement about our recent study, which functionally and quantitatively analyzed the contribution of contractility and adhesion to the compaction and extrusion processes on few but multiple embryos. Moreover, this is backed up by several other studies of the same vein that we have carried out on mouse embryos over the last 10 years (https://doi.org/10.1038/ncb3185 ; https://doi.org/10.1038/nature18958 ; https://doi.org/10.7554/eLife.68536) and more generally more in line with our current knowledge about morphogenesis of animals.	Thank you very much for this important input. It should be clarified that the said paper in Nature was published way after the submission deadline. Nevertheless, this new insight in the compaction process certainly deserves to be referenced. The text was amended accordingly.
Patricia Fauque	Page 31 - Line 950	The chances of birth are nevertheless lower. Is it possible to give some data (an order of magnitude) between J5/J6 and J7?	Giving concrete numbers of the live birth chances is difficult, however, the group developed a statement on this in line 1043.
Danilo Cimadomo	Characteristics associated with blastocyst implantation potential	Regarding the reproductive competence of (euploid) blastocysts, you may also refer to Cimadomo et al, Human Reprod Update, 2023. In this study, we scrutinized and summarized through qualitative and quantitative meta-analytical approaches, the associations between several embryonic features and euploid blastocyst live birth rate per transfer or miscarriage rate per clinical pregnancy.	This reference has been added in the last chapter of the manuscript.
Chapter 6			
Andrea Abdala	Morphological features to consider for blastocysts assessment	Day 7 blastocysts assessment is one of the most important topic added on the updated version of the Istanbul Consensus, hence new literature have been published supporting the clinical value of day 7 blastocysts.	Abdala et al., 2023 has been added as reference to day 7 blastocyst section.

Andrea Abdala	Page 38 - Line 977/978	Healthy live births have been reported with Day-7 blastocysts, therefore extending embryo culture until day 7 must be a decision based on patient's characteristics and the cycle. As we have published (Abdala et al., 2023)*, I would recommend to add to the sentence that "these embryos may be of particular importance for patients with few embryos available and advance maternal age" since from our publication we have noticed that the older the patient the higher the chances to have delayed embryo development in vitro, a finding that correlates with Su et al., 2016 and Cimadomo et al., 2019, 2022. Nevertheless, few embryos available, advanced maternal age and low AMH would be related somehow. *Abdala A, Elkhatib I, Bayram A, El-Damen A, Melado L, Nogueira D, Lawrenz B, Fatemi HM. Reproductive outcomes with delayed blastocyst development: the clinical value of day 7 euploid blastocysts in frozen embryo transfer cycles. Zygote. 2023 Dec;31(6):588-595. doi: 10.1017/S0967199423000485. Epub 2023 Nov 13. PMID: 37955175.	The reference has been added to day 7 blastocyst section.
Marco Sbarcia	1116- Table 8	The committee reported the stage of expansion for blastocysts. They have any suggestion for which stage is not good to transfer? For instance, in my experience the transfer of blastocyst with expansion of stage 5 or 6 the outcome is poor. May they give suggestions?	While there is some indication that fully hatched blastocysts MIGHT have a lower implantation rate, the available evidence is limited and poor. There is no evidence that stage 5 blastocysts have poorer implantation potential.
Daniel Brison	1193	Risk of pre-term birth after blastocyst transfer. A large UK-based study of 75,000 treatment cycles in the HFEA database showed that: "blastocyst culture had the largest observed effect on odds of LBR (odds ratio (OR) = 1.35, CI: 1.29-1.42), increased the risk of pre-term birth even when controlling for oxygen tension (MLR; OR = 1.42, CI: 1.23-1.63), and gestation-adjusted BW (MLR, β = 38.97 g, CI: 19.42-58.53 g) when compared to cleavage-stage embryo culture." This study is noteworthy because we contacted clinics and collected data on oxygen level, which is not available on the HFEA database, and so were able to confirm that the risk of pre-term birth from blastocyst culture exists in both low and high oxygen. It is also noteworthy because we had research approvals to access the full HFEA dataset, and so were able to include many of the usual ART confounding factors in the analysis. I acknowledge that this reference is from my group in collaboration with Joyce Harper, carried out via the auspices of the then UK Association of Clinical Embryologists; Castillo et al 2020. https://doi.org/10.1093/hropen/hoz031 Our study should be cited alongside the only UK studies currently cited, of Marconi 2023, which analysed the anonymized HFEA database which does not include full patient details. This may be a reason why their study differed in terms of pre-term birth from blastocyst culture. And from the same group, Raja et al 2023. This latter study is of particular interest because the authors accessed the HFEA register, and included cycles from post-2009, when the UK introduced specific consent to disclosure (CD) for use of information for research. We have recently shown (Fiskani 2024) that the very low rates at which patients provided consent (CD) after 2009 means that this subpopulation are not representative of the full register ART population and therefore studies using this subset are at risk of bias. Also a reason why their results on pre-term birth differ from ours. In confidence, I am aware of a large prospective RCT on blastocyst transfer making its way through final stages of peer review at a top journal. This shows a small increase in live birth rate following 3 transfers, compared to cleavage stage transfer, and a more than doubling in the risk of pre-term birth. It would be worth keeping an eye out for this paper as the guidelines go through final review. Again, these comments are in no way a criticism of the document, which is excellent and much needed!	The text has been modified accordingly.

