

## The origin and significance of additional aneuploidy events in couples undergoing PGD for translocations by aCGH

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## Abstract

Diagnostic application of array-CGH in PGD for reciprocal and Robertsonian translocations has revealed 45-55% embryos with additional aneuploidies with or without translocation related imbalances. The occurrence of these extra abnormalities with the balanced form of the translocation reduces the number of embryos suitable for transfer. This study followed up 83 embryos on day 5-7 of development from 23 infertile or sub-fertile carriers for whole chromosome and segmental aneuploidies present in addition to the balanced or unbalanced translocations detected on aCGH diagnosis. Embryos were analysed by FISH (63) and aCGH (20). Meiotic aneuploidy affected 35% of embryos and 47% had mitotic events; 15% had both types. In total, meiotic and mitotic events were almost equal (60 versus 64), 97 affected whole chromosomes (58 meiotic, 39 mitotic) and 27 were segmental (2 meiotic, 25 mitotic). Aneuploidies of meiotic origin and the majority of abnormalities of mitotic origin were widespread in the embryos followed up. All embryos diagnosed as abnormal (translocation balanced or unbalanced) after aCGH diagnosis at cleavage stage would have remained unsuitable for transfer if tested at later stages of development. Additional aneuploidies are thus confirmed as significant findings and merit full consideration when considering the choice of embryos to transfer.

**Keywords:** aCGH; reciprocal translocations; aneuploidies; mitotic; meiotic

**Summary for lay readers :** One partner in about 1 in 500 couples is the carrier of a chromosome rearrangement known as a translocation. For such couples that experience fertility problems or several miscarriages, preimplantation genetic diagnosis (PGD) is available. The parents need to undergo IVF treatment so that a few cells can be taken from the embryo when it is 3-5 days old, before it implants in the womb. The cells are then tested using array-CGH that tests for all the 23 chromosomes, and not only detects abnormalities from the translocation but also any others affecting the other chromosomes. Testing in this way has revealed half of embryos contain additional chromosomal imbalances with or without translocation related abnormalities. The significance of these extra anomalies has been debated as their origin was not known nor how widespread they were in the embryo. This study followed up abnormalities present in addition to the balanced or unbalanced translocations detected on diagnosis in embryos from infertile or sub-fertile carriers of translocations. In 85% of the embryos it was found that the extra abnormalities were either present in all cells or in more than 50% of cells. The majority of errors affecting whole chromosomes were present throughout the embryo, showing that they were derived from the egg or the sperm. The remainder, affecting just a proportion of cells, occurred as a result of mistakes while the embryos were growing in the first few days. Additional abnormalities are thus confirmed as significant when considering the choice of embryos to transfer.

## Introduction

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3 According to cytogenetic studies of newborn infants carried out on 63,000  
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5 individuals from six different countries the combined incidence of balanced  
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7 Robertsonian and reciprocal translocations lies between 1.5 and 1.7 per  
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9 thousand (Jacobs, 1977; Maeda et al., 1991). As adults, most of these  
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11 individuals are able to reproduce successfully, with the help of prenatal  
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13 diagnosis where necessary. A minority will experience overt fertility problems  
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15 leading them to present for investigation and to consider preimplantation  
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17 genetic diagnosis (PGD). PGD was first made available in 1995 for couples at  
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19 risk of chromosomally abnormal conceptions arising from the translocation  
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21 carrier status of one partner (Conn et al., 1995). The approach employed the  
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23 technique of fluorescence in situ hybridisation (FISH) with chromosome  
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25 probes specific to regions that had been translocated. Other publications soon  
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27 followed (Conn et al., 1998; 1999; Munné et al., 1998; Scriven et al., 1998;  
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29 Iwarsson et al., 2000). Later in some centres, additional probes were added to  
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31 screen for common aneuploidies, but the main focus was on the translocated  
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33 chromosomes. The development of techniques to allow the application of  
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35 comparative genomic hybridisation (CGH) analysis to single cells paved the  
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37 way to the comprehensive screening of all chromosomes (Wells et al., 2000;  
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39 Voullaire et al., 2000). Initially, this approach required a high degree of skill  
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41 and a lengthy period of hybridisation, so it was the advent of single cell CGH  
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43 analysis via microarrays (aCGH) that opened up the field and reports of its  
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45 application in translocation PGD cycles rapidly appeared (Alfarawati et al.,  
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47 2011; Fiorentino et al., 2011). It quickly became apparent that additional  
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49 aneuploidies were as common as translocation imbalances (Alfarawati et al.,  
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1 2011; Fiorentino et al., 2011). At that time, most cases involved day 3 biopsy  
2 of blastomeres, a minority were diagnosed on trophectoderm (TE) samples.  
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4 Most centres avoided the transfer of any embryo with a chromosomal  
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6 anomaly but a few decided to consider only the translocation imbalances. The  
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8 reasoning behind this was that the true extent of any additional aneuploidies  
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10 was unknown since most were diagnosed on a single cell. There have been  
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12 few reports of any follow up analyses to answer this question. In a validation  
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14 study, Colls et al. (2012) re-analysed by FISH embryos diagnosed as  
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16 abnormal by aCGH; of 102 embryos (not all from translocation cases) 52 were  
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18 uniformly aneuploid and 45 were aneuploid mosaic or chaotic mosaic – but  
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20 with all cells abnormal. Follow up analysis was also carried out by Fiorentino  
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22 et al. (2011); embryos diagnosed as abnormal were re-biopsied on day 6 and  
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24 the cells subjected to analysis identical to that employed for diagnosis. In all,  
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26 57 embryos with additional aneuploidies, with or without translocation  
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28 imbalance, were re-analysed and the diagnoses confirmed. Aneuploidy  
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30 mosaicism was seen in 24/57 embryos but all were confirmed as abnormal  
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32 despite the mosaicism; no details were given.  
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41 The aim of our study was to investigate in detail the significance of  
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43 aneuploidies that occur in addition to translocation imbalances in couples  
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45 undergoing PGD via aCGH and to determine whether the origin was meiotic  
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47 or mitotic.  
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51 We found that overall meiotic and mitotic events were almost equal. For the  
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53 whole chromosome aneuploidies meiotic errors predominated whereas for  
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55 segmental anomalies, almost all were mitotic.  
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## Methods

### Patient information and details of PGD cycles

PGD via aCGH was performed on embryos from couples that included 16 carriers of reciprocal translocations and 7 carriers of Robertsonian translocations. For all the treatment cycles, ICSI (intracytoplasmic sperm injection) was carried out to limit paternal contamination. One or two blastomeres were biopsied on day 3 post-fertilisation in 29 PGD cycles and TE cells were biopsied on day 5 in two cycles. 24Sure™ arrays (BlueGnome, Cambridge, UK) were used for the detection of Robertsonian translocations and 24Sure+™ (BlueGnome Ltd, Cambridge, UK) arrays were used for reciprocal translocations. Embryos with balanced forms of the translocation and no other aneuploidies were recommended for transfer. Embryos unbalanced for the translocation and/or with additional aneuploidies were considered unsuitable for transfer and were collected on day 5-7 post-fertilisation for follow-up analysis. The average maternal age was  $35 \pm 4.2$  years. Table 1 provides the patient details, which include the karyotypes of the carriers along with the reproductive histories of the couples and the maternal ages. Information about the number of embryos biopsied and the number of transferrable embryos for each PGD cycle is also provided.

### Processing of untransferred embryos and allocation of embryos for follow up.

Embryos unsuitable for transfer were processed immediately after collection. The morphology of the embryos was noted at the time of processing. Whole embryos were either spread on microscopic slides for FISH or tubed (in  $0.2\mu\text{l}$

1 microcentrifuge tubes) for aCGH analysis. Untransferred embryos with one to  
2 five distinct additional abnormalities were followed up; embryos with multiple  
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4 chromosome abnormalities (> five aneuploidies) and embryos with no  
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6 diagnostic results were excluded.  
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### 10 11 **Clinical and research approvals**

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14 Diagnosis and follow up was covered by the treatment licence from the HFEA  
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16 (Human Fertilisation and Embryology Authority) held by the CRGH (Centre for  
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18 Reproductive and Genetic Health). UCL Centre for PGD is an accredited  
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20 laboratory (Clinical Pathology Accreditation, reference no. 2920).  
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### 26 27 **Spreading of untransferred embryos and FISH procedure**

28  
29 Whole untransferred embryos were spread using the Tween-HCl method as  
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31 described in Harper et al. (1994). The FISH protocol was performed as  
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33 previously described with slight modifications (Harper et al., 1994). FISH  
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35 probes were chosen to follow up those chromosomes, unrelated to the  
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37 translocation, that were identified as aneuploid on diagnosis. A combination of  
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39 CEP (chromosome enumeration probes), Sub-tel (sub-telomeric probes) and  
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41 LSI (locus specific identifier probes) in different fluorochromes was suitably  
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43 selected. All probes were from Abbott Molecular, UK.  
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### 48 49 **Microscope analysis and scoring of FISH signals**

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51 The slides were examined under an epifluorescence Olympus microscope  
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53 (Olympus BX 40) equipped with suitable filters. Specific signal scoring criteria  
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55 were applied to interphase nuclei for uniform evaluation of FISH signals, in  
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57 accordance with Hopman et al., 1988 and Mantzouratou et al., 2007.  
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### **Tubing of untransferred embryos for aCGH analysis**

Whole untransferred embryos were tubed under sterile conditions. Under a dissecting microscope using 0.3 $\mu$ m microcapillary (Sartorius, UK), the embryos were transferred from the IVF culture dish into drops of 1XPBS (phosphate buffered saline)/0.1%PVA (Polyvinyl alcohol) (Sigma, UK) solution in a petri dish. By repeated pipetting using a smaller 0.2 $\mu$ m microcapillary the embryonic cells were detached from the zona pellucida. The cells were then washed in a fresh drop of PBS/0.1%PVA and transferred into sterile 0.2ml microcentrifuge tubes with a minimal amount of solution.

### **Whole Genome Amplification (WGA) and aCGH analysis**

The whole genome of the embryonic cells was amplified using the Sureplex™ amplification kit (BlueGnome Ltd, Cambridge) following the manufacturer's protocol. Amplification efficiency was checked by gel electrophoresis.

Samples that successfully amplified had DNA smears of fragment size ranging between 100 and 1000 bp (base pairs) with a median size of 400 bp.

The untransferred embryos were subjected to follow-up by aCGH using two different array platforms. Some embryos were analysed by BlueGnome 24Sure™/24Sure+™ BAC (bacterial artificial chromosome) arrays. 24Sure+™ high density BAC arrays include ~4800 clones spaced at 623Kb intervals across the human genome. The remaining embryos were subjected to follow-up by Agilent's 8x60K high-resolution oligonucleotide arrays (Agilent Technologies, UK). These high-density arrays included ~55,000, 60-mer

1 oligonucleotides spaced at an average of 41kb intervals across the human  
2 genome (Design ID 021924). BlueGnome 24Sure/24Sure+ arrays were  
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4 previously validated in our laboratory for clinical use. The 8x60K  
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6 oligonucleotide arrays were also validated in house before employing them for  
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8 follow-up purposes.  
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### 11 **Array-CGH diagnosis and follow up using BlueGnome 24Sure/24Sure+** 12 **BAC arrays**

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14 Array-CGH using 24Sure (v2 and v3) or 24Sure+ arrays was performed  
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16 according to the manufacturer's instructions. Briefly, test samples were  
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18 labeled with Cy3 and reference samples with Cy5 fluorophores. Labeling was  
19  
20 carried out for 16-18 hours. Combination, ethanol precipitation and  
21  
22 denaturation were performed on the following day and the samples were  
23  
24 hybridized for 3-4hrs. The slides were washed and scanned using InnoScan  
25  
26 700 (Innopsys SA) or ScanArray Express (Perkin Elmer) or Agilent SureScan  
27  
28 microarrays (Agilent Technologies) scanners at a resolution of 10 $\mu$ m and  
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30 scanned images were analysed and interpreted using the BlueFuse Multi  
31  
32 software (BlueGnome Ltd, UK). The BlueFuse algorithm is based on  
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34 calculating the median log<sub>2</sub>ratio of all the chromosomes for detection of gains  
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36 and losses. A median log<sub>2</sub>ratio value  $\geq 0.3$  indicated chromosome gains  
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38 whereas values  $\leq -0.3$  indicated chromosome losses. Gains and losses of  
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40 segmental (partial) aneuploidies unrelated to the translocation were identified  
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42 when a minimum of 10 consecutive BAC clones crossed the normality  
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44 thresholds.  
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### **Array-CGH follow up with Agilent's 60-mer oligonucleotide microarrays**

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2 Eight embryonic samples after WGA were tested using both the high-  
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4 resolution Agilent (8x60K) 60-mer oligonucleotide microarrays and  
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6 BlueGnome BAC (bacterial artificial chromosome) 24Sure/24Sure+  
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8 microarrays. The manufacturer's protocol was used to perform the Agilent  
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10 8x60K oligo microarrays with certain modifications. Briefly, test samples were  
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12 labeled with Cy5 and reference samples with Cy3 fluorophores. Labeling of  
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14 the amplified DNA was carried out for 2.5 hours. Preparation for hybridization  
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16 included combination, ethanol precipitation and denaturation, performed on  
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18 the same day. Hybridisation was carried out overnight for 16 hours.  
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23 Microarray washing was performed the following day. The arrays were  
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25 scanned using Agilent SureScan Microarray Scanner at 3 $\mu$ m and the TIFF  
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27 images analysed using Agilent Genomic Workbench 7.0 (Agilent  
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29 technologies). All samples were analysed by a single cell analysis method  
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31 recommended by the manufacturer. The analysis method was configured with  
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33 ADM-2 (Aberration Detection Method 2). This algorithm identified all aberrant  
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35 intervals in the sample with consistently high or low  $\log_2$ ratios based on a  
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37 statistical score. The software identified the aberrations if the average  
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39  $\log_2$ ratio of the sample over the reference exceeded a specific threshold;  
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41  $\log_2$ ratio of  $\geq 0.3$  for gains and  $\geq 0.55$  for losses.  
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### **Classification of embryos according to their mechanism of origin of aneuploidy post FISH and aCGH follow-up analysis**

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51 The embryos were classified as having mitotic or meiotic aneuploidy events  
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53 based on the extent of aneuploidy seen in the embryos either by FISH or  
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55 aCGH follow-up. Aneuploidy was recorded based on the number of  
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1 aneuploidy events per chromosome per embryo analysed excluding the  
2 chromosomes associated with the translocation.  
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### 7 **Follow up by FISH**

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9 Embryos were classified as having a mitotic aneuploidy event when the  
10 embryos were mosaic with at least two cell lines, a diploid cell line and an  
11 aneuploid cell line or two different aneuploid cell lines. The imbalance of the  
12 aneuploid cell line could be either concordant with that detected at clinical  
13 diagnosis (day 3/day 5) or a complementary gain or loss. The percentages of  
14 nuclei with the diploid and aneuploid cell lines for a single aneuploidy event  
15 were calculated.  
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26 Embryos were classified as having a meiotic aneuploidy event when over  
27 90% of nuclei were scored in concordance with the aneuploidy scored at  
28 diagnosis.  
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### 36 **Follow up by aCGH**

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38 If the aneuploidy event was seen on aCGH diagnosis and not seen on follow-  
39 up or if the aneuploidy event was seen on follow-up and not seen on biopsy, it  
40 was considered as being mitotic in origin. If the aneuploidy event was seen on  
41 diagnosis and on follow-up with no indication of mosaicism (deviation of all the  
42 clones/oligos belonging to one chromosome between the  $\log_2$  ratio of zero  
43 and the normality thresholds in either direction), it was considered as being  
44 meiotic in origin.  
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Table 1 Karyotype of carriers of reciprocal and Robertsonian translocations, along with their reproductive histories

Patient ID	Karyotype of translocation carrier	PGD Cycle No.	Maternal Age (years)	Sample type tested	No of embryos biopsied	No. of transferrable embryos	Reproductive History
<b>Reciprocal translocation - male carriers</b>							
A	46,XY,t(1;13)(p34.3;q34) **	1	23	Blastomeres	14	4	No previous attempts at IVF
		2	23	Blastomeres	9	1	
B	46,XY,t(1;14)(p21.1;q11.2)	1	28	Blastomeres	8	1	No previous attempts at IVF
C	46,XY,t(1;19)(p34;q13.1)	1	34	Blastomeres	20	1	No previous attempts at IVF
		2	34	Trophectoderm cells	8	0	No previous pregnancies
D	46,XY,t(16;20)(q13;q13.3)	1	32	Blastomeres	7	1	Primary infertility No previous attempts at IVF Low AMH
		2	32	Blastomeres	4	2	
E	46,XY,t(9;15)(q33.2;q11.2)	1	35	Blastomeres	5	2	Primary Infertility 1 failed IVF attempt 1 TOPFA 1 natural pregnancy with balanced translocation
F	46,XY,t(1;6)(p22;q15)	1	35	Blastomeres	22	3	2 miscarriages Previous triplet IVF pregnancy reduced and birth of a child with balanced translocation Normal birth from previous PGD cycle

Patient ID	Karyotype of translocation carrier	PGD Cycle No.	Maternal Age (years)	Sample type tested	No of embryos biopsied	No. of transferrable embryos	Reproductive History
G	46,XY,t(1;3)(q31;p13)	1	36	Blastomeres	11	0	4 failed IVF attempts 2 miscarriages 1 previously failed PGD-FISH cycle
H	46,XY,t(4;12)(q25;q24.31)	1	37	Blastomeres	8	0	2 failed IVF attempts 1 ectopic pregnancy Normal birth from previous PGD cycle
		2	37	Blastomeres	17	2	
I	46,XY,t(4;10)(q33;q24.1)	1	38	Blastomeres	10	2	3 first trimester miscarriages
J	46,XY,t(3;4)(q27;q27)	1	40	Blastomeres	4	0	2 failed IVF attempts 2 miscarriages Female with polycystic ovaries.
K	46,XY,t(11;13)(q21;q14.1)	1	41	Blastomeres	8	1	7 miscarriages
L	46,XX,t(5;15)(p15.31;q22)	1	34	Blastomeres	5	1	Primary infertility (duration 9 years)
<b>Reciprocal translocation - female carriers</b>							
M	46,XX,t(5;7)(q14;q11.2)	1	35	Blastomeres	19	0	3 failed IVF attempts
		2	35	Trophectoderm cells	6	2	
N	46,XX,t(12;14)(q24.1;q32.1)	1	38	Blastomeres	4	0	2 failed IVF attempts Female with polycystic ovaries.
O	46,XX,t(11;19)(q14.2;q13.3)	1	39	Blastomeres	3	0	1 miscarriage (8/40), 1 TOPFA with balanced karyotype but with cloacal abnormality,

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Patient ID	Karyotype of translocation carrier	PGD Cycle No.	Maternal Age (years)	Sample type tested	No of embryos biopsied	No. of transferrable embryos	Reproductive History
							Suboptimal ORT
P	46,XX,t(11;22)(q23.3;q11.2)	1	39	Blastomeres	9	0	3 miscarriages 1 TOPFA after abnormal CVS result with an unbalanced form of translocation
		2	39	Blastomeres	13	2	
<b>Robertsonian translocation - male carriers</b>							
Q	45,XY,der(13;14)(q10;q10)	1	34	Blastomeres	9	2	Primary infertility (duration 2 years) 3 failed IVF attempts
R	45,XY,der(13;14)(q10;q10)	1	37	Blastomeