2022

ESHRE Working Group
Chromosomal Mosaicism

ESHRE survey results and good practice recommendations on managing Chromosomal Mosaicism

European Society of Human Reproduction and Embryology

REVIEW REPORT

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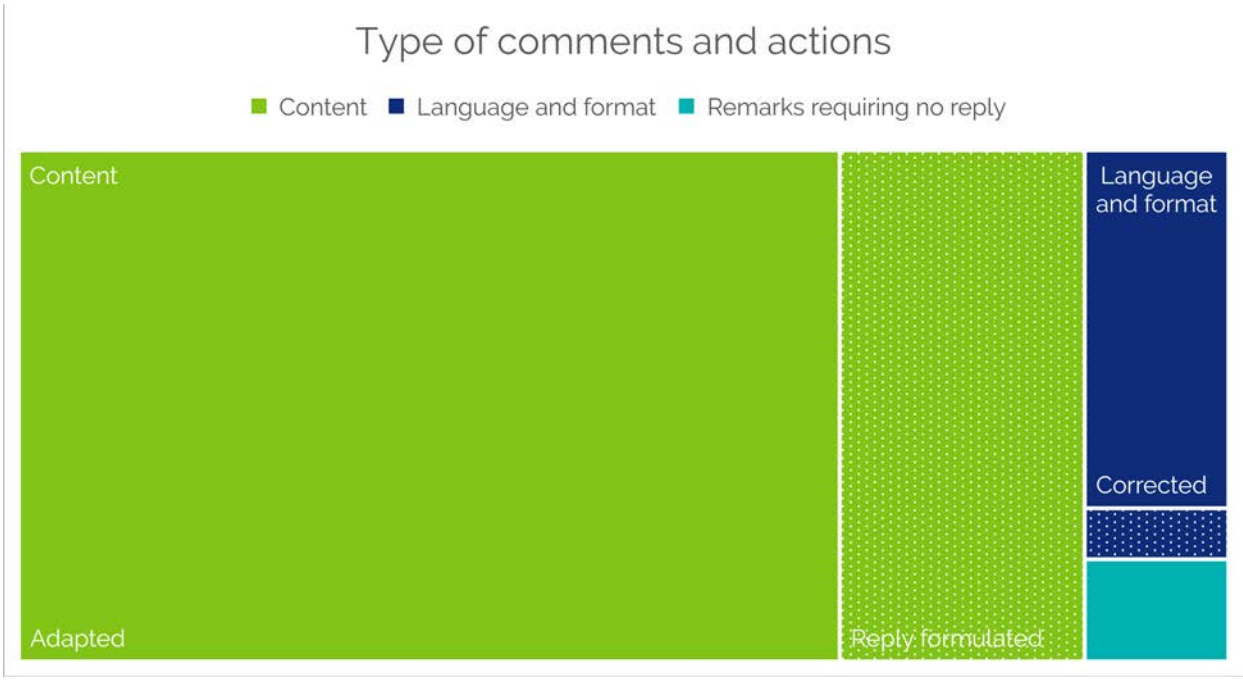
The draft of the paper "ESHRE survey results and good practice recommendations on Chromosomal Mosaicism" was published for public review for 4 weeks, between 15 February and 16 March 2022.

This report summarizes all reviewers, their comments and the reply of the working group and is published on the ESHRE website as supporting documentation to the paper.

During the stakeholder review, a total of 84 comments (including 0 duplicates) were received from 16 reviewers.

The comments were focussed on the content of the document (74 comments), language and style (8 comments), or were remarks that did not require a reply (2 comments). 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 paper (n= 57; 77 %). Adaptations included revisions and/or clarifications of the text, and amendments to the recommendations. For a number of comments, the working group considered them outside the scope of the paper or not appropriate/relevant (n= 17; 23 %)



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.

Representatives of professional organisations

Organisation	Country	Representative
Fertility Genetics and ARGC	UK	Valerie Shaikly
DiNA science	Spain	Diana Campos
CARE Fertility UK and Fertility Genetics	UK	Karen Sage
Zouves Foundation for Reproductive Medicine	USA	Manuel Viotti

Individual experts

Reviewer	Country
Paul N Scriven	UK
Aşina Bayram	United Arabian Emirates
Maria José De los Santos	Spain
Marco Sbracia	Italy
Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff	Austria
Elpida Fragouli	UK
Amanda Odell-West	UK
Cristina Albanese	Italy
Francesca Spinella and Ermanno Greco	Italy
Sebastiaan Mastenbroek	The Netherlands
Cristina Magli	Italy
Liborio Stuppia	Italy

Reviewer comments and replies

Reviewer	General comments	Action / Reply
Paul N Scriven	In my opinion this manuscript suffers from statements that are substantially correct but technically inaccurate.	We have addressed the specific comments by the reviewer and revised the manuscript significantly in reply to this comment.
Paul N Scriven	In my view it would be helpful to provide numbers (numerator and denominator) for percentages.	To keep the text readable and manageable, we have opted to use percentages with 1 decimal throughout the text. The numerator and denominator are included in the accompanying figures. We have adapted to ensure the numbers are included in the figures or the text throughout the paper .
Marco Sbracia	These recommendations seem in general useful, the only think that can be observed is they are little bit late, after that several other groups have stated their observations.	Thank you for this comment. We trust that these recommendations will still have an added value and be useful.
Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff	The statement that PGT-A is increasingly applied as stand-alone test or in addition to PGT-M/SR, despite controversy surrounding its use (line19, 20) is rather weak and does not reflect the reality that the PGT-A is an expansive und elaborate Add-on procedure without proven advantage of application. • While ESHRE survey has evaluated (i) developmental stage where embryo is biopsied (ii) the procedure of zone pellucida opening the number of cell biopsied they missed to evaluate (I) minimum blastocyst quality biopsied; (ii) location of biopsy (polar, mural) (iii)details on biopsy technique -as well as (iiii) whether excluded cells were strictly excluded from biopsy. There are all important issues that may influence the results. Additionally, ESHRE survey missed to evaluate the % of yielding no or inconclusive results i.e., due to WGA failure. In general, this uncertainties of PGT-A should be highlighted. In principle, we suppose, that it is in general to early to give recommendations in regard to chromosomal mosaicism, yet. This holds true for the fate of chromosomal mosaicism, different subtypes (segmental & numerical) -and most important the uncertainties whether the biopsied material truly reflect the chromosomal constitution of the embryo.	We agree with the reviewers that the ESHRE survey missed to evaluate the % of yielding no or inconclusive results i.e., due to WGA failure. We have rewritten the introduction and the paper to be more general in terms of PGT and to highlight the current unknowns. Still, we consider it relevant to provide some guidance to practitioners on how to deal with mosaicism when detected, even if this guidance may change when more information and data become available.
Elpida Fragouli	The phrasing of this entire section is vague. It is not clear how the presence of mosaicism would affect the accuracy of the PGT-M diagnostic results. Do the authors refer to embryos that are unaffected/ carriers of recessive mutations (transferrable), but have a mosaic abnormality? Ideally this section should be revised to be made clearer to assist in the decision making in PGT laboratories and the IVF clinics using them.	We have clarified the recommendations on PGT-M by adding the PGT-M specific recommendations in a recommended PGT-M workflow.
Elpida Fragouli	The statements about genome amplificiations are very general. Published data clearly show that certain genome amplificiation methods are more vulnerable to artefacts than other. Ideally the statements should be rephrased to reflect this variability. It would also be good to provide a range of artefact frequency related to the diferrent types of genome amplification methods	The different PGT methods, including their strength and limitations are covered in previous ESHRE publications. We have added a reference to guide the reader towards more detailed information.