Daniela Zuccarello	Page 37-Line 920	In Chapter 6. Blastocyst stage (Days 4 - 7) I think it would be useful to add a paragraph discussing the classification of blastocysts according to their 'state of health', referring to the article published in JARG in 2021 by the two Italian scientific societies (SIERR and SIGU) of embryology and genetics. This article should also be cited in the bibliography in the paragraphs on embryo classification. Bibliography: Cimadomo D, Capalbo A, Scarica C, Sosa Fernandez L, Rienzi L, Ciriminna R, Minasi MG, Novelli A, De Santis L, Zuccarello D. When embryology meets genetics: the definition of developmentally incompetent preimplantation embryos (DIPE)-the consensus of two Italian scientific societies. J Assist Reprod Genet. 2021 Feb;38(2):319-331. doi: 10.1007/s10815-020-02015-x. Epub 2020 Nov 24. PMID: 33236289; PMCID: PMC7884494. The possibility of defining embryos as DIPE after pre-implantation diagnosis (PGT) is very important because it would open the way, in countries where it is not yet allowed, for the donation of embryos for scientific research.	The working group considered that this is a valid point that merits inclusion - though this topic is far from fully developed. Thus - the reference is included merely to recognize this topic is important and merits further development.
Jessica Eastick	Page 41-Line 1073	Title: cytoplasmic strings. Eastick et al 2023 assessed 1152 blastocysts in culture and found cytoplasmic strings present in 77% of blastocysts (this included blastocysts that were transferred, cryopreserved and discarded).	This reference was already listed - and is addressed with the statement that 55-85% of blastocysts have strings.
Jessica Eastick	Page 41-Line 1074	Title: cytoplasmic strings. Eastick et al 2021 identified that blastocysts that resulted in a clinical pregnancy had a higher number of cytoplasmic strings present.	This reference was added in the text: "cytoplasmic strings are positively associated with implantation"
Jessica Eastick	Page 41-Line 1075-1078	Title: cytoplasmic strings. Eastick et al 2021 performed a multivariate analysis controlling for the potential confounding effect of the developmental stage. This finding indicated that it was still an independent predictive factor of clinical pregnancy. Similar studies by Ma et al 2021 and Ebner et al 2020 also found that blastocyst stage of expansion was related to cytoplasmic string presence. Eastick et al 2023 performed a multivariate analysis to determine the predictive role of cytoplasmic strings for a clinical pregnancy while controlling for confounders blastocyst development stage, blastocyst quality (grade). It was found that blastocyst quality (grade) was significantly associated with a clinical pregnancy. Through a bivariate regression analysis, it was found that top quality blastocysts and blastocysts in the more advanced stages had a significantly higher chance of having cytoplasmic strings present compared to their poorer quality counterparts and in the earlier stages of development.	The reference as cited does indeed control for blastocyst grade when analyzing pregnancy rate - this has been corrected. The study did not, however, control for blastocyst age (d5/6/7).
Liliana Ramos	Page 46-Line 1191-1201	The results from the TOF study (large RCT, day 3 of 5 transfer; trail registration NTR7034) have been presented at ESHRE 2023 (oral presentation; Cornelisse et al.) and the manuscript has been submitted for publication (we are waiting the final decision at any moment). In this study, we observe differences in the preterm birth of the blastocyst transfer. Because of the impact of this trail, I suggest to include these results by the time this guideline is finalized and ready for publication.	A reference ha been added: Cornelisse et al., 2024 https://doi.org/10.1093/humrep/dead093.005.
