Copy number analysis of meiotic and postzygotic mitotic aneuploidies in trophectoderm cells biopsied at the blastocyst stage and arrested embryos

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Abstract
Preimplantation genetic testing for aneuploidy (PGT-A) by copy number analysis is now widely used to select euploid embryos for transfer. Whole or partial chromosome aneuploidy can arise in meiosis, predominantly female meiosis, or in the postzygotic, mitotic divisions during cleavage and blastocyst formation, resulting in chromosome mosaicism. Meiotic aneuploidies are almost always lethal, however, the clinical significance of mitotic aneuploidies detected by PGT-A is not fully understood and healthy live births have been reported following transfer of mosaic embryos. Here, we used single nucleotide polymorphism genotyping of both polar bodies and embryo samples to identify meiotic aneuploidies and compared copy number changes for meiotic and presumed mitotic aneuploidies in trophectoderm cells biopsied at the blastocyst stage and arrested embryos. PGT-A detected corresponding full copy number changes (≥70%) for 36/37 (97%) maternal meiotic aneuploidies. The number of presumed mitotic copy number changes detected exceeded those of meiotic origin. Although mainly in the mosaic range, some of these mitotic aneuploidies had copy number changes ≥70% and would have been identified as full aneuploidies. Interestingly, many arrested embryos had multiple mitotic aneuploidies across a broad range of copy number changes, which may have arisen through tripolar spindle and other mitotic abnormalities.

1 | INTRODUCTION

Abnormal chromosome copy number, or aneuploidy, is a major cause of IVF failure, pregnancy loss and rarely, abnormal pregnancy and live births.1-3 Most aneuploid embryos are not viable and fail to implant or are lost through miscarriage later in pregnancy. A few aneuploidies, however, mainly of the small acrocentric chromosomes and the sex chromosomes, are compatible with development to term, though the incidence of recognised aneuploidies at birth is rare (0.3%-0.5%).4 Hence, preimplantation genetic testing for aneuploidy (PGT-A) is now widely used to select viable euploid embryos following trophectoderm biopsy at the blastocyst stage, vitrification and transfer in later managed cycles.5,6

The incidence of aneuploidy in the human preimplantation embryo is high, with some studies indicating an overall incidence of 50%.7 Most aneuploidies associated with miscarriage or affected live births are caused by errors in meiosis, predominantly female meiosis, though there is variation among different chromosomes, and these increase exponentially in women over the age of 35 years.1,8 In addition, however, chromosome segregation errors, including non-disjunction, anaphase lag and chromosome breaks, occur in mitosis during the early cleavage divisions following fertilisation, resulting in chromosome mosaicism, both
for whole and partial chromosomes.\textsuperscript{9-11} Furthermore, we recently reported that abnormal tripolar mitosis in early cleavage results in dispersal of chromosomes among the three daughter cells, which can continue to divide, forming clones of cells with reduced chromosome number, including frequent nullisomy.\textsuperscript{12} If this occurs early, the whole embryo is affected and fails to form a blastocyst, whereas, at later stages, the affected cells are excluded from the blastocyst.

Various methods for genome-wide chromosome copy number analysis for PGT-A have been developed, including microarray comparative genomic hybridisation (array CGH) and, more recently, next generation sequencing (NGS) and mapped fragment counting. The higher resolution and linear relationship between copy number and fragment count of NGS-based testing allows accurate detection of aneuploidies. However, both methods have also highlighted that, in addition to full aneuploidies, chromosomal mosaicism, indicated by intermediate copy number changes, and partial (or segmental) chromosome copy number abnormalities are relatively common even among trophectoderm cells biopsied at the blastocyst stage. Following an initial report of healthy live births resulting from transfer of embryos with only mosaic changes detected by array CGH,\textsuperscript{13} there is now increasing clinical evidence that many of these embryos are viable and should be considered for transfer.\textsuperscript{14-16} However, pregnancy rates are lower and genetic counselling is recommended.\textsuperscript{17}

As aneuploidies of meiotic origin are inherited through aneuploid gametes at fertilisation, primarily aneuploid oocytes, all the cells of the developing embryo following fertilisation are affected and these errors predominate in the products of conception (POCs) following miscarriage.\textsuperscript{1,3} Whereas, mosaic changes, by their very nature, are of uncertain clinical significance, since they may only affect an unknown proportion of the trophoderm and/or the inner cell mass (ICM) cells of the blastocyst, from which the fetus is derived.\textsuperscript{18} Hence, it is clinically important for PGT-A to discriminate between these two types of aneuploidy, if possible.