Valerie Shaikly	Recommendations in this paper reference that counselling should include the discussion of chromosome mosaicism as an inherent biological phenomenon in human preimplantation embryos but also that risk assessment should include false results and risk of unbalanced/mosaic offspring (when to date this has not been demonstrated) this information is generally included in patient counselling already but leaves patients faced with contradictory facts, taking a 'leap of faith' for transferring a mosaic embryo. It would be helpful to include in risk assessment a note for provision of review of new data on the outcomes of mosaic transfers for patients to put decision making in the context of the emerging evidence base.	We have added a statement at the start of the recommendations that policies should be updated in relation to emerging data on the topic
Valerie Shaikly	Overall, the recommendations appear to cover aspects of mosaicism faced by PGT laboratories and leave the policy making regarding transfer and storage to clinics. This area should be acknowledged if not addressed in these recommendations; further guidance for clinics is also required, so patients have a standardised experience.	we have added a sentence at the start of the recommendations stating that recommendations formulated are aimed to provide guidance to PGT centres and be a basis for the centres' own policy with regards to 'mosaic' embryos.
Karen Sage	Clinicians counselling patients on the transfer of mosaic embryos need to understand that the reference ranges are for mosaic calls are not standardized across different laboratories, that settings for PGTA calls are configurable and that technologies used for PGTA are different. Especially important for those clinicians working in multi-centre IVF units where the laboratory provision may be different. This is also relevant for patients wishing to transport PGTA-analysed embryos from clinic to clinic for transfer. Pre-embryo transfer discussions/ genetic counselling with patients already includes the potential impact of variable biopsy techniques, the introduction of DNA amplification 'noise' and technical artifacts associated with different PGT-A technologies, the increasing sensitivity of testing and whether the mosaic result is meaningful in a genetic condition/syndrome context. The latter being the utmost concern for patients and the most challenging for decision-making. Currently data suggests there is no direct correlation with the genetic result (apart from the one reported case in the literature and referenced in these guidelines (page 318 Kahraman et al 2020). It would be beneficial for clinicians, if standardized reporting can be agreed and adopted by laboratories across the numerous technological platforms deployed for PGT-A mosaic calls. A simplification of reporting, stratifying results into 3 clear categories (as the guidelines and literature are moving towards) for example: • euploid (to include the 'low level' mosaic) Reference = 0-50% ? • mosaic*(remove the word "high") Reference = = 50-80% ? • aneuploid Reference = >80% ? *mosaic including whole chromosome and segmental aneuploidies.	We agree with the reviewer that a standardised reporting would be helpful for clinicians and patients, and specifically those using services from different PGT centres. However, with different techniques used in practice, and unequivocal data, such standardised reporting system is currently not feasible. We consider this is covered by recommending that laboratories state their approach towards the reporting of mosaicism in the consent form for genetic testing and that it is discussed during counselling sessions prior to initiating PGT.
Karen Sage	The guidelines produced for clinical use seem to lack the clinician's voice. They appear to be written for laboratory use, and are therefore confused by the lack of standardization across the platforms. Clinical guidance would be more meaningful if reporting simplified. In my experience, currently clinicians are overwhelmed with low/high mosaic calls involving single, two or three whole chromosomes +/- segmental aneuploidies without a euploid embryo in a cycle and many are struggling with providing advice and counselling for their patients. Additionally, in the UK, PGTA is classified as an "add on" by the regulator (HFEA) and has a 'red light' for caution grading on use of PGTA in treatment cycles, given the lack of robust clinical trials. Lack of clear standards and guidelines is raising concerns about reporting of mosaicism in PGTA embryos.	The paper focusses primarily on genetic testing and reporting, rather than informing and counselling patients. As genetic testing and reporting are activities for the PGT centre, we did not include their voice in the working group. However, PGT centres are considered to be multidisciplinary and clinicians should have a voice in the development of the centre's policy. We have clarified this in the paper. With regards to PGT-A, describing the relevance of PGT-A is outside the scope of the current paper, but we have thoroughly rewritten the paper to put less emphasis on PGT-A.
Manuel Viotti	I would like to clarify an incorrect reference to a study of which I am the corresponding author. Specifically, I am referring to the following passages (lines 305-308): Another large retrospective study, including mostly poor prognosis couples that did not have euploid embryos	Thank you for alerting us of these incorrections. We have adapted the sentence in line with the comment and the original paper.

	available for transfer, reported slightly lower live birth rates based on some specific putative mosaic patterns. Miscarriage rates were not different (Viotti, et al., 2021b). This is incorrect on three counts: 1) 'including mostly poor prognosis couples that did not have euploid embryos available for transfer'. This is misleading. In fact, that is only true for 51.7% of cases. The rest did have euploid embryos available, which either implanted in previous cycles or failed to implant. See the exact quote in the original paper (Viotti et al 2021b, analysis of 1,000 mosaics): "To investigate that possibility, we analyzed outcomes for mosaic embryo transfers from cycles with no euploid embryos, in which mosaic embryos were the first to be transferred from within a cohort. That "no-euploid" mosaic group (n=517) experienced significantly lower rates of implantation (44.1%) and OP/B (35.4%) compared with the euploid group (Supplemental Fig. 1A, available online)." 2) What the authors are calling 'slightly lower' is deceptive and should be changed to 'lower in a statistically significant manner'. See original paper Figure 1C. 3) 'Miscarriage rates were not different' This is just plain wrong. See original paper Figure 1C, these are massive highly statistically significant differences.	
Manuel Viotti	In general terms, I really enjoyed the survey part of the manuscript, which provides a valuable overview of current stances and the existing climate around mosaicism.	Thank you for this comment.
Cristina Albanese	I was expecting a more technical document; it looks like a general report of the current literature but it doesn't emerge a clear position	We have clarified the aim of the paper and adapted the format. Based on the uncertainties, it was impossible to formulate strict recommendations, but we were able to provide guidance to PGT centres, specifically towards development of their own policy with regards to 'mosaic' embryos. This was clarified in the paper. We further changed the format of the recommendations in a PGT-A and PGT-M/PGT-SR workflow, which should make it easier for centres to compare the recommendations with their current workflow, or to start their centre specific policy for managing 'mosaic' embryos in PGT-A and PGT-M/PGT-SR .
Francesca Spinella and Ermanno Greco	The document aims to provide guidance on mosaic embryo transfer strategies in clinical practice. It was created starting from a Survey and from the analysis of the literature. The ESHRE survey is well done and provides insight into the mosaic embryo identification and management strategies adopted by different IVF clinics. The key findings of the report are that: 1. In PGT including PGT-A, blastocyst stage biopsy on 5 to 10 cells is the most applied approach among the different IVF canters. 2. Importantly, of the centres performing PGT (either PGT centres of ART/PGT centres), 88.1% indicated that they had validated the technology for PGT in-house, independently from manufacturer validation. 3. Forty percent of centres use a cut off level of ≤20% abnormal cells to designate a euploid embryo and≥ 80% abnormal cells for an aneuploid embryo; 4. 71% of centres will specify the degree of mosaicism. 5. 90% of centres for prioritization of mosaic embryo considered the level of mosaicism, the type of mosaicism, the type of chromosomes involved, severe intrauterine growth retardation or liveborn syndromes and the number of chromosomes involved, either as sole criterion or in combination. 6. In the majority of centres when no euploid embryos are available for transfer, the preferred option is to transfer mosaic embryos. 7. Following the transfer of a mosaic embryo, prenatal diagnosis is recommended in 95% of centres.	We have made several adaptations in reply to this analysis and comments. Firstly, we corrected the errors in the representation of the Viotti study. Still, most data support the conclusion that mosaic embryos may lead to lower implantation, but not to genetic/congenital abnormalities in the offspring and therefore, we confirm the recommendations allowing the transfer of mosaic embryos in specific cases. we have modified the recommendations in such a way that it is up to the centres to develop and document their own policy, and communicate this with the patients. Some centres may base themselves on published literature and assign a lower priority for low grade mosaic embryos, compared to euploid embryos whereas other centres may have their own data