Verena Nordhoff	Page 74 - Fig.4 stage of fresh embryo transfer	It is a pity that ET on day 5 and 6 are combined in one column, as exactly this is a matter of debate among embryologists and physicians. Is there any chance to separate them? This would be very useful.	Unfortunately, the survey's design does not allow distinguishing the results between Day 5 and Day 6 fresh embryo transfer (see Supplementary data SI - survey questions - Q9 and 10)
Patricia Fauque	Page 38-Line 966	Typo : add a space	This was corrected in the text
Patricia Fauque	Page 38 - Line 966	Add the CIs	This was added in the text

Patricia Fauque	Page 38 - Line 967	Typo : Day-4 blastocysts	This was corrected in the text
Patricia Fauque	Page 38 - Line 980	In FET there are additional parameters : e.g. the day of replacement on the endometrium side	The working group believes that is an important point, but is beyond the scope of laboratory KPIs.
Klaus Wiemer	Page 38 - Line 958	Insert: TLT and AI offer additional benefits for selection of embryos based upon timing on morphokinetic events that led up to and include blastulation. AI in conjunction with TLT can report a blast score which is based upon a series of morphokinetic events and other variables that can only be assessed with TLT. This information can be utilized for embryo selection which is not possible with conventional culture systems (reference). Source: S Sharma, A Doshi, C Bhatia, A Zepeda, A Brualla, C Hickman, P-741 Morphokinetic Goldilocks: assessing the morphokinetic range to identify embryos with the optimal chance of being euploid, Human Reproduction, Volume 38, Issue Supplement_1, June 2023, dead093.1060, https://doi.org/10.1093/humrep/dead093.1060 Lucio, C. M., Lozano, M., Mora, A. B., Sakov, A., & Hickman, C. (2022b). CAN CHLOE EQTM, AN AI-BASED EMBRYOLOGIST ASSISTANT TOOL, AUTOMATICALLY PREDICT WHETHER AN EMBRYO WILL BLASTULATE, BE UTILISED AND/OR IMPLANT? Fertility and Sterility, 118(4), e116. https://doi.org/10.1016/j.fertnstert.2022.08.344	This is a study presented as an abstract - which is not suitable for inclusion.
Klaus Wiemer	Page 38 - Line 969	Insert: In a recent study, Ten et al., (2024), identified optimal developmental ranges for morphokinetic parameters with the use of an AI tool. Specifically, the morphokinetic events associated with improved live birth were t6, t8, tB, t8-t4 and t8-t2. Embryos exhibiting optimal morphokinetic events for these time periods demonstrated a 5% increase in live birth when compared to embryos exhibiting suboptimal morphokinetic developmental ranges (reference). Source: Jorge Ten, MariCarmen Tio, Pedro Pini, Alexa Zepeda, Adriana Brualla, Cristina Hickman. Embryos developing within the optimal morphokinetic range have a 5% higher chance of leading to a Live birth (LB) (Accepted abstract, ESHRE 2024)	This is a study presented as an abstract - which is not suitable for inclusion.
Klaus Wiemer	Page 40 - Line 1031	Insert: A recent study confirms with AI that speed of blastocyst expansion is significantly associated with euploidy and live birth. Of interest, significant discordance among embryologists existed between embryo expansion grades and AI score. AI in conjunction with TLT may be able to further reduce the subjectivity associated with blastocyst expansion and therefore grading even amongst experienced embryologists (reference). Source: Federica Innocenti, Anabella Marconetto, Samuele Trio, Tamara Canosi, Marilena Taggi, Gaia Saturno, Viviana Chiappetta, Daria Maria Soscia, Laura Albricci, Ben Kantor, Michael Dvorkin, Anna Svensson, Giovanni Coticchio, Laura Rienzi, Danilo Cimadomo. Blastocyst expansion speed assay: a putative artificial intelligence-powered tool for embryo selection. Retrospective study involving 2184 blastocysts and 786 PGT-A cycles. (Accepted abstract, ESHRE 2024)	This is a study presented as an abstract - which is not suitable for inclusion.
Klaus Wiemer	Page 40 - Line 1051	Insert: Innocenti et al., (2024) demonstrated that euploid embryos had higher blastocoele expansion rates than aneuploid blastocyst as assessed by an AI model in conjunction with TLT (761±465 µm2/hour vs 667±449 µm2/hour; p<0.01) (reference). Source: Federica Innocenti, Anabella Marconetto, Samuele Trio, Tamara Canosi, Marilena Taggi, Gaia Saturno, Viviana Chiappetta, Daria Maria Soscia, Laura Albricci, Ben Kantor, Michael Dvorkin, Anna Svensson, Giovanni Coticchio, Laura Rienzi, Danilo Cimadomo. Blastocyst expansion speed assay: a putative artificial intelligence-powered tool for embryo selection. Retrospective study involving 2184 blastocysts and 786 PGT-A cycles. (Accepted abstract, ESHRE 2024)	This is a study presented as an abstract - which is not suitable for inclusion.

