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ORIGINAL RESEARCH

Concordance of various chromosomal errors among different parts of the embryo and the value of re-biopsy in embryos with segmental aneuploidies

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ABSTRACT: Chromosomal mosaicism detected during preimplantation genetic testing for aneuploidy (PGT-A) and its impact on embryo implantation have been widely discussed, and healthy live births from mosaic embryos were reported by many groups. On the other hand, only very few studies have focused on segmental chromosome aneuploidies and their clinical impact. Eighty-nine embryos with various PGT-A results (trophectoderm 1: TE1) were re-analysed using a second trophectoderm biopsy (TE2) and the rest of the embryo (RE) for testing. Of 19 euploid TE1 biopsies, 18 were concordant across TE2 and RE. Similarly, whole chromosomal aneuploidies were concordant in 59 of 62 TE1-TE2 and 58 TE1-RE. In contrast, from 31 segmental aneuploidies detected in TE1, only 15 were observed again in TE2 and 14 in RE. If a TE1 segmental abnormality appeared again in TE2, it was almost always present in RE (17/18) as well. Moreover, when a TE1 segmental abnormality was not detected in TE2, in 12 out of 13 cases RE was also unaffected. Similarly, only 1 of 26 TE1 whole chromosome mosaics were repeated in TE2 and 7 in RE. Our study confirms that euploid and whole chromosomal aneuploidy results are highly predictive of the embryo. In contrast, mosaicism has a very low concordance rate. Most importantly, re-biopsy of embryos with segmental aneuploidies demonstrated that they are mostly not uniform across the embryo. Finally, in the case of segmental aneuploidy, the second biopsy enables an accurate prediction of the real status of the embryo and could be offered to patients undergoing PGT-A.

Key words: preimplantation genetic testing for aneuploidy / segmental aneuploidy / mosaicism / embryo re-biopsy / massive parallel sequencing / concordance of chromosomal errors / infertility / ART

Introduction

PGS, newly termed preimplantation genetic testing for aneuploidy (PGT-A), has become the gold standard in IVF treatment especially in patients with advanced maternal age. It has been shown that an increasing number of couples are postponing pregnancy until an advanced age, leading to an increasing number of women suffering from infertility problems (Cooke *et al*., 2012; Johnson *et al*., 2012). It is well known that the number of whole chromosomal aneuploidies in embryos increases rapidly with maternal age (Munné *et al*., 2007; Franasiak *et al*., 2014) and can lead to development arrest, implantation failure, miscarriage or even the birth of an affected child (Hassold and Hunt, 2001; Dahdouh *et al*., 2015; Maurer *et al*., 2015).

In recent years, methods used for identifying euploid embryos have evolved rapidly from fluor escent *in situ* hybridisation (FISH), through quantitat ive PCR and array-based comparative genome hybridisation to the now widely used next generation sequencing (NGS). Single blastomere biopsies have been widely replaced with 5–10 cell trophectoderm (TE) biopsies, leading to an increased resolution and an enhanced view of embryo quality, enabling more reliable and precise PGT-A results (Scott *et al*., 2013). An undeniable benefit of PGT-A using NGS lies in the high dynamic range analysis, providing improved detection of chromosomal mosaicism compared to other techniques (Fiorentino *et al*., 2014; Kung *et al*., 2015). However, the phenomenon of mosaicism has become challenging for interpretation, with occurrence of these errors being reported in up to ~25% of IVF embryos

(McCoy et al., 2015). Many groups have focused on this phenomenon recently, providing data about implantation rates, ongoing pregnancy rates and birth rates after transferring mosaic embryos (Fragouli et al., 2017; Munné et al., 2017b; Victor et al., 2019b).

Together with mosaicism detection, NGS-based PGT-A has been reported as a good tool for segmental (sub-chromosomal) aneuploidy detection with a higher resolution than alternative methods (Lai et al., 2017). However, compared to mosaicism, only a few studies have focused on this issue and the possible potential of an embryo with segmental errors. The incidence of these errors varies between 4 and 58%, depending on the method used and the data set size (Treff and Franasiak, 2017). Interestingly, in light of recent studies, we know that the majority of segmental errors in TE biopsies are of mitotic origin, originating in the first few cell divisions in cleavage stage embryos and are therefore not present in every cell of the embryo (Vera-Rodríguez et al., 2016). This finding suggests that many potentially viable embryos are unknowingly being called segmental aneuploid or segmental mosaic and are being discarded based on the small cell population in the biopsy.