	The authors claim they used the survey results, published data, and currently good practice documents on PGT to implement these recommendations. However, some recommendations do not seem to be reflected either in the survey results or in the published articles and the scientific data on which the authors based their recommendations are not very clear. Indeed, recommendation N.15 (line 416) for managing mosaicism, recommends to consider embryos with a low degree of mosaicism in the same way as euploid embryos. Furthermore, the same recommendation suggests co-evaluating mosaicism with morphology, suggesting that a mosaic embryo can be given priority when its morphology is superior to that of a euploid embryo. It is not clear from which scientific data and from which studies these conclusions are drawn. This seems in contrast to those reported by the survey in which the majority of centres prefer to transfer a euploid embryo when both euploid and mosaic embryos are available for transfer. These two recommendations are also at odds with that proposed by the PGDIS and by many studies that demonstrated that mosaic embryos have lower clinical success than that of euploids (Fragouli et al. 2011, 2017, Munnè et al., 2017 and 2020, Spinella et al., 2018, Viotti et al., 2020 and 2021). Viotti's study analyzed 848 embryos with low mosaicism, demonstrating that Ongoing Pregnancy / Birth rates was statistically lower than that of euploid embryos (n=5,561) (40% vs 52.3% p <0.01). On the contrary, one study (Capalbo et. al, 2021) showed equivalent live-birth rates and miscarriage rates across 484 euploid, 282 low-grade mosaic, and 131 medium-grade mosaic embryos. It would appear that ESHRE working group recommendations are derived from only on this study. If this were the case, the recommendations would lose substance, as they would base two important points on a single study that has many weaknesses. First of all the study was conducted on just 484 cases. The second is that the NGS technology used to identify mosaic embryos has a low resolution for the identification of low mosaicisms. In fact, the Ion Torrent platform is unable to detect chromosomal mosaicism below 30% (Biricik et al., 2021). The third is that the percentage of implantation (55.8%) obtained after the euploid embryo transfer is very low. This is probably due to the transfer of embryos with a low level of mosaicism not detected by the NGS platform used. This led to a result similar to that obtained after the transfer of the embryos with a mosaicism of 30 to 50%. The fourth point is that only 14% of pregnancy were with prenatal follow-up. The most serious criticism of these recommendations is that many studies demonstrating the poor outcome of mosaic embryos compared to euploid are completely ignored, while others are discarded without any valid reasons. In addition, results presented in some of these studies are not correctly reported by the authors and the conclusions reached by the authors are misleading. The Viotti 2021 study carried out on 1000 mosaic was deemed to be compromised by the fact that mosaic embryos were transferred as a last option in women with previous euploid embryo transfer failures. This is not entirely true as the study also includes mosaic embryo transfers from cycles with no euploid embryos, in which mosaic embryos were the first to be transferred from within a cohort. These embryos (n = 517) experienced significantly lower rates of implantation (44.1%) and OP / B (35.4%) compared with the euploid group. In addition, the study also analyzed 16.4% of cases in which the embryo was transferred under supposition of euploidy but post-transfer re-evaluation of the sequencing profile led to the embryo being assigned to the mosaic category. In any case, even if the analysis was done in a population with no euploid embryo, there is a significant reduction in implantation and pregnancy success after mosaic embryos (low- and high level) transfer compared to euploid, and this should be taken into consideration. Indeed, a large proportion of patients may result after IVF treatment, in no euploid available for transfer and the results obtained in this study are very relevant for the management and prioritization of mosaic embryos. In the comments on the article, many results are reported incorrectly: the sentence on line 306 is incorrect: Viotti's study reports a significant difference in the outcome of mosaic embryos compared to that of euploid embryos;	showing that there is no difference in clinical outcome between euploid and low grade mosaic embryos, and they will develop a different policy. We also added a recommendation that it is not acceptable to consider a mosaic embryo per definition as aneuploid and discard it. With regards to prenatal testing, we have added an umbrella statement recommending genetic counselling for prenatal diagnosis after PGT.

	sentence 307 is incorrect: miscarriage rates were 8% in euploid versus 25% in mosaic embryos. Another aspect that is completely ignored by the recommendations is the type of mosaicism and the effect on the embryo's outcome. The authors of the recommendations state that information on the risk associated with the different types of mosaicism is still missing or inconsistent. Today there are dozens of articles, such as that of Fragouli of 2011, 2017, Munnè 2016 and 2020, Spinella 2018, Victor 2019, Viotti 2021, in which it was shown that mosaic type (nature of the aneuploidy implicated in mosaicism) affects outcomes, with a significant correlation between number of affected chromosomes and unfavorable outcomes. Despite this, none of these data is taken into account in the recommendations. Finally, no recommendations (recommendation N 11) are given on whether or not to do prenatal testing when transferring a low grade mosaic embryos. This could be a big risk, also considering that the only case of mosaicism reported at birth to date was a mosaic of 35% (Kaharman et al., 2020). In addition, other cases in which the chromosomal mosaicism was not corrected during development and was also present in the fetus have been found and will be published soon. These recommendations could have serious repercussions from a medical / legal point of view when a child suffering from chromosomal mosaicism is found after the transfer of a mosaic embryo. In conclusion, the recommendations as they are written contain many problems: Prioritizing embryos with a low potential of implantation when a euploid embryo is available exposes patients to an increased risk of spontaneous abortion, of prolonging time to pregnancy, and of obtaining a child with mosaic aneuploidies. I highly recommend reviewing recommendations # 11 and # 15	
S. Mastenbroek	Comment on the draft paper 'ESHRE good practice recommendations on Chromosomal Mosaicism': A clear statement on the appropriateness of PGT-A is needed. In short: there is no high level evidence of any clinical benefit of PGT-A, while there is evidence of harm (Cornelisse, et al., 2020, Mastenbroek, et al., 2021, Yan, et al., 2021). On top of this, the rationale of PGT-A can seriously be doubted now (Mastenbroek, de Wert and Adashi, 2021). Such doubt should not to be taken lightly. To illustrate: in the past 25 years hundreds of thousands of women have been harmed by PGT-A, as their chances of live birth were lowered by discarding their mosaic embryos, while these embryos have now been proven to result in equivalent live birth rates as euploid embryos (Capalbo, et al., 2021). If anything, the ESHRE survey results that are presented by the working group in the current document actually confirm this is still an ongoing issue in routine practice. Note that besides mosaicism there are other issues that are suggested to undermine PGT-A efficacy (Mastenbroek, de Wert and Adashi, 2021). It is unethical to present good practice recommendations, or a document that examines 'how to perform' PGT-A, even if it only discusses part of the treatment, while avoiding to mention that the actual treatment has no proven benefit and has actually been proven harmful in the past. The working group should include a statement that given the lack of high-level evidence of the effectiveness for PGT-A and the potential for adverse consequences, the use of PGT-A should at present be limited to the research setting (Mastenbroek, de Wert and Adashi, 2021). Related to the above: the working group does mention there is "controversy surrounding the use of PGT-A" (page 1, line 20) and does mention that "the discussion of clinical benefit and utility of such genetic tests is not the aim of the current paper" (page 11, line 265). This really is insufficient. Providing 'good practice recommendations' suggests that PGT-A can be 'good practice'. No lay reader (such a patients) will assume otherwise. Even professionals working in ART, that are less familiar with the controversy surrounding PGT-A, will assume so. While the use of PGT-A should at present be limited to the research setting (Mastenbroek, de Wert and Adashi, 2021). By avoiding the topic of the efficacy of PGT-A, the working group, especially as representatives of ESHRE, too easily disregard their responsibility, and that of ESHRE, here (Dondorp and de Wert, 2011). A conflict of interest statement is missing from the document that was available for review. From the author list it	We have addressed this comment by rewriting the paper being less focussed on PGT-A, but would like to stress that this document does not examine 'how to perform' PGT-A, but rather how to handle embryos with biopsies indicating chromosomal mosaicism. The controversies around PGT-A are well known and documented, but describing them in detail is outside the scope of the current paper, which aimed to provide guidance on how professionals should responsibly proceed with embryos indicating chromosomal mosaicism. The conflict of interest statement was not included in the draft for review, as this is not something that can be adapted based on the stakeholder review, but will be added to the final paper.