To investigate this for both array CGH and NGS-based PGT-A, we have quantified all of the copy number changes in trophectoderm biopsies at the blastocyst stage and in arrested embryos (whole and part). Both polar bodies were biopsied for all embryos, SNP genotyped and analysed both by meiomapping and karyomapping to identify female meiotic errors.\textsuperscript{19-23} Furthermore, SNP genotyping and karyomap analysis of the same amplified DNA used for copy number analysis in the embryo samples was SNP genotyped and analysed by karyomapping to confirm the female meiotic errors and detect any additional paternal errors of meiotic origin.

## 2 Methods

### 2.1 Source of human embryos

Three couples presented with either an inherited genetic disorder and/or previous failed IVF treatment and requested either PGD of a monogenic disease (PGT-M) by SNP genotyping and karyomapping or PGT-A by array CGH or NGS based copy number analysis. Full details of ovarian stimulation, IVF/ICSI, polar body and trophectoderm biopsy and whole genome amplification, single nucleotide polymorphism (SNP) genotyping and microarray comparative genomic hybridisation (array CGH) and NGS-based copy number analysis for PGT-Amethods have been reported previously.\textsuperscript{12}

### 2.2 Meiomapping of polar bodies

Following whole genome amplification and SNP genotyping of the first and second polar bodies (PB1 and PB2), meiomap analysis was performed using a dedicated VBA macro in Microsoft Excel as previously described.\textsuperscript{21-23} Abnormal patterns of chromosome segregation in the two meiotic divisions were based on analysis of the maternal haplotype patterns in PB1 and PB2. As meiomap analysis of the polar bodies alone cannot distinguish the presence of three or four chromatids in PB1, the segregation pattern was identified by reference to the karyomap of the corresponding embryo. Comparison of the patterns of recombination in polar bodies and embryo samples was analysed by karyomapping and the predicted pattern was always concordant with the karyomap of the maternal chromosome(s) in the corresponding embryo.

### 2.3 Karyomapping of embryo samples

Karyomaps of polar bodies and embryo samples were processed from SNP genotype data using a dedicated VBA macro as previously described.
described. Karyomaps for all 22 autosomes and the X chromosome were then displayed using a second macro to analyse the pattern of recombination in the chromosomes for 46 chromosome fingerprinting. This was based on the proportion of informative SNPs for the four parental haplotypes in successive groups of 35 SNPs across each chromosome.

2.4 Analysis of copy number changes

Copy number changes were assessed manually and calculated as a percentage of the theoretical full trisomy or monosomy for array CGH and NGS-based testing. There is currently no consensus on the classification of euploid/mosaic/aneuploid copy number changes since this may vary between different methods. However, at the time these embryos were tested, most labs had adopted thresholds at 30% and 70% and those have been used to classify the changes in all the embryo samples (Table S1).

2.5 Informed consent

All of the data used in this study were generated in the course of clinical treatment involving the genetic testing of human embryos for a single gene defect and/or aneuploidy screening in a private IVF clinic licensed by the Human Fertilisation and Embryology Authority in the UK and in accordance with all relevant regulations and legislation. Follow up analysis of genetically tested embryos is established best practice for quality control purposes. All patients and their partners were counselled both by the clinician managing their treatment and a genetic counsellor and provided written informed consent for the genetic testing and follow-up analysis.

3 RESULTS

3.1 Polar body analysis

Three patients undergoing IVF for preimplantation genetic testing of monogenic disease (PGT-M) and/or aneuploidy (PGT-A), had four cycles of treatment. The first polar body was biopsied from all mature oocytes, which were then inseminated by ICSI and following fertilisation, had the second polar body biopsied (Figure 1). All the polar bodies from 51 normally fertilised embryos with two pronuclei were successfully genotyped for genome-wide SNP along with parental DNA and reference samples and meiomap and karyomap analysis performed. A total of 107 meiotic segregation errors were identified, with a majority caused by premature sister chromatid separation (PSSC) or reverse segregation (RS), which after the second meiotic division, resulted in 58 maternal meiotic aneuploidies (20 trisomies and 38 monosomies) in 21/51 (41%) embryos (for details see Tables S1 and S2).

3.2 Trophectoderm biopsy and arrested embryo analysis

The normally fertilised embryos were cultured for 4 to 7 days post insemination and 26/51 (51%) embryos reached the blastocyst stage when the trophectoderm was biopsied for whole genome amplification. Whole or part of euploid/mosaic/aneuploid chromosome copy number analysis and SNP genotyping and karyomapping were performed.
amplification, SNP genotyping and karyomap analysis in parallel with PGT-A by either microarray comparative genomic hybridisation (array CGH) or NGS-based testing (Figure 1). In one case, a poor-quality blastocyst with excluded cells was tested as a whole. The remaining 25 embryos arrested at cleavage stages on days 4 to 5 were either disaggregated to single cells (n = 8) and tested by SNP genotyping and karyotyping (as previously reported; Ottolini et al12) or the whole or part(s) of the arrested embryos amplified and tested by karyomapping and PGT-A. Including the consolidated single cell results, karyomapping was successful in 50/51 (98%) of these follow-up samples, all 58 maternal meiotic aneuploidies were confirmed and no paternal meiotic aneuploidies were identified (Table S1). In two embryos identified as euploid by polar body analysis, however, karyomapping of embryo samples identified missing maternal chromosomes: in one blastocyst, analysed whole, maternal chromosome 19 was missing and, in an arrested embryo, 12 maternal chromosomes were missing (Table S1).

At the embryo level, 11/27 (41%) embryos with no meiotic aneuploidies were identified as euploid by PGT-A and copy number analysis of trophectoderm biopsies and whole or part arrested embryos, 8 (29.5%) were identified as aneuploid with one or more full aneuploidies (defined as copy number changes ≥70%) and 8 (29.5%) had only mosaic and/or partial chromosome abnormalities (defined as copy number changes 30%-70%; Table 1 and Figure 2). In contrast, none of the 16 embryos with one or more maternal meiotic aneuploidies were identified as euploid by PGT-A and all had full aneuploidies with or without additional mosaic and/or partial chromosome abnormalities.

At the chromosome level, all 19 maternal meiotic aneuploidies in 23 trophectoderm biopsy samples were confirmed as full aneuploidies by PGT-A (Table 2). In addition, however, there were 13 full aneuploidies and 17 mosaic copy number changes, presumably of mitotic origin. In whole or part arrested embryo samples (n = 20), 15/19 (79%) meiotic aneuploidies were confirmed as full aneuploidies and 3 (16%) as mosaic. Also, there were an additional 23 full aneuploidies and 34 mosaic copy number changes, presumably of mitotic origin. The only maternal meiotic aneuploidy, which did not result in a copy number change in the corresponding embryo, was a trisomy 10 in an embryo with multiple meiotic and postzygotic aneuploidies. This embryo developed into a poor-quality blastocyst with some excluded cells and was analysed as a whole embryo (Figure 3). In this case, the trisomy is clearly identified by karyomapping but copy number is normal.

The distribution of copy number changes detected by PGT-A, calculated as a percentage of full trisomies or monosomies, in aneuploidies of meiotic compared with presumed mitotic origin is

| TABLE 1 | Chromosome copy number analysis by array CGH or NGS-based testing for preimplantation genetic diagnosis of aneuploidy (PGT-A), in trophectoderm biopsies or whole or part arrested embryos, following polar body analysis to identify maternal meiotic errors |
|---|---|---|---|
| Polar body meiomap/karyomap analysis | n | PGT-A result | Array CGH (%) | NGS (%) | Total (%) |
| TE biopsy (day 5 to 7) | | | | | |
| Euploid | 14 | Euploid | 8 (57) | - | 8 (57) |
| | | Aneuploid | 2 | - | 2 |
| | | Aneuploid/partial | 1 | - | 1 |
| | | Mosaic | 2 | - | 2 |
| | | Mosaic/partial | 0 | 1 | 1 |
| Aneuploid | 9 | Euploid | 0 (0) | 0 (0) | 0 (0) |
| | | Aneuploid | 1 | 1 | 2 |
| | | Aneuploid/mosaic | 1 | 3 | 4 |
| | | Aneuploid/partial | 1 | 1 | 2 |
| | | Aneuploid/mosaic/partial | 0 | 1 | 1 |
| Whole or part arrested embryo (day 4 to 5) | | | | | |
| Euploid | 13 | Euploid | - | 3 (23) | 3 (23) |
| | | Aneuploid | - | 2 | 2 |
| | | Aneuploid/mosaic | - | 2 | 2 |
| | | Aneuploid/partial | - | 1 | 1 |
| | | Mosaic | - | 3 | 3 |
| | | Mosaic/partial | - | 1 | 1 |
| | | Partial | - | 1 | 1 |
| Aneuploid | 7 | Euploid | - | 0 (0) | 0 (0) |
| | | Aneuploid | - | 3 | 3 |
| | | Aneuploid/mosaic | - | 3 | 3 |
| | | Aneuploid/partial | - | 1 | 1 |
presented in Figure 4. In trophectoderm biopsies analysed by array CGH, all 5 meiotic aneuploidies and 8/15 (53%) of presumed mitotic aneuploidies had full copy number changes ≥70%. Similarly, in those analysed by NGS-based PGT-A, all 13 meiotic aneuploidies had full copy number changes ≥70% (range 70%-134%), whereas only 4/25 (16%) mitotic aneuploidies were ≥70%, 13 (52%) were in the mosaic range (30%-69%) and a further 7 changes were <30% (range 19%-134%). With whole or part arrested embryo samples, there was a similar pattern. However, the overlap between the two types of aneuploidy was more pronounced with 3/17 (18%) meiotic aneuploidies in one embryo with copy number changes of about 50% (range 50%-107%) and 19/70 (27%) mitotic changes of ≥70% (range 29%-179%). Copy number changes for partial chromosome aneuploidies of presumed mitotic origin were mainly in the mosaic range in trophectoderm biopsies but more spread in arrested embryos.

TABLE 2 Comparison of chromosome copy number analysis by array CGH or NGS-based testing for preimplantation genetic diagnosis of aneuploidy (PGT-A) in trophectoderm (TE) biopsies

| PGT-A method | No of TE biopsy samples | No of maternal meiotic aneuploidies identified by polar body analysis | Meiotic aneuploidies confirmed by TE biopsy and PGT-A<sup>a,b</sup> | Additional mitotic aneuploidies identified by PGT-A<sup>a</sup> |
|--------------|-------------------------|-----------------------------------------------------------------|---------------------------------------------------------------|----------------------------------------------------------|
|              |                         |                                                                  | Full | Mosaic | Total | Full | Mosaic | Total |
| Array CGH   | 16                      | 6                                                               | 6    | 0      | 6 (100)| 9    | 6      | 15    |
| NGS         | 6                       | 13                                                              | 13   | 0      | 13 (100)| 4    | 11     | 15    |

<sup>a</sup>Full aneuploidies are defined as >70% copy number change and mosaics as intermediate copy number changes between 30% and 70%.

<sup>b</sup>No paternal meiotic aneuploidies were detected by SNP genotyping and karyomapping in these TE samples.

4 | DISCUSSION

A high incidence of chromosome abnormalities in human eggs and embryos following IVF identified by karyotyping metaphase chromosomes was first reported and suggested as a possible cause of low implantation and live birth rates in the mid-1980s and early 1990s. These observations were later extended using multicolour fluorescence in situ hybridisation (FISH) with combinations of chromosome-specific probes to interphase nuclei and highlighted that, in addition to aneuploidies affecting all cells and presumed to be of meiotic origin, cleavage stage embryos were commonly mosaic with combinations of diploid, aneuploid and haploid or polyploid cells. Maternal meiotic segregation errors were also identified by multi-colour FISH in the first and second polar bodies and these increased with advanced maternal age.
More recently, whole genome amplification has enabled genome-wide chromosome copy number analysis for all 24 chromosomes by a range of methods including SNP analysis, microarray-based comparative genomic hybridisation (array CGH) and NGS-based tests. These methods have confirmed the high incidence of chromosome aneuploidy, increasing with maternal age, and trophectoderm biopsy at the blastocyst stage for PGT-A is now widely used to select euploid embryos for transfer. With NGS-based testing, in which there is a
linear relationship between chromosome copy number and mapped DNA fragments, intermediate copy number changes between the normal two copies and full trisomies or monosomies are also commonly detected. These copy number changes are presumed to result, in most cases, from true chromosome mosaicism of postzygotic mitotic origin among the biopsied trophectoderm cells. However, in some cases, undetected sample contamination, polyploidy or technical artefacts, or the bioinformatic algorithms used to normalise copy number data across the genome, may also result in artefactual intermediate copy number changes. Furthermore, when embryos identified by PGT-A as having only mosaic copy number changes are transferred clinical pregnancies and healthy live births have been reported.\textsuperscript{13,15,16} The challenge for PGT-A is therefore to distinguish full aneuploidies from mosaic copy number changes, of whatever origin, to avoid discarding potentially viable embryos particularly in poor prognosis patients.\textsuperscript{32,33}

In this small dataset (n = 51), copy number analysis of trophectoderm biopsies by NGS-based testing broadly discriminated between full changes, mainly of meiotic origin, and mosaic changes of presumed mitotic origin but there were a few exceptions, when the threshold was arbitrarily set at 70%. Most of the full changes of mitotic origin in trophectoderm biopsy samples were in one embryo with multiple meiotic and mitotic changes. In that situation, it is difficult for the software to normalise the data and establish a two-copy baseline since the assumption is that most chromosomes will have two copies. It may therefore be necessary to treat such samples separately and some testing labs refer to these as "complex" aneuploidies. Reducing the threshold to, for example, 50% could have the advantage that any mosaic mitotic changes below that threshold could be discounted and embryos with only changes below this threshold reported as 'euploid', whereas those with any changes above 50% reported as 'aneuploid'. The risk of increasing the false positive rate for meiotic aneuploidies in the aneuploid group, and conversely, the risk that embryos with mosaic changes may have mitotic aneuploidies in the euploid group, may be minimal. However, more data will be required to make these judgements and thresholds need to be validated for any quantitative copy number test.

In poor prognosis patients with fewer normally fertilised embryos and blastocysts, identifying even a single euploid blastocyst for transfer can be critical. Therefore, in cycles in which one or two of the embryos have been identified by PGT-A and copy number analysis as mosaic for whole and/or partial chromosome abnormalities, while all the others are aneuploid, advising the patient on the possibility of transferring them is problematical and genetic counselling is advised.\textsuperscript{17} To avoid discarding these potentially viable embryos, however, it is clinically important to follow up on the initial testing (See Summers in Rosenwaks et al\textsuperscript{34}). For example, a second biopsy can be
performed and the embryo retested or the amplified DNA from the original biopsy (with DNA samples from both parents) can be used for reanalysis using a different approach such as SNP genotyping and karyomapping. Concordance among multiple trophectoderm biopsies and the ICM, from which the fetus is derived, is high for full whole chromosome aneuploidies detected by copy number analysis but much lower for mosaic changes. However, for mosaic partial (or segmental) chromosome copy number changes, rebiopsy and confirmation of a change identifies serious imbalances of meiotic origin and is clinically useful.

We have routinely used SNP genotyping and karyomapping analysis for follow up of embryos with only mosaic and/or partial copy number changes and have found that a significant proportion are confirmed to have meiotic aneuploidies. For example, in a recent series of clinical trophectoderm biopsy samples identified by NGS-based PGT-A as having only mosaic whole and/or partial chromosome aneuploidies (n = 21), 5/11 (45%) mosaic whole chromosome aneuploid samples were confirmed as maternal meiotic trisomies or monosomies and 2/11 (18%) as digynic triploid by SNP genotyping and karyomapping analysis (Table S3). Whereas only 1/10 samples with full or mosaic partial chromosome abnormalities were confirmed and the rest were euploid. These data may explain the reduced clinical pregnancy and live birth rates and increased miscarriage rates reported following transfer. Also, triploid changes and have found that a significant proportion are confirmed to have meiotic aneuploidies. For example, in a recent series of clinical trophectoderm biopsy samples identified by NGS-based PGT-A as having only mosaic whole and/or partial chromosome aneuploidies (n = 21), 5/11 (45%) mosaic whole chromosome aneuploid samples were confirmed as maternal meiotic trisomies or monosomies and 2/11 (18%) as digynic triploid by SNP genotyping and karyomapping analysis (Table S3). Whereas only 1/10 samples with full or mosaic partial chromosome abnormalities were confirmed and the rest were euploid. These data may explain the reduced clinical pregnancy and live birth rates and increased miscarriage rates reported following transfer. Also, triploid embryos, which are sometimes misclassified as normally fertilised two-pronucleate zygotes, can only be identified by copy number analysis alone in male embryos by mosaic loss of the X and Y chromosomes and not in 69 XXX females. We recently confirmed this as the cause of an early miscarriage in a patient having single vitrified warmed blastocyst transfer with PGT-A, first by karyotyping of the POC and subsequently by karyomapping analysis of both the trophectoderm biopsy and DNA extracted from the POC, which confirmed identical patterns of digynic triploidy for all chromosomes. In contrast, targeted NGS and SNP analysis allows accurate detection of all triploid embryos. Similarly, NGS-based SNP analysis also detected a minority of samples with abnormal ploidy and conversely some embryos identified as having 0 or 1 pronuclei, which were normal diploid. Finally, 4/11 (36%) of these samples with mosaic whole chromosome changes were shown to have normal biparental inheritance, although not necessarily in all the biopsied cells, and because of absence of evidence of meiotic aneuploidies, could be considered for transfer.

Alternatives to trophectoderm biopsy and PGT-A by copy number testing include copy number or SNP analysis of polar bodies to identify only meiotic aneuploidies of maternal origin and, SNP analysis of embryo samples for meiotic and mitotic aneuploidies of paternal or maternal origin. Array CGH of polar bodies accurately predicted maternal aneuploidies in cleavage stage embryos although there was a significant false positive rate. An RCT in women of advanced maternal age using this approach failed to show an improvement in cumulative live birth rates although implantation and clinical pregnancy rates per transfer were increased. However, there was a high failure rate with one or both polar bodies, which may be improved with newer NGS-based methods. SNP genotyping of both polar bodies and maternal DNA and meiomapping enables high resolution, maternal haplotype recombination analysis, which allows identification of all four types of missegregation in both meiotic divisions. Although this approach has not been used clinically, in this study, all the polar bodies from 51 normally fertilised embryos were successfully genotyped and follow up karyomapping analysis confirmed the presence of 58/58 (100%) of the predicted maternal aneuploidies in embryo samples (Table S1). The main disadvantages of polar body analysis are the increased cost of testing both polar bodies and the need to biopsy all mature oocytes and all normally fertilised zygotes even though only about half of the latter will develop to the blastocyst stage. Microarray-based SNP genotyping and karyomapping analysis of embryo biopsies has the advantage that both paternal and maternal meiotic trisomies and monosomies of either origin can be identified accurately. Furthermore, parallel analysis by standard quantitation of SNP allele intensities can identify full or mosaic, meiotic and mitotic aneuploidies of whole or partial chromosome abnormalities. This approach has been used extensively, mainly on single blastomeres biopsied from cleavage stage embryos and high clinical pregnancy rates have been reported. Finally, NGS-based SNP genotyping and high-resolution analysis of all types of chromosome abnormalities is now possible with improved bioinformatics algorithms. The main disadvantage of these methods for PGT-A is that the cost of SNP genotyping either by microarray or NGS is higher than copy number analysis and genotyping of both parents is required. Recently, however, a lower cost, high-resolution microarray-based SNP analysis method with improved quantitation has been reported for comprehensive PGT, including polygenic risk scoring for common polygenic diseases.

Choosing which of these alternative approaches will provide maximum clinical benefits for all patients needs to be informed by knowledge of the evolution and consequences of the different types of aneuploidy for both preimplantation and postimplantation development. All of the maternal meiotic aneuploidies identified by polar body analysis in this study were present on days 4 to 7 in trophectoderm cells biopsied from blastocysts and arrested embryos and the proportion of embryos developing to the blastocyst stage was similar in those with or without these meiotic aneuploidies (Table 1). Thus, the genetic imbalance caused by meiotic aneuploidies does not appear to compromise preimplantation development. However, the number of meiotic aneuploidies per oocyte increases with maternal age and in women aged 43 to 45 years reached 86%, with a maximum of seven aneuploidies in one oocyte. As embryos with multiple aneuploidies are rarely present in POCs following implantation, it may be that the preimplantation development of some of the corresponding embryos with multiple aneuploidies may be affected. In contrast, the incidence of aneuploidies detected in embryos of both meiotic and presumed mitotic origin decreases between cleavage stages on day 3 (analysed in single blastomeres) and in trophectoderm cells biopsied on days 5 to 7 and this is associated with the loss of karyotype-wide aneuploidies associated with abnormal mitosis and developmental
arrest. This is supported by detailed single cell analysis following tripolar mitosis demonstrating that even severely subdiploid blastomeres with karyotype-wide monosomies and nullisomies can continue to divide for the first four cleavage divisions forming clones of cells with closely similar chromosome sets but then arrest prior to blastocyst formation. Recently, extended culture and outgrowth of biopsied blastocysts to days 8 or 12 followed by reanalysis of both trophectoderm and ICM samples has allowed examination of the effects of chromosome copy number changes on peri-implantation development. This demonstrated that the blastocysts which outgrew successfully by day 12 were mainly euploid or those with trisomies, duplications or mosaic changes. Whereas those blastocysts which detached and failed to develop had monosomies, deletions and complex aneuploidies. Furthermore, there was 100% concordance of the outgrowth samples with full changes identified in the original biopsy and 60% of blastocysts with only mosaic changes remained viable on day 12.

Following transfer of single blastocysts, a large-scale cytogenetic study of the retained POCs (n = 1030) following missed abortions between 7 and 10 weeks gestation (average maternal age 39 years) reported that 80.6% were aneuploid. Most of the aneuploidies detected, however, were single and double trisomies, 62.3% and 7.8%, respectively. Other frequent cytogenetic abnormalities included polyploidy (1.0%), embryonic mosaicism (1.1%) and structural abnormalities (2.4%). Also, the incidence of trisomies increased with maternal age over 35 years and this correlated with a decrease in the proportion of euploid samples. Similarly, another report of a large-scale cohort of POCs following natural conception and miscarriage by microarray-based SNP analysis identified chromosomal abnormalities in about 60% of samples. Most of these samples (n = 1106) had single aneuploidies (78%), mainly maternal trisomies, or were triploid (10%). Whereas only 5% and 1% had double and triple aneuploidy of maternal origin, respectively. Finally, microarray-based SNP analysis of another large cohort of POCs has also highlighted a significant incidence of molar pregnancies with paternal triploidy (2.8%) or full paternal uniparental disomy (0.3%), respectively, which can be associated with persistent gestational trophoblastic disease. Thus, polar body analysis alone should identify most embryos with multiple maternal meiotic aneuploidies, which are rarely observed in POCs beyond 7 weeks gestation as well as those with single or double maternal aneuploidies, many of which are associated with early miscarriage. NGS-based copy number analysis of trophectoderm cells at the blastocyst stage provides a more comprehensive analysis of chromosome abnormalities but does not directly distinguish meiotic and mitotic aneuploidies which may have different clinical consequences for later development. In conclusion, therefore, fully comprehensive analysis of all types of aneuploidies, including abnormalities of fertilisation, will require a combination of high-resolution copy number analysis and genotyping.

ACKNOWLEDGEMENTS
We would like to thank Leoni Xanthopoulou, David Chrimes and Tony Gordon (Coopers Genomics) for their support.

CONFLICT OF INTEREST
A.H.H. is Scientific Advisor to Vitrolife Sweden AB, Gothenburg, Sweden.

DATA AVAILABILITY STATEMENT
All the anonymised detailed relevant data has been provided in the Supplementary Tables for online publication.

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**SUPPORTING INFORMATION**
Additional supporting information may be found online in the Supporting Information section at the end of this article.