In our study, we selected euploid and affected embryos with various chromosomal errors for evaluation of the concordance rate between two different TE biopsies (TE1 and TE2) and the rest of the embryo (RE), including the inner cell mass (ICM). Thirty-one samples reported as containing segmental aneuploidies were included to investigate the true origin of these errors and their concordance rates. The results obtained provide increased insight into the actual chromosomal status of an embryo, but most importantly we have investigated the benefits of TE re-biopsy to help confirm the uniformity of segmental errors.

Material and Methods

Embryos and NGS analysis

For the purpose of this comparative study, a total of 89 PGT-A blastocysts were donated by 65 couples undergoing infertility treatment. All original trophectoderm biopsies (TE1) were obtained following IVF-ICSI and an embryo culture protocol previously described (Kubicek et al., 2019) followed by SurePlex (Vitrolife, Gustaf Werners gata 2 SE-421 32 Västra Frölunda, Sweden) whole genome amplification (WGA) and VeriSeq (Vitrolife) based PGT-A. To increase the reproducibility of PGT-A findings in this study and to discount any effects of using an alternate library preparation and analysis software, all original TE1 Sureplex (Vitrolife) amplification products were re-processed with the PG-Seq (PerkinElmer, 940 Winter Street, Waltham, MA, 02451, USA) library preparation protocol and Nexus Copy Number software (BioDiscovery, Inc., 715 North Douglas Street, El Segundo, CA 90245, USA) according to the manufacturer's protocol.

Ethical approval for this study was sought and a waiver was obtained on 1 August 2019 from the Ethical Commission for Assisted Reproduction Techniques and Preimplantation Genetic Diagnosis (IRB reference number: 012019).

All selected embryos were de-vitrified, and the second TE2 biopsy was obtained according to the same protocol as TE1, with the consent of patients. In all cases, the RE, including the ICM, was tubed separately. All TE2 biopsies and RE were subjected to DOPlify (PerkinElmer) WGA and PG-Seq based PGT-A, according to the manufacturer's protocol. All copy number variant (CNV) profiles were analysed independently by three analysts using the Nexus Copy Number software (BioDiscovery). All embryos in this study were selected based on PGT-A results to achieve equal distribution of chromosomal abnormalities over chromosomes in order to generalize the results of this study. The status of all analysed embryos and a summary of all individual errors detected in TE1 biopsies via PG-Seq based PGT-A re-analysis is shown in the Table I.

Table I Analysed human embryos and all individual errors detected in the first trophectoderm biopsy.

Status of embryos			
Euploid	19	True euploid	5
		Unbalanced	14
Aneuploid	65	Whole chromosome	42
		Segmental	23
Mosaic	5	Whole chromosome	2
		Segmental	3
Individual errors			
Aneuploidies	62		
Segmentals	31		
Mosaics	26		
Segmental mosaics	7		

Aneuploidy and mosaic calling thresholds

The threshold for aneuploidy detection was set $>80\%$ (0.48 for trisomy and -0.72 for monosomy on a logR plot) and the mosaic interval was set from 30 to 80% (0.18 to 0.48 for gains and -0.27 to -0.72 for losses on logR plot). Samples with copy number values $<30\%$ were designated as euploid.

Validation

A manufacturer's validation study was performed on a total of 250 five-cell and 192 single-cell samples to confirm the accuracy of the PG-Seq kit workflow prior to commencement of this study. Cell line samples with known chromosomal abnormalities (Coriell Institute, 403 Haddon Avenue, Camden, NJ 08103, USA) along with peripheral lymphocytes from a male and female donor of proven fertility were manually isolated as described previously (Hussey et al., 1999) before analysis with PG-Seq according to the manufacturer's instructions. Cell karyotypes included in the validation data set were as follows: 47,XY,+9, 47,XY,+13, 47,XY,+15, 47,XX,+18 47,XX,+21, 47,XY,+22, 48,XY,+16,+21, 48,XXY,+21, 46,XX and 46,XY. Additionally, cell lines with known segmental errors ranging in size from 7 to 31 MB were obtained and included to confirm the theoretical resolution of the kit.