	can be concluded that several working group members do have a direct or indirect (through their employer) conflict of interest. Such statement should accompany documents like this. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, Cimadomo D, Benini F, Farcomeni A, Cuzzi J et al. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. Am J Hum Genet 2021;108: 2238-2247. Cornelisse S, Zagers M, Kostova E, Fleischer K, van Wely M, Mastenbroek S. Preimplantation genetic testing for aneuploidies (abnormal number of chromosomes) in in vitro fertilisation. Cochrane Database Syst Rev 2020;9: CD005291. Dondorp W, de Wert G. Innovative reproductive technologies: risks and responsibilities. HumReprod 2011;26: 1604-1608. Mastenbroek S, de Wert G, Adashi EY. The Imperative of Responsible Innovation in Reproductive Medicine. N Engl J Med 2021;385: 2096-2100. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, Sun X, Ling X, Li H, Hao C et al. Live Birth with or without Preimplantation Genetic Testing for Aneuploidy. N Engl J Med 2021;385: 2047-2058.	
Cristina Magli	The document is very well written. Nice literature review and excellent report of the survey results. There is no doubt that this part represents a useful support for the user. My opinion is different for the recommendation section. I couldn't get any relevant message here, no practical indication about how to handle with this issue. I was expecting some guidance or at least opinion, even a suggestion towards some direction. Maybe a suggestion to assess mosaicism in the hope of collecting more data. Maybe some consideration on the used platform or on the size of the biopsy. I do understand that the evidence is very scarce, but precisely for this reason I am wondering whether we should release such a document as a GPR where recommendations are not really relevant. For me, this is a state of the art paper with at the end some suggestions or indications for the user - not enough for a GPR. In other words, I support the publication of this document, but not in the format of a GPR. Should this be the case, some revision is obviously needed. But you are the expert here, so no reason to say more. I hope that I could expressed my thought.	We have clarified the aim of the paper and adapted the format. Based on the uncertainties, it was impossible to formulate strict recommendations, but we were able to provide guidance to PGT centres, specifically towards development of their own policy with regards to 'mosaic' embryos. This was clarified in the paper. We further changed the format of the recommendations in a PGT-A and PGT-M/PGT-SR workflow, which should make it easier for centres to compare the recommendations with their current workflow, or to start their centre specific policy for managing 'mosaic' embryos in PGT-A and PGT-M/PGT-SR .
Liborio Stuppia	First of all I would like to express my congratulations with all the authors for the excellent job. In my opinion this document will provide useful information to all people involved in this field of studies. I have just a few specific comments, reported in the “specific comments” section.	Thank you for this comment.

Reviewer	Page	Line	Comment	Action / Reply
Paul N Scriven	1	Lines 10-12	Polar bodies are not cells and the second polar body is extruded after fertilisation; it is DNA that is tested from biopsied polar bodies and cells. Zegers-Hochschild et al. 2017 (cited by the authors) define PGT to be "a test performed to analyze the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for HLA-typing or for determining genetic abnormalities."	We have adapted the sentence in relation to the exact definition provided by Zegers-Hochschild, et al., 2017.
Paul N Scriven	1	Lines 15-16	Aneuploidy is defined by Zegers-Hochschild et al. 2017 to be "an abnormal number of chromosomes in a cell." PGT-A tests for gain and loss of chromosomal material and might detect whole-chromosome aneuploidy and segmental imbalance in a DNA sample, which may or may not reflect the true potential of the embryo to be viable. Embryos with an abnormal test result are typically excluded from transfer.	By rewriting the introduction, based on other comments, we have removed the sentence, and consider this comment resolved.
Paul N Scriven	1	Lines 23 – 26	Mosaicism is defined by Zegers-Hochschild et al. 2017 to be "a state in which there is more than one karyotypically distinct cell population arising from a single embryo." Others have argued that mosaicism is by international consensus, the "presence (anywhere) in an individual of normal and abnormal cells that are genotypically distinct and are derived from a single zygote" and that "PGT-A does not determine whether an embryo is mosaic (i.e., exhibits anywhere within the embryo two or more unique cell lineages). PGT-A only determines whether a single random 5–6 cell biopsy of trophectoderm at blastocyst-stage contains two or more distinct cell lineages". [https://doi.org/10.1186/s12958-021-00716-1 (not cited by the authors)]. I suggest making it clear at least once that the issue at hand concerns embryos with test results consistent with aneuploid/diploid mosaicism, which becomes an issue for clinical decision makers and patients when there are no embryos available with a normal test result (euploid, and a presumed diploid chromosome complement with no detectable imbalance).	We have added the definition of Chromosomal mosaicism by Zegers-Hochschild, et al., 2017 in the text and added it is an inherent biological phenomenon in human preimplantation embryos.
Paul N Scriven	1	39	I suggest following transfer of embryos with putative mosaic (aneuploid/diploid) test results. Euploidy is defined by Zegers-Hochschild et al. 2017 to be "the condition in which a cell has chromosomes in an exact multiple of the haploid number; in the human this multiple is normally two. Thus, a normal embryo that is euploid is also diploid." The authors should consider specifying at least once that a euploid test result is presumed to be a diploid (normal) chromosome complement with no detectible chromosomal imbalance.	We have added a sentence clarifying that euploid is presumed diploid.
Paul N Scriven	3	98 – 117	Consider giving numbers with percentages: 53.6% of 239 is 128.104 centres; there were 128 (54%) centres located in Europe.	To keep the text readable and manageable, we have opted to use percentages with 1 decimal throughout the text. The numerator and denominator are included in the accompanying figures. (Figure 1)
Paul N Scriven	3	107 – 111	Consider denominating thousands with a comma and not a period (i.e. 10,000 not 10.000). I doubt the activity is normally distributed and you can't have a fraction of a centre or a cycle: consider providing the median and range for the number of cycles per year and the percentage with genetic testing.	We have kept the median already mentioned for this outcome. As the sentence explained the variation, the range did not add any information. We removed the mean. With regards to the comma, this was adapted in line with editorial guidelines.
Paul N Scriven	4	Figure 1	Technically the Russian Federation (which spans Eastern Europe and Northern Asia) and Turkey (located mainly on Anatolia in Western Asia) are transcontinental countries. Fig. 1E, 50 cycles is not included; i.e. I suggest up to 50 cycles or fewer than 51 cycles rather than "less than 50 cycles".	We have not considered that some countries are transcontinental. As we did not analyse any differences between continents, we do not think this is a major item to be highlighted in the paper. We have corrected the figure.