Ashleigh Storr	Page 37 - Line 945	Without TLT, observations might also be dictated by patient treatment in order to optimize culture conditions. For example, if a patient is undergoing a freeze all cycle and freezing is performed in the afternoon, would you assess freeze all cases in the morning alongside patients being assessed for transfer?	The working group considered that consistency of timing within a clinic is recommended to allow development of clinic-specific KPIs. The new consensus provides "ideal" timings for assessment based on TLT. Assessments outside of these timeframes influence comparability between clinics.
Ashleigh Storr	Page 38 - Line 983	"....though the relative importance of each remains to be fully resolved."	Corrected in the text
Jessica Eastick	Page 41- Line 1071-1078	Title: cytoplasmic strings. To date, cytoplasmic strings assessments have limited information on how the assessments were performed and the number of assessors and are yet to be classified. Eastick et al 2022 investigated the inter- and intra-observer agreement when assessing day 5/6 human blastocysts for cytoplasmic strings and their vesicles. A moderate level of inter and intra-observer agreement was seen when assessing cytoplasmic strings in blastocysts, while only a slight to moderate level of agreement was seen when the specific characteristics of cytoplasmic strings (i.e. number, vesicles) were assessed. This study highlights the need for further training of embryologists to improve overall observer agreement to allow studies to be performed to assess its value in embryo selection. https://doi.org/10.1007/s43032-022-01151-2	This was added in Cytoplasmic String section
Etienne Van den Abbeel		Blastocysts: There is consensus that blastocyst grading is difficult and subjective. The Gardner grading is recommended in the paper . It is very well known that the Gardner grading is in many ways somewhat suboptimal due to ill described definitions, for example the definitions for grade A, B and C ICM are extremely subjective , some viable blastocyst may not be graded correctly and discarded. It should be recommended to characterize blastocysts in a more correct way to transfer nd cryopreserve also C grade blastocysts in order to better document potential positive outcomes after transfer. Furthermore in the consensus paper the authors refer to Gardner scoring system which might be misleading when mostly "grading / ranking "systems are discussed	The WG agrees that blastocyst grading is subjective and have stated as such, and a brief statement about subjectivity of grading in the introduction of this manuscript. While we also agree that a more objective method beyond the Gardner system is an goal, evidence for an alternate approach is lacking. A statement is included to this effect. We do use grading and scoring interchangeably.
Chapter 7			
Danilo Cimadomo	page 46 - Line 1184	I would add Tiegs et al, Fertil Steril, 2021 as well here. Perhaps you can also refer to the ESHRE good practice recommendations about embryo biopsy for PGT published by Kokkali et al, Human Reprod Open, 2020	The reference of the study by Tiegs et al 2021 was added in the text. The ESHRE Biopsy Recommendation paper is already cited.

Daniel Brison	Page 46 - Line 1189	"Initial concerns about extended embryo culture due to the possible prolonged influence of environmental factors on embryonic epigenetics are decreasing (White et al., 2015, Ghosh et al., 2017, Ji et al., 2018)". As a researcher active in this area, I do not believe this statement is true or represents the consensus view. Just because studies have so far failed to identify the mechanism behind the impact of extended culture, does not mean that concern is decreasing, it actually means researchers are continuing to look. "Absence of evidence is not evidence of absence…". In light of this ongoing research effort, he authors would need references more recent than 6 years old to justify their point. For example, in my lab, we have recently found that glucose level in medium has a profound impact on the methylome of the human embryo; so far this study is only at pre-print stage but I am happy to make it available for scrutiny if it would help. Also, the paper in Nature Medicine describing reduced telomere length predicting reduced lifespan in babies born from blastocyst culture should be cited here. Wang et al 2022 https://doi.org/10.1038/s41591-022-02108-3	We thank the reviewer for his comment. The phrasing of the sentence in question has been modified to reflect the uncertainty over the effects of extended embryo culture. In addition, the paper by Wang et al 2022 is now mentioned and cited. However, the reviewer will be able to see that references published up to 2023 are cited in this paragraph, presenting the latest data on the effect of blastocyst culture on offspring health. In addition, the working group felt that the paper by Wang et al., 2022 was not of sufficient scientific calibre to include in this document.