Unbalanced embryos derived from translocation cases ($n=14$) were included in this study for clinical validation of segmental aneuploidy detection and evaluation of the resolution of the PG-Seq kit (PerkinElmer) and Nexus Copy Number software (BioDiscovery). The size of segmental errors in this group varied from 4 to 100 MB. These

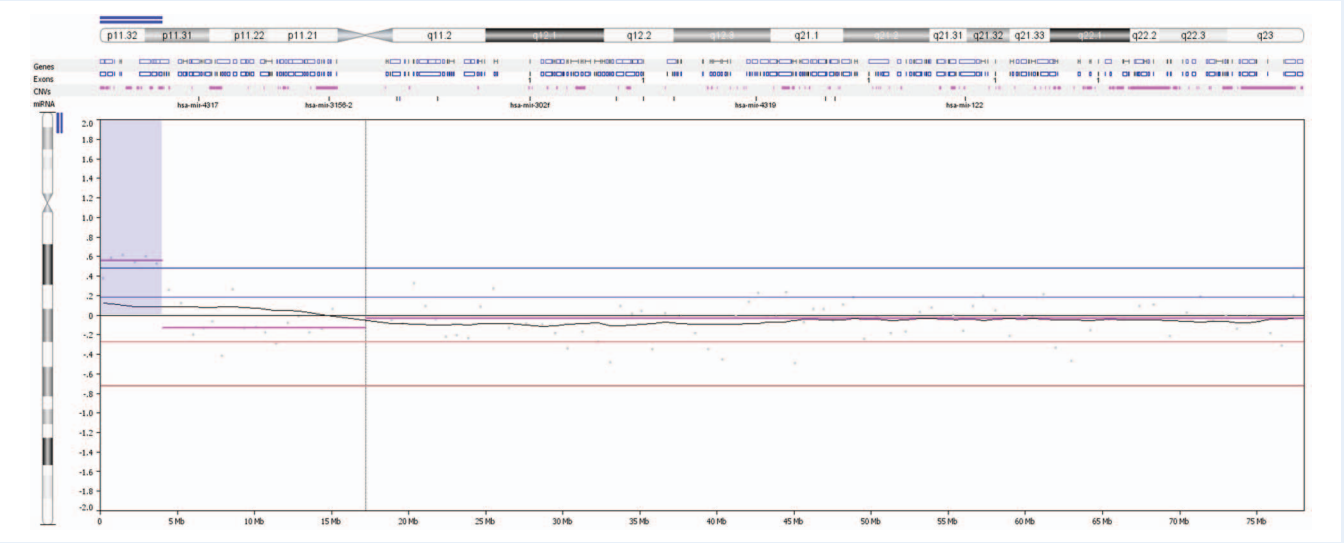

Figure 1 Copy number variant plot of detected 4 Mb segmental gain on chromosome 18 (position 0-4.038.924) in a human embryo detected using PG-Seq.

unbalanced embryos were included as part of the euploid embryo group to increase the sample size of euploid results based on the fact that segmental errors on derivative chromosomes observed in unbalanced embryos are always of a meiotic origin and are present in every cell of the embryo.

Data analysis

Concordance rates of different chromosomal error types were evaluated between the first and second trophectoderm biopsy (TE1–TE2), between the TE1 and the rest of the embryo (TE1-RE) and between TE2 and the rest of the embryo (TE2-RE). GraphPad Prism (GraphPad, 2365 Northside Dr #560, San Diego, CA 92108, USA) was used for statistical analysis. Results were considered as statistically significant if the P value was < 0.05.