Paul N Scriven	5	127	Provide the numbers of centres that 10% relates to.	The sentence was rephrased. The 10,0% relates to the average over the different PGT indications
Paul N Scriven	5	144	Provide the number of centres performing PGT, and the number of centres outsourcing their genetic analysis. (or provide the numerator and denominator for percentages as you have done for the in-house validation).	The nominator/denominators were added as these were not explicitly included in the figure.
Paul N Scriven	6	149	Provide the number of centres.	Information is added in the paper.
Paul N Scriven	7	167 – 172	Specify the number of centres the percentages relate to. Segmental imbalance is not aneuploidy by definition (see above). Consider "segmental imbalance" and/or "segmental gain/loss" and also reiterating that "mosaicism" relates to intermediate test results indicating aneuploid/diploid mosaicism.	The numbers were not added in the text, but they were clarified in the figure. We have modified "segmental aneuploidies" to "segmental imbalances (gain/loss) in the respective sentence and throughout the paper.
Paul N Scriven	7	173 – 190	Specify the number of centres the percentages relate to.	The nominator/denominators were added as these were not explicitly included in the figure.
Paul N Scriven	7	198	Specify the number of centres.	We have added some numbers to the figures. The text remarks were not quantified.
Paul N Scriven	11	244	Provide the total number of centres that 95% relates to.	An explanation is added to the text.
Paul N Scriven	11	264 – 265	Consider "these embryos with abnormal test results" rather than "these abnormal embryos".	This was corrected in the paper.
Paul N Scriven	11	274	Consider "segmental imbalance" rather than "segmental aneuploidy".	This was corrected throughout the paper.
Paul N Scriven	12	291	Define positive predictive value; I suspect that it could be the likelihood that an abnormal test result is correct, or the likelihood that an embryo with an abnormal test result is not viable.	We have rephrased the paragraph and removed the term "positive predictive value" as indeed it could be interpreted in different ways.
Paul N Scriven	12	298-311	Offered only as a comment. It is interesting to consider the diagnostic odds ratio (dor) for viability comparing testing to morphology alone (where 1 indicates no power to discern a viable embryo) for several recent studies: In the STAR trial (NGS, trophectoderm sampling) [https://doi.org/10.1016/j.fertnstert.2019.07.1346] embryos diagnosed to have mosaic, segmental or polyploid imbalance of uncertain clinical significance were not transferred. The dor can be estimated to be 1.359 for women aged 25 – 40 y, and 2.476 for women aged 35 – 40 y. Yan et al, 2021 (women aged 20 – 37 y) [https://doi.org/10.1056/nejmoa2103613] excluded from transfer embryos with an aneuploid, intermediate copy number "mosaic", or segmental imbalance test result. The doi can be estimated to be 2.341. Viotti et al. 2021 [https://doi.org/10.1016/j.fertnstert.2020.11.041] examined mosaicism with a 50% cut-off and the doi can be estimated to be 2.371 for whole-chromosome mosaicism and 1.103 for segmental imbalance. For comparison, Yang et al, 2012 (women aged < 35 y, aCGH, trophectoderm sampling) [https://doi.org/10.1186/1755-8166-5-24]. The doi can be estimated to be 25.529.	Thank you for this comment and interesting approach. While we would encourage further exploration and publication of this analysis with regards to the clinical utility of PGT-A, we consider this to be outside the scope for the current paper.
Paul N Scriven	13	358	The purpose/meaning of "[segmental]" in this statement is not clear to me. Presumably these mixtures are intended to be aneuploid (trisomy, monosomy)/diploid.	We agree with the reviewer and have removed the word "segmental" in this specific recommendation.

Paul N Scriven	13	366 – 367	I suggest that an abnormal “mosaic” test result might not reflect the true potential of an embryo to be viable.	We have added a sentence to the recommendation stating that "Detection of chromosomal mosaicism in a TE biopsy may not reflect the constitution of the entire embryo or embryo viability."
Paul N Scriven	15	430-434	It is not clear to me what statements 18 and 19 mean. PGT-M: a test result indicating mosaicism for the chromosome where the gene of interest is located. PGT-M/A: excluding embryos with test results indicating mosaicism for unrelated chromosomes (which might not reflect the true potential to be viable) adversely affecting the cumulative unaffected live birth rate; although, the detriment might be considered to be marginal when several embryos unaffected for the monogenic condition of interest are available for transfer. The authors have not offered recommendations for PGT-SR and PGT-SR/A cycles.	We have adapted the layout of the recommendations, which provided background to these recommendations and clarifies their relevance in the PGT-M workflow.
Aşina Bayram	5	137	“A minority of centres aims for less than 3 cells (3.7%)”. After this sentence, I have found it contradictory for the mosaicism detection. These centers, how do they accept the range of mosaicism considered diagnostically indicative of an aneuploid, euploid embryo or mosaic embryo?	The question in the survey and the reply are independent of whether the centres report mosaicism. This question and its replies merely provide insight on how blastocyst biopsy is performed, i.e on which day and the number of cells the operator aims to collect. It is clarified in the text further down that these centres did not consider reporting of mosaicism.
Maria José De los Santos	11	243	Is there any data with regards of genetic analysis of POC??	We did not include a question in the survey on whether genetic analysis of products of conception is performed.
Maria José De los Santos	14	400	Any recommendation in doing genetic analysis of POC? Should it be explored only under a research context?	We have added a sentence on genetic analysis of the POC in the research recommendations section.
Marco Sbracia	14	line 385 / 5th rec	I do not think that at this point is "acceptable" for a lab to report or not report mosaicism in their framework. All Lab must report more information than possible about these procedures that are still experimental, even though largely marketed.	We have adapted this recommendation to: It is acceptable for a PGT centre not to report mosaicism, provided that the centre has a strategy for classifying embryos and acts on it.
Marco Sbracia	14	line 403 /11th rec	About no recommendations can be made at present with regard prenatal follow up for mosaic pregnancy, at least should be suggested to follow up them after the birth to know if there is in the infants soe mosaicism. This should be really fair in order to establish the real risk for newborn of a preimplantation diagnosis of mosaicism.	We have inserted two previous recommendations from ESHRE reading " ART/PGT centres should be encouraged to obtain follow up data on babies born after treatment" and "genetic counselling for prenatal diagnosis should be offered to all women who become pregnant following PGT", but have retained the recommendation regarding the uncertainties on prenatal follow up specifically for mosaicism.

Marco Sbracia	15	line 416/ 15th rec	In the selection of embryo for transfer after biopsy of TE and mosaicism diagnosis the use of embryo morphology does not seem correct, this point should be amended that only in case after having transferred all the euploid embryos and with more embryos diagnosed with mosaicism, morphological criteria should be used to choose which embryo to transfer	Centres may either rank mosaic embryo with a lower priority than euploid embryos, and then they will automatically use morphology to select an embryo among euploid embryos, and when there are no further euploid embryos, again morphology is used for selection among mosaic embryos. The recommendation is specific for centres considering low level mosaic and euploid embryos as similar and now reads: ' when selecting embryos for transfer among euploid and low-range mosaics, the TE biopsy PGT-A result should be co-evaluated with embryo morphology and preferably not be assessed on its own.
Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff		15-17	PGT-A with deselection of aneuploid embryos for transfer is mostly offered to specific ART patient groups, mainly patients of advanced maternal age, with repeated ART failure or with recurrent miscarriages. Comment : It should be clearly stated that there is still no proven benefit of PGT-A for these patient clientele. It should be mentioned that mostly RCT on PGT-A were performed in good responder patients, -also revealing mostly no benefit -especially in regard to life birth (1-3). Second, the latest Cochrane Review on PGT-A stated that there here is insufficient good-quality evidence of a difference in cumulative live birth rate, live birth rate after the first embryo transfer, or miscarriage rate between IVF with and IVF without PGT-A (4). This should be acknowledged in the Introduction and Background. The statement that clinical benefit and utility is not aim of the current paper (line 267) ignores the fact that clinical benefit, (in)consistency of different NGS platforms in the diagnosis of embryo mosaicism and many other uncertainties are closely entwined. They might influence the physician's decision to propose a PGT-A approach as well as the patient to decide for or to refuse PGT-A. Therefore, these issues cannot be seen as separate aspects.	The current paper focuses on chromosomal mosaicism, which is increasingly a challenge in genetics due to the wide application of PGT-A. We do not advocate for or against PGT-A. Such position is outside the scope of the current paper, but will be covered in another project to be finalised in 2022. We have also substantially rewritten the paper to put less focus on PGT-A
Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff		263-265	PGT-A and PGT-SR are widely performed with the goal of detecting chromosomally abnormal embryos and withholding them from embryo transfer under the hypothesis that these abnormal embryos would result in implantation failure, miscarriage or ongoing aneuploid pregnancy/birth. Comment: This sentence is somewhat misleading. While PGT-SR has a proven indication (i.e., patients of balanced translocations) and benefit- PGT-A has not. PGT-SR aims to withhold those embryos from transfer that reveal unbalanced translocations leading to miscarriage, stillbirth or severe disabilities of children born but not ongoing aneuploid pregnancy/birth. Aneuploidy per definition is the presence of an abnormal number of chromosomes	We have clarified that PGT-M and PGT-SR are performed upon indication and have added a section describing the imbalances in study data from PGT-A, but that mosaicism applies both to PGT upon indication and PGT-A
Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff		287-391	Studies have suggested that high-range mosaicism detection (approx. >50% aneuploid cells) in the original TE biopsy is associated with whole chromosome aneuploidy in a significant proportion of cases (Capalbo, et al., 2021, Handyside, et al., 289 2021, Wu, et al., 2021), meaning that high-range mosaic TE biopsies might actually represent technical variation from the uniform aneuploidy range. Comment: This is however misleading. First, the detection of a at least second cell line fulfils the criteria of a mosaic constitution. Second, it should be noted, that all cut-off values of euploid, mosaic or aneuploid are set due to technical but not biological reasons (1 aneuploid cell of 5 cell-probe = 20%). Additionally, PGT-A only determines a single random 5–6 cell biopsy of trophectoderm, while this can mathematically never reflect the true chromosomal constitution of an embryo (5) The lack of clinical significance must be highlighted. Therefore, all the recommendations 11, 13-17 are highly questionable	We have reworked the paragraph on clinical validity, with reference to technical factors and highlighting of the uncertainties

Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff	378	Informed consent and counselling of patients Comment: The informed consent and counselling of patients should also include the high proportion of embryo loss, the increased risks for embryo waste due to non-biopsable embryos, inconclusive or false results and the high risks of lacking embryos for transfer. The high financial burden is also an important aspect that should be included in the counselling information.	The statement informed consent and counselling is related only to the topic under discussion, being genetic testing and mosaicism. More detailed information on patient counselling in the context of ART and PGT has been previously published (ESHRE PGT Consortium good practice recommendations for the organisation of PGT. Human Reproduction Open. 2020;2020.)
Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff	396	If reported in PGT-A analysis, the terminology should reflect the fact that a TE biopsy cannot provide an absolute determination of mosaicism in the remainder of the embryo. Therefore, genetic findings consistent with the detection of mosaicism can be designated in wording such as "putative mosaic"," indicating mosaicism" or "suggestive of mosaicism". Comment: As afore mentioned, a TE biopsy cannot predict the whole chromosomal constitution of an embryo-therefore we recommend the replacement of "can be designated" to "should be designated"	We have adapted the sentence as suggested by the reviewer.
Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff	413-415	Re-biopsy of embryos with a mosaic TE biopsy result for the purpose of confirming chromosomal/ploidy status is not recommended since there is no evidence that this has diagnostic value. Comment: It should be noted that biopsy per se might interfere with course of pregnancy. There are increasing hints that TE biopsy could have an impact (6-9). This should be also mentioned in patient counselling. As well as that re-biopsy may increase the risk of implantation failure	In a previous paper, we have addressed rebiopsy in more detail (ESHRE PGT Consortium and SIG Embryology good practice recommendations for polar body and embryo biopsy for PGT. Human Reproduction Open. 2020;2020.). For the current paper, we have added "and it may have a possible negative impact on further embryonic development and implantation" as suggested by the reviewer.
Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff	489-494	It is currently unknown to what extent the aneuploidy in a TE biopsy reflects the status of the inner cell mass. More studies are needed to investigate both the concordance/discordance between the mosaicism status in trophectoderm/inner cell mass and the fate of mosaic cells later in pregnancy. Since it could be hypothesized that different chromosomal aneuploidies have different developmental potential amongst different tissues, it would be valuable to pay attention to potential differences between the different chromosomes. Comment: It is not only unclear whether a TE biopsy reflects the status of the inner cell mass, it should be also mentioned that from model systems aneuploid cell in TE and ICM might have a different fate with regard to their persistence (10,11). Moreover, the possible inconsistency between different NGS platforms should be mentioned (12)	We have adapted the text in reply to this comment, but we have not included the suggested sentence "Moreover, the possible inconsistency between different NGS platforms should be mentioned (12)", as this is related to validation rather than the question at hand (To which extent does the TE biopsy reflect accurately the genetic status of the remainder of the embryo?)
Maximilian Murtinger; Barbara Wirleitner; Maximilian Schuff	492-494	The sentence "Since it could be hypothesized that different chromosomal aneuploidies have different developmental potential amongst different tissues, it would be valuable to pay attention to potential differences between the different chromosomes" is striking. This implies that this issue should also be recognized, also this is not reflected in the recommendations. Mosaicism, embryo transfer policies, prenatal testing, and children follow-up. In facts there are strong hints that different aneuploidies have different fate and persistence during human embryogenesis (see 13). Even more serious is the complete neglection of segmental chromosomal aberrations in transfer policy. Here, the risks that such chromosomal aberrations might persist are many times greater than in numerical aberrations.	We have adapted the text in reply to this comment, specifically with regards to "different aneuploidies having different fate and persistence during human embryogenesis". The comment that the difference between different chromosomes is not reflected in the recommendations is correct. We considered there are insufficient data to make any recommendations in this respects, and hence considered it relevant to list this as a topic for future research.

Elpida Fragouli	15	431-434	Please consider rephrasing to clearly explain what is meant by "the impact of mosaic results on the accuracy of PGT-M diagnostic resuts"	We have clarified the recommendations on PGT-M by adding the PGT-M specific recommendations in a recommended PGT-M workflow.
Elpida Fragouli	16	453-455	Please consider briefly explaining how these "Novel amplification methods which can address some of those issues" differ to the ones currently used?	We have clarified in the text that this refers to methods that can generate genomic analysis without preamplification
Valerie Shaikly	1	13	The word 'Separate' here with 'modalities' gives the impression that the types of testing exist separately. PGT-A is usually included with PGT-M, and PGT-SR uses the same methodology and almost the same reporting as PGT-A. Removal of the word 'separate' would avoid any confusion here at the start of the paper for readers. The above is clarified in line 20.	We agree with removing "separate" in this introductory sentence and have adapted this.
Valerie Shaikly	3	92	Acknowledgement should be given here that published data since the time of the survey (Feb to April 2020) and Pubmed review (to 2020) do not include critical papers considered in the recommendations that were published later (Capalbo 2021 Viotti 2021); this knowledge could have led to different responses than those given up to April 2020 if the survey was repeated.	We added a sentence stating "In considering the survey results, readers should be mindful that the replies were collected in 2020, and may be different following recently published data relevant to mosaicism." The literature search was updated in september 2021, this was corrected in the paper.
Valerie Shaikly	14	393	The data informing 50% mosaicism as a threshold has been published by a single PGT testing laboratory that uses one methodology. Therefore caution should be given to applying this cut-off, which encourages a binary allocation of euploid/high mosaic or aneuploid across the sector; evidence is not available to show that it is appropriate for other laboratories. This is addressed to some degree at line 474 but would be beneficial if referenced here.	We acknowledge that the 50% cut off is supported by some, but not all studies. This is reflected in the phrasing "can be used" rather than "should be used"
Valerie Shaikly	14	401	Transporting embryos between centres is common, and patients may seek to do this when a clinic policy for mosaic transfer does not fit with their wishes. Therefore, the recommendations should include that clinic policy should include careful interpretation of PGT-A reporting at the time of testing for embryos transported between centres.	we have added a recommendation to add technical information in the PGT report, in line with previous ESHRE recommendations, which will allow careful interpretation of the data
Valerie Shaikly	15	410	Professionals will also seek guidance for managing high-level CNV so the paragraph at 425 would be more easily accessed by giving the text as a recommendation rather than a side comment. I.e., There is currently insufficient data for the management of high range mosaic embryos, and centres should ensure they have access to the emerging evidence base to inform management.	We have added a note that policies regarding high-mosaic transfer should be adapted based on emerging knowledge, and we have changed the text on high-range mosaics to a recommendation rather than text.
Diana Campos	14	11	A recommendation should always be added if mosaicism is reported. The final decision whether or not to perform prenatal diagnosis rests with the patient and what we have to ensure is that she has all the necessary information about risks and benefits so that she can make an informed decision. On the other hand, the elective prenatal diagnostic technique after the transfer of a mosaic embryo should be amniocentesis since it avoids the risk of confined placental mosaicism (CPM). CVS has a higher risk of CPM and maternal contamination. NIPT analyzes free fetal DNA from trophoblast cells, so the risk of CPM is not ruled out. Moreover, the sensitivity and specificity of the technique is reduced in chromosomes other than 21,18,13, X and Y, and it does not allow the study of UPD in case of mosaicism of an imprinted chromosome. Finally, being a screening technique, a positive result must be confirmed by an invasive procedure. In case of pregnancy of a mosaic embryo, the prenatal diagnostic center should be informed so that the sample can be treated following the recommendations for mosaic samples.	We have inserted a previous recommendation from ESHRE reading "Genetic counselling for prenatal diagnosis should be offered to all women who become pregnant following PGT", but have retained the recommendation regarding the uncertainties on prenatal follow up specifically for mosaicism.

Diana Campos	15	13	Mosaic embryos have a higher probability of implantation failure and miscarriage and, in addition, there is a risk of an affected pregnancy due to the presence of an aneuploid cell line that, a priori, it is not known how it could affect the ICM. Therefore, low-range mosaic embryos should not be included in the same category as euploid embryos, as these are considered to be free of chromosomal abnormalities. Considering low-range mosaic embryos as euploid has a negative effect on the results of IVF with PGT-A, which would result in a decrease in PGT-A reliability.	The major recommendation is that centres should develop and document their own policy, and communicate this with the patients. The policy should state how embryos are classified and how the centre acts on these different groups of embryos. A centre's policy may be that low range mosaic embryos and euploid embryos are considered as different groups, whereas other centres may develop a different policy. We also added a recommendation that it is not acceptable to consider a mosaic embryo per definition as aneuploid and discard it).
Karen Sage	1	13	The word 'Separate' here with 'modalities' suggests the individual testing is mutual exclusive and could potential be misleading. Suggest replace 'separate' with 'distinct' to indicate that PGT-A/SR/M are distinct analytical methods which can be deployed separately or in conjunction – clarified later in lines 19-20.	We agree with removing "separate" in this introductory sentence and have adapted this.
Karen Sage	2	42	'handle these embryos' is a colloquial expression. Given this is the crux of the guidelines it should be clearly stated here exactly what the aim is. Suggestion How to guide clinicians and patients on the management of embryos scored as 'mosaic.'	We have adapted the sentence accordingly
Karen Sage	2	58-59	"deal with" is a colloquial expression. Suggestion: This paper aims to provide good practice recommendations for clinical use of embryos scored as 'mosaic'.	We have adapted the sentence to remove "to deal with"
Karen Sage	7	177-178	9/93 responses stating need for genetic counselling for prioritization, implies that for all others genetic counselling was not required? Can this be clarified or elaborated on?	9.7% (9/93) emphasized the need for genetic counselling for prioritization without providing any further details. The information provided by the other replies were summarized in the following sentences. We made a minor adaptation to the first sentence to clarify.
Karen Sage	14	392-393	This is unclear. Does this mean that laboratories should specify their reference ranges for making mosaic calls? As in? 20%-50% or 30%-50% = low level mosaicism 50%-70% or 50%-80% = high level mosaicism Can this be further clarified	We have rephrased the recommendation to clarify.
Karen Sage	15	411-412	Should the range of CNV detecting mosaicism but reporting as euploid be defined here? Ie <20% or < 30% or <50%? given that low level mosaics are being categorized as similar in potential to euploid embryos (from the literature). This is an essential requirement of guidelines to assist clinicians counselling patients on the transfer of embryos and their potential outcomes. Signposts to recent and emerging data sources would be welcomed	The paper includes a cut off to distinguish low from high level mosaics, which is based on biological factors. However, there is also a strong technical factor influencing the range of detecting mosaicism, which is why we recommend upfront that each centre should determine these technical cut offs and include them into their centre's policy with regards to mosaicism.

Karen Sage	15	410	seeking guidance on transferring 'high' level mosaics with insufficient data.. Clinicians will need some reassurance here that although data is insufficient, centres are transferring these embryos and data is emerging. There needs to be a distinction between clinical and research. Introducing the most recent publications to assist with clinical decision-making would be helpful here. Genetic counselling provision for managing uncertainty would be an essential component of the recommendations in this instance.	We have adapted the paragraph with respect to high range mosaic ET with respect to adaptation in case of emerging data and genetic counselling as a *conditio sine qua non*.
Amanda Odell-West	1	23-24	It may not be correct to say, 'technical advances have in fact led to the frequent detection of mosaicism,' when its true incidence and accurate classification in preimplantation embryos is disputed (e.g., Gleicher et al 2020, Treff and Marin 2021). It is reported that NGS demonstrates high sensitivity to numerically uniform normality or abnormality (100%) but when uniformity is absent, it is a challenge with whole genome analysis protocols to distinguish technical bias from biological variability, limiting, '… the accuracy of diagnosing mosaicism in clinical practice. As such a certain proportion of euploid embryos will inevitably be reported as clinically unsuitable' (Popovic et al, 2019).	By rewriting the introduction, based on other comments, we have rephrased the sentence, and consider this comment resolved.
Amanda Odell-West	1	25	'The presence of cells with different karyotypes (two or more genetically distinct cell lineages) in a preimplantation embryo' may be a better working definition without the words '… due to postzygotic errors'.	We have adapted the definition of chromosomal mosaicism in the paper to the definition of Zegers-Hochschild, et al., 2017.
Amanda Odell-West	1	38	It may not be correct to suggest that a 'clear consensus emerged that implantation rates are lower and miscarriage rates are higher following transfer of a mosaic embryo compared with euploid embryo transfer' in the study by Viotti et al (2021) as none of the transferred embryos were confirmed as mosaic. Vera-Rodriguez et al (2017) showed diagrammatically how the possible cell mixes obtainable by one biopsy, from various mosaic typologies, results in misdiagnosis in all but 2 out of 9 cases. Takahashi et al (2021) have showed that chromosomal status analysis based on trophectoderm biopsy does not accurately reflect the chromosomal status of the whole "mosaic" blastocyst. In one systematic review of studies, 57% of re-biopsied embryos originally classified as mosaic, were wrong (Marin, Xu and Treff (2021)).	The paragraph has been adapted and we removed the sentence saying there was a clear consensus. We have further adapted our terminology, referring to 'embryos with putative mosaic results', and included in the previous section that mosaicism in the biopsy sample may not be representative for the embryo.
Amanda Odell-West	2	53	The date of the PGDIS position statement was 2019, not 2021. Criticism of the CoGEN statements and PGDIS position/practice statements should be included for balance. For example, in one study by Popovic et al (2019), 25 embryos originally classified as either "euploid/mosaic" or aneuploid were re-checked using next generation sequencing (NGS) at eight- or twelve-days culture. Seven embryos originally classified as "mosaic" were re-classified as euploid and reported viable at 12 days culture, even in cases where the degree of mosaicism exceeded 50%. These data are at odds with the view expressed in the PGDIS 2019 statement that high levels of mosaicism (>40-80%) in a first biopsy led to similar levels of mosaicism in the trophectoderm and inner cell mass upon subsequent analysis and that the "relative percentage of mosaicism seems a better predictor of outcome rather than the specific chromosome(s) involved". Other research that conflicts with the PGDIS and CoGEN guidance includes an important study on 100 mosaic embryos which reported the degree or level of mosaicism should not be used to prioritise mosaic embryos (Victor et al, 2019). The International Do No Harm in IVF group was formed in response to the 2019 PGDIS statement. The group is highly critical of what it views as a "bewildering ability" of PGDIS to influence worldwide IVF/PGT-A practice badly, causing concerns about patient autonomy, safety and efficacy, and the need to minimise viable embryo wastage (N. Gleicher et al. 'The 2019 PGDIS	We have corrected the reference to the PGDIS statement, also including the most recent version. As the aim of the introduction is to provide a state-of-the-art, we have listed the available statements and recommendation. Making an interpretation or judgement on these documents is outside the scope of this paper.