Han Wei	Page 43 - Line 1116-1117	Regarding the scoring of TE (trophectoderm) and ICM (inner cell mass), the criteria are based on the number of cells and the tightness of their connections, categorized into grades A, B, C, and D. However, the use of non-specific numerical expressions such as "many cells," "several cells," "moderate number of cells," and "few cells" can lead to significant subjectivity in the scoring of blastocysts, resulting in substantial differences in scoring by different embryologists for the same blastocyst. Can we establish specific cutoff values, for example, on the maximum equatorial plane, if the number of TE cells is clearly 15 or more (or another specific number), it is rated as grade A; or to evaluate the ICM based on the average of its maximum and minimum diameters?	We agree with this comment. Unfortunately, there is limited evidence for defining the number of cells for the different TE grades. Lacking this, quantitative measures are not included in the consensus.
Patricia Fauque	Page 46 - Line 1170	I propose to add « in a good prognosis population »	This was added in the text
Patricia Fauque	Page 47 - Line 1209	Larger studies and assessments on live birth rates taking into account the e women age are needed to provide a conclusive answer to the above question.	The reviewer's comment was taken into consideration and addition in the test was made.
Keerti Singh	Page 46 - Line 1178	Only information regarding PGT-A testing has been included even in table 10 & 11. Although it was not a part of the questionnaire. Suggest including information from recent articles regarding: PGT-SR in embryos caused by de novo or at risk when parents are carriers for balanced translocations or inversion. SNP array methodology PGT-M testing in disorders due to pathogenic germline variants in a single gene. eg: sickle cell dis. As some centres use these	It is not clear to which part the reviewer refers to, although adding details on PGT-SR and PGT-M is not entirely relevant to this paper.
Keerti Singh	Page 48 - Line 1230-1231	Please include recent references	The reviewer does not suggest any specific references
Keerti Singh	Page 48 - Line 1235-1236	Please include recent references	The reviewer does not suggest any specific references
Keerti Singh	Page 48 - Line 1240-1243	Please include recent references	The reviewer does not suggest any specific references

Ashleigh Storr	Page 48 - Line 1234	It could be good to place more emphasis on the fact that dishware, culture drop size and oil overlay all play a crucial role here.	The reviewer's comment was taken into consideration and addition in the test was made.
Dan Zang	line 1278	the recommendations section should be more explicit in guiding embryologists on how to implement the new criteria. For instance: Training and Calibration: Suggest regular training and calibration exercises for embryologists to ensure consistency in assessments.	The existing sentence "The collated recommendations (Table 10) aim to promote standardized embryo evaluation practices to better predict viability and optimise embryo selection for transfer and cryopreservation. " entails the meaning of the reviewer's suggestion.
Knowledge gaps			
Iman Halvaei		Note to gap of knowledge in each section: I recommend to describe how the quality of studies were evaluated	The quality of studies was evaluated using the GRADE Pro software. This was added in the methodology section.
Iman Halvaei		Note to gap of knowledge section: It is better to suggest not to inject into SER clusters in the oocytes In the oocyte abnormality, Bull's eye should be added Size of vacuoles in the oocyte is not discussed	The text has been modified accordingly.
Iman Halvaei		Note to gap of knowledge: Z scoring is not clearly mentioned	The Z scoring cannot be object of specific description
Iman Halvaei		Note to gap of knowledge: Type of fragmentation (scattered vs concentrated, pseudo fragmentation) is not noted Reverse cleavage is not fully discussed	To be noted for a future version. Not much evidence at present.
Dan Zang	line 1282	Consider adding: 1) Impact of Environmental Factors: More research on how culture conditions (e.g., oxygen levels, temperature) affect embryo development (morphology or morphokinetics). 2) By addressing these points, the Istanbul Consensus update for 2024 will not only offer more robust and practical guidelines for assessing oocyte and embryo morphology but also significantly enhance consistency across clinical practices.	This manuscript aims to describe oocyte and embryo assessment. Technical impacting factors were addressed briefly in the first and last chapter of this manuscript.
Recommendations and Supplementary material			
Patricia Fauque	Page 50- Recommendations	I'm not sure that the current data could allow such recommendation. I will remove that from "recommendations" as it needs further researches	The reviewer did not specify which recommendation is mentioned.
Etienne van den Abbeel	Conclusion	General conclusion: The consensus document should be considered for publication provided (maybe?) if some minor comments / clarifications are added. To discard or use, an embryo remains a dilemma for embryologists. Tools like AI could help in standardization of assessments. Standardization of assessment should a global approach.	Thank you for your comment. The group agree with your comment and this was added in the conclusion of the manuscript.
Zhongwei HUANG	Page 73 - Line 2275	Spelling for blastocyst was incorrect	This was corrected in the text