Results

PG-Seq validation

The manufacturers' validation study of the PG-Seq kit showed 98.4% accuracy for five-cell samples and 95.8% accuracy for single-cell samples. Segmental errors of 7–31 Mb were detected with 98.3% sensitivity and specificity in five-cell samples (https://perkinelmer-appliedgenomics.com/home/preimplantation-genetic-testing/pg-seq-kit-2-0/). In the group of 62 segmental error samples, 3 (4.84%) were found to contain false-positive segmental errors in addition to the expected deviations. These results were obtained from cell lines that are known to contain low level mosaicism from karyotyping data and confirmed acceptable performance of the kit.

In the sample set of unbalanced embryos derived from translocation cases that were tested in the current study, all expected segmental errors were detected in both TE biopsies (TE1 and TE2) and in RE. Two unbalanced embryos with a derivative chromosome 18 containing a 4-Mb gain (chr18:0-4,038,924) were successfully detected (Fig. 1), demonstrating clinical resolution greater than the manufacturer's claims of 7 Mb.

Table II Overview of all individual errors detected in TE1, TE2 and RE.

	TE1	TE2	RE
Euploid	19	31	31
Aneuploidies	62	66	63
Segmentals	31	15	17
Mosaics	26	9	20
Segmental mosaics	7	7	6

TE1: first trophectoderm biopsy, TE2: second trophectoderm biopsy, RE: rest of the embryo

Analysis of TE2 and RE

All 89 TE2 and RE samples were successfully amplified and analysed and passed all quality control metrics with an average number of reads ~500 000 per sample. All biopsies and the corresponding PGT-A results are given in the summary Table II, with more detailed data in Supplementary Table SI.

Assessment of whole chromosome concordance rates

Concordance of all 62 whole chromosomal errors was established between TE1 and TE2, TE1 and RE and TE2 and RE. Eighteen out of 19 (94.7%) euploid TE1 results were concordant in TE2 and RE. There were an additional 12 euploid samples in TE2 that were not euploid in TE1. Of the total 31 euploid results from TE2, 26 (83.9%) of them were concordant in the RE. Of these TE2-RE non-concordant results, none of the RE results were concordant with TE1.

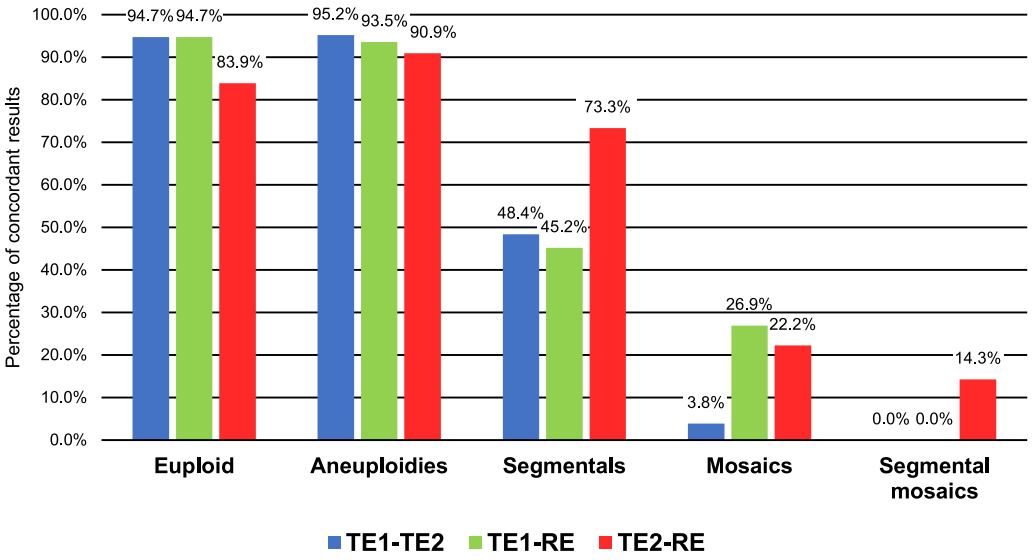

Figure 2 Concordance of different error types and euploid results between trophectoderm biopsy 1 and 2, and the rest of the embryo. TE1: trophectoderm biopsy 1, TE2: trophectoderm biopsy 2, RE: rest of the embryo.

Table III Changes of whole chromosome aneuploidies and whole chromosome mosaics detected in TE1.

TE1	TE2			RE		
Aneuploidy	Aneuploidy	Mosaic	Normal	Aneuploidy	Segmental	Normal
62	59	1	2	58	1	3
Mosaic	Mosaic	Aneuploidy	Normal	Mosaic	Aneuploidy	Normal
26	1	4	21	7	1	18

The data show all individual whole chromosome aneuploidies and whole chromosome mosaics detected in the TE1 biopsy and results of re-analysis of these errors in TE2 and RE.

All concordance rates are shown in Figure 2, with more detailed data about the karyotype results of all biopsies shown in Supplementary Table SI. In the case of TE1 whole chromosomal aneuploidies, 59 out of 62 (95.2%) were concordant in TE2 and 58 (93.5%) in RE. Concordance between TE2 and RE reached 90.9% (60 out of 66). Results of the whole chromosomal aneuploidies and mosaics detected in TE1 are shown in Table III.

Reproducibility of segmental errors

There were 31 segmental errors found in TE1, ranging in size from 5 to 150 Mb and found on 18 different chromosomes. Only 15 out of 31 (48.4%) segmental errors in TE1 were also detected in TE2 and only 14 (45.2%) in RE. Out of the 15 segmental errors detected in TE2, 11 (73.3%) were also concordant in RE (Fig. 2).

The reproducibility of segmental errors and whole chromosomal mosaics was assessed based on results from the TE re-biopsy (TE2) and RE analysis. For segmental errors, 17 aneuploid TE2 samples manifested as segmental or whole chromosomal aneuploidies again when analysing RE, and in one case the original segmental was not detected in any form making the true positivity rate of the re-biopsy analysis 94.5% ($P < 0.001$). If the original TE1 segmental error was not found in TE2 in any form (13 discordant results), 12 REs showed normal results as well, making the true negativity rate of the re-biopsy results 92.3% ($P < 0.001$). Detailed data are shown in Figure 3. The size of all individual segmentals in the group of 17 true positive TE2 concordant results range from 5 to 150 Mb, and in the group of 12 true negative TE2 the discordant segmentals range from 7 to 105 Mb. Detailed data about the correlation between concordance and size of the segmental aneuploidy is shown in Supplementary Table SII, with more detailed data about segmental aneuploidies in Supplementary Table SI. No correlation was observed between the concordance of individual segmental aneuploidies and particular chromosomes or chromosomal regions.

Reproducibility of whole chromosome mosaicism

A single whole chromosomal mosaic in TE1 was concordant with TE2 (1 of 26, 3.8%) and seven of these results (26.9%) were concordant in RE. Comparing TE2 and RE, two out of nine (22.2%) whole chromosomal mosaics were concordant (Fig. 2).

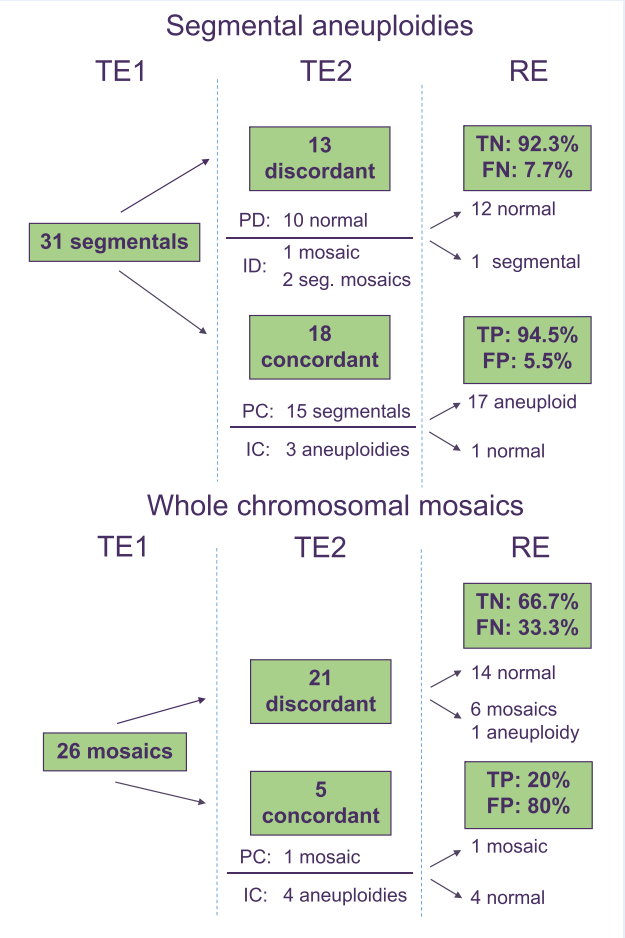

Figure 3 Reproducibility of segmental errors and whole chromosome mosaics. Thirty-one segmental errors detected in TE1 were divided into two groups in TE2 based on the observed result. In 13 cases, results of TE2 analysis were discordant with TE1. Ten of them were perfectly discordant (PD, diploid chromosomes) and three were imperfectly discordant (ID, segmental and whole chromosome mosaics). Eighteen TE2 results were concordant with TE1 segmental findings. Fifteen of them were perfectly concordant (PC, original segmentals), and three segmentals manifested as a whole chromosome loss in TE2 (imperfectly concordant results, IC). Further analysis of RE showed the true negativity rate (TN) of TE2 re-analysis was 92.3% (false negativity, FN: 7.7%), and the true positivity rate (TP) was 94.5% (false positivity, FP: 5.5%). When reviewing the reproducibility of samples reported as whole chromosome mosaic, 26 detected in TE1 were similarly divided into concordant or discordant groups. From five concordant TE2 results, one was perfectly concordant (PC, mosaic) and four were imperfectly concordant (IC, whole chromosome aneuploidy). RE analysis revealed the true negativity rate (TN) of 21 discordant (normal) TE2 re-biopsies as 66.7% (false negativity; FN: 33.3%) and true positivity rate (TP) of five concordant TE2 re-biopsies as 20% (false positivity, FP: 80%).

For whole chromosome mosaics, one concordant TE2 sample was again concordant when analysing RE, illustrating one embryo with apparent embryo-wide cellular mosaicism not confined within cell populations. In four cases, the original mosaic result in TE1 was aneuploid in TE2 and euploid in RE, making the true positivity rate of re-biopsy analysis 20% ($P = 0.094$). From 21 discordant mosaic TE1 and normal TE2 results, only 14 were again normal in RE making the true negativity rate of re-biopsy analysis 66.7% ($P = 0.036$). Detailed data are shown in Figure 3. Results of the whole chromosomal aneuploidies and mosaics detected in TE1 are stated in Table III. Detailed data of all TE1, TE2 and RE mosaic percentages are stated in Supplementary Table SI.

Reproducibility of segmental mosaics

None of the TE1 segmental mosaics were detected again in TE2 and RE and only one out of seven (14.3%) was concordant between TE2 and RE. Of the six non-concordant segmental mosaic results in TE2, one was segmental aneuploid for the same chromosome in both TE1 and RE and another was segmental aneuploid for the same chromosome in TE1 and euploid in RE. There were also five segmental mosaic aneuploidies detected in RE, four of which were not found in TE1 or TE2 and one was a segmental aneuploidy for the same chromosome in TE1. There were no mosaic segmental aneuploidies found in all three samples from the same embryo. Summary results are in Table III.

Discussion

Concordance of the second TE biopsy (TE2) and the rest of the embryo (RE)

The reliability of PGT-A based on the sampling of very few TE cells and its representation of the whole embryo is questioned by several groups (Esfandiari *et al.*, 2016; Orvieto *et al.*, 2016; Gleicher and Orvieto, 2017; Gleicher *et al.*, 2017). Some studies have even gone so far as to describe live births after transferring PGT-A aneuploid embryos, suggesting the possibility that viable embryos are being discarded based on false positive PGT-A results (Gleicher *et al.*, 2015; Darilek *et al.*, 2018). In the present study, we performed re-biopsy of 89 embryos originally analysed with an NGS-based PGT-A protocol. We found that whole chromosome aneuploidies detected in TE biopsies are highly predictive for the rest of the embryo, with a concordance rate for aneuploidy of the same chromosome of 93.5%. The same was seen for euploid results, with 94.7% embryo concordance. These results correlate with observations of other studies (Huang *et al.*, 2017; Victor *et al.*, 2019a) suggesting the undeniable accuracy of NGS-based PGT-A using TE biopsy for euploidy or whole chromosome aneuploidy detection. It is possible that false positive PGT-A results and reported live births after the transfer of aneuploid embryos in published studies could be impacted by different methodologies and aneuploidy evaluation criteria across PGT laboratories. It is crucial in a clinical setting that full aneuploidies are correctly distinguished from high percentage mosaics, and further study on this matter is required.