			position statement on transfer of mosaic embryos within a context of new information on PGT-A' Reprod Biol Endocrinol 18 (2020), 57.	
Amanda Odell-West	13	364	Counselling should include information about how PGT-A testing and the phenomenon of chromosome mosaicism is likely to affect clinical diagnosis and embryo selection.	We have added that the counseling should include the centre's policy on transfer and cryopreservation of mosaic embryos.
Amanda Odell-West	14	385	The rationale for it being acceptable for a lab not to report mosaicism rates, is unclear.	We have adapted this recommendation to: It is acceptable for a PGT centre not to report mosaicism, provided that the centre has a strategy for classifying embryos and acts on it.
Amanda Odell-West	14	368	Risk assessment should include psychological morbidity which is well recognized following IVF failure (Bryson and Traub (2002); Martins and Rackowski (2018)). This will be of particular importance as informed consent to PGT-A must encompass the risk of viable embryos being misclassified as "mosaic" and deprioritized/ discarded. Risk assessment should also include risks to the viable preimplantation embryo and risks to a child born following PGT-A. Evidence that assisted conception technologies are clearly associated with a range of adverse early life outcomes including rare imprinting disorders exist, of importance considering the emerging links between epigenetic variation and a range of adverse adult-onset cardiometabolic, neurodevelopmental, and respiratory conditions (Novakovic et al (2019). An increased incidence of autism spectrum disorders is reported in ART-conceived persons (Lui et al, 2017). There are no data on the long-term consequences of TEB for children born following PGT-A; potential effects on children born after prolonged culture and/or cryopreservation are similarly under-investigated (Sciori and Dattilo (2020); Verpoest et al (2018).	The statement on risk assessment is related only to the topic under discussion, being genetic testing and mosaicism. Patient counselling should also cover the risks of ART treatments, embryo culture etc, but it is outside the scope of the current paper to elaborate on this topic. More detailed information on patient counselling in the context of ART and PGT has been previously published (ESHRE PGT Consortium good practice recommendations for the organisation of PGT. Human Reproduction Open. 2020;2020.)
Manuel Viotti		410-429	However, the 'recommendations' part of the manuscript (particularly lines 410-429) are heavily biased to highlight a single study using a particular PGTA methodology, mostly due to its design (prospective, non-selection) rather than its sample size, technical prowess, or execution – thereby dismissing dozens of peer-reviewed publications simply because they are retrospective in nature and have a potential for bias. This also nonchalantly ignores the analyses performed that control for such potential bias (see for example Viotti et al 2021a, Fig.1) The following point is misleading and should be revised: 'However, retrospective studies are affected by the fact that mosaic embryos are transferred as last option in women with previous failed transfers with euploid embryos. This introduces a significant selection bias, where mosaic embryo reproductive outcomes are measured on a poor prognosis population of patients.' (lines 44-46). For example, in the 1,000 mosaic embryo transfer study, in 51.7% of cases there was no previous euploid embryo available, so there could not have been a previous failed transfer. Of the remaining 48.3% of cases that did have previous euploid embryo transfers, a proportion resulted in babies.	We have adapted the text and corrected the errors in the representation of the Viotti study, putting less emphasis on the prospective, non-selection trial.
Francesca Spinella and Ermanno Greco		Rec 11 and 15	it is not clear from which scientific data and from which studies these conclusions are drawn.	We have created a section with further information on the studies and data considered in the recommendations.
Liborio Stuppia	3	104:	independent PGT centres not linked to ART centres"- This sentence can be confusing. A PGT centre should be connected in some way with an ART centre. Does the sentence means that analysis are carried out in outsourcing labs as a service? In this case, what about the protocols of shipment of the embryo samples?	We have clarified this in the text by adding "independent PGT centres not linked to a specific ART centre (i.e. performing PGT for several ART centres)"

Liborio Stuppia	3	107-108	There is a huge variability in the number of ART cycles performed by the different centres. (from less than 50 to more than 10.000. for year). Could this induce a bias in the statistical analysis ?	There is a huge variability in the number of ART/PGT cycles performed by the different centres both in the survey and in practice. The results of the survey include descriptive only, and should be considered with respect to the background information of the centres, with regards to their location, their size etc.
Liborio Stuppia	7	167	About half of the centres include full information on aneuploidy results in their PGT reports: Does this mean that in the remaining half of centres full information on aneuploidy results are provided? Which kind of results are provided by these centres?	We have corrected the sentence, now reading "About half of the centres include full information on aneuploidy results in their PGT reports (including whole chromosome aneuploidy, segmental imbalances (gain/loss) and intermediate copy number results and segmental aneuploidies and mosaicism), over all indication groups, while most of the others report aneuploidy (but not mosaicism). '
Liborio Stuppia	7	178	"9.7% (9/93) only emphasized the need for genetic counselling for prioritization " - Did these centres have an internal service for genetic councelling?	We have adapted the sentence. 9.7% (9/93) emphasized the need for genetic counselling for prioritization without providing any further details, the others provided further information which was already included in the text
Liborio Stuppia	9	220-221	"It is unclear whether this decision is taken in consultation with the patient" - This is a very important issue. Centres should be encouraged to discuss with patients about such critical decisions	We agree with the reviewer on the importance of patient discussion and shared decision making, but unfortunately the survey did not allow us to comment on current practice on this aspect.
Liborio Stuppia	10	Line 230:	"Patient counselling occurs at one timepoint, i.e. before the start of the PGT cycle (46.7%)" - Genetic counselling as a rule must be performed before and after testing.	The section the reviewer is referring to is related to the survey, showing clinical practice, and in this case showing points were adaptations to clinical practice may be warranted based on the recommendations
Liborio Stuppia	11	244	"Following the transfer of a mosaic embryo, prenatal diagnosis was recommended in 95% of centres." - Although 5% is a minimal part of the investigated sample, I anyway think that prenatal diagnosis must be performed in any case after the transfer of a mosaic embryo.	The section the reviewer is referring to is related to the survey, showing clinical practice, We have made a recommendations for prenatal diagnosis