For segmental aneuploidies, the concordance rate decreased significantly compared to whole chromosome aneuploidies, suggesting a different origin of these errors. This observation supports the theory that whole chromosome aneuploidies are mainly of meiotic origin and are derived from oocytes (Nagaoka *et al.*, 2012), while segmental errors on the other hand are more often of mitotic origin and likely to arise during

the first few mitoses after fertilisation (Vera-Rodríguez et al., 2016; Babariya et al., 2017). Moreover, as a previous study described, full and mosaic segmental losses are most frequently of paternal origin and could be caused by fragmentation during spermatogenesis (Kubicek et al., 2019). Owing to the fact that the human embryonic genome is not active during the first mitotic divisions (Braude et al., 1988), the speed of mitosis and laxness of the cell cycle control mechanism may be the reason for the occurrence of these errors in cleavage stage embryos (Babariya et al., 2017). It has also been suggested that artefacts introduced by WGA may be misinterpreted as segmental errors (Treff and Franasiak, 2017). This could account for several of the non-concordant results observed between biopsies of the same embryo in the current study, particularly when the segmental errors present in one biopsy are euploid in another. The PG-Seq manufacturer's validation study found that up to 5% of cell line samples contained unexpected false positive segmental errors. WGA artefacts might be introduced by S-phase DNA replication (Van der Aa et al., 2013), or as an effect of the smoothing algorithm on chromosomal copy lines used in CNV software (Treff and Franasiak, 2017) and could be a reason for false-positive segmental mosaic calling, especially related to smoothing of low-quality and noisy data. WGA artefacts require further study and should be considered when reporting segmental mosaic or segmental aneuploidy with regard to their size and mosaic level.

An even more striking difference in concordance rates was shown in the group of whole chromosomal mosaics. Whole chromosomal mosaic findings in a TE biopsy or rebiopsy appear to have a very low predictive value towards the status of the rest of the embryo. Interestingly, 13 whole chromosomal mosaics detected in the RE were not detected in the original TE biopsy (TE1) and only one was detected in TE2. Once again, it is possible that some apparent mosaicism is actually caused by a technical artefact(s), with WGA phenomena such as the wave and step change artefact and ramping chromosome artefacts being previously described (https://support.illumina.com/downloads/veriseq-pgs-technical-guide-to-aneuploidy-calling.html). The step change or wave artefact can be especially difficult to distinguish from low level mosaicism. If misinterpreted as mosaicism, it would impact the accuracy of statistical analysis when reviewing implantation rates and ongoing pregnancy rates after transferring apparently mosaic embryos, which could in fact have been euploid embryos in the first place. It is known that several groups have described live births after transferring mosaic embryos, however with lower implantation rates and higher miscarriage rates (Fragouli et al., 2017; Munné et al., 2017b). Our data showing low concordance of mosaic results across the embryo has confirmed that the transfer of mosaic embryos could provide a chance for patients without any euploid embryos to conceive a healthy child. It is essential that proper counselling is carried out based on the Preimplantation Genetic Diagnosis International Society recommendations (http://www.pgdis.org/docs/newsletter_071816.html), together with correct mosaic calling and interpretation.

The accuracy of mosaic PGT-A results from TE biopsy compared to the ICM is an area of intense investigation as we try to understand how to interpret these results and decide on the best clinical approach. For example, a recent study described two embryos reported as high percentage mosaics (60–70%) from TE biopsy and the ICM was aneuploid, suggesting that lower prioritisation of these embryos is required (Chuang et al., 2018). However, the data herein shows that the TE1 mosaic percentage is not predictive for the rest of the embryo (Supplementary Table SI). For example, one mosaic over 60% in TE1 was not detected in TE2 or RE and another 35% mosaic manifested as a full aneuploidy in RE. Nevertheless, both the published study (Chuang et al., 2018) and the data herein have small sample sizes and the correlation between the mosaic percentage of a TE biopsy and embryo potential should be further investigated. However, the hypothesis herein of incorrect sorting of embryos by the mosaic percentage is supported by the observations on full segmental errors presented in this study, as almost half of the non-mosaic segmental aneuploidies detected in the TE1 are in fact mosaics and yet they are not present in the rest of the embryo.

Interestingly, a different concordance rate was observed when comparing TE2 and RE in the group of euploid results and segmental errors. Only 83.9% (26/31) of euploid results in TE2 were concordant when analysing RE. This discrepancy is likely caused by preselection of embryos with segmental errors in TE1 for the current study which, as is already described, are frequently mosaic. These euploid results in a second biopsy are also, for the same reason, more likely to be discordant when analysing the rest of the embryo and do not reflect the true concordance rate for euploid results in embryos that are euploid in TE1. Similarly, preselection in the current study also affected the concordance in the group of segmental errors. An increased percentage (73.3%) of concordant results when comparing segmental errors found in TE2 and RE is likely to be due to a decreased total number of segmental errors detected in TE2 ($n = 15$) compared to TE1 ($n = 31$). These segmentals found in both TE1 and TE2 are more likely to be present in every cell of the embryo, as reflected by RE analysis.

Reproducibility of segmentals and whole chromosomal mosaics

According to our observations, segmental errors detected in TE1 were frequently absent when analysing TE2 and RE. Our results show that 45.2% (14/31) of segmentals detected in TE biopsies are in fact mosaics. However, and most importantly, our data have confirmed that re-biopsy and an additional round of PGT-A provides an excellent insight into the true chromosomal state of the embryo. This is consistent with other studies which described the possibility of the re-biopsy of embryos with segmental errors (Victor et al., 2019a; Victor et al., 2019b). Despite the small sample size in the group of true positive and true negative re-biopsy (TE2) results, reproducibility of segmental finding seems not to be affected by its size, since the sizes range from 5 to 150 Mb, and 7 to 105 Mb, respectively. However, the impact of segmental size on its reproducibility should be further investigated on larger data sets. Interestingly, applying a similar re-biopsy approach in the group of whole chromosome mosaics provided very different results. Naturally, the group of whole chromosome mosaics is small and could be biased by internal heterogeneity, but re-biopsy and re-analysis of embryos with whole chromosome mosaics appeared to be of no benefit considering the very low predictive value. This is in stark contrast to segmental aneuploidies.

The viability of re-biopsied embryos with repeated thaw cycles could be compromised (Bradley et al., 2017), but our own unpublished data and a recent study performed on 104 re-biopsied embryos showed

no impact on implantation and pregnancy rates (Neal *et al*., 2017). Since the ART treatments differ significantly between IVF clinics and it is known that this has a strong impact on embryo ploidy (Munné *et al*., 2017a), clinical and operator experience could also impact the viability of re-biopsied embryos. An internal analysis of the possible impact on the embryo together with the potential benefits should be considered before routine re-biopsy becomes practice, along with proper counselling of patients. The data herein suggest that re-biopsy of embryos with segmental aneuploidies could be offered to PGT-A patients. More supporting data on a larger scale are necessary, but our results and other consistent studies suggest the possible inclusion of re-biopsy practice into the future PGT-A recommendations and PGT-A patient clinical management.

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Authors' roles

R.N.—conception and design of the study, acquisition of data, analysis and interpretation of data, author of the manuscript. J.H.—substantial contributions to conception and design of the study, analysis of data. M.H.—substantial contributions to conception and design of the study, analysis of data. D.K.—substantial contributions to conception and design of the study, analysis of data. M.B.—substantial contributions to conception and design of the study, analysis of data. G.T.—final approval of the version to be published. P.T.—final approval of the version to be published. K.V.—final approval of the version to be published.

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Conflict of interest

We declare no conflict of interests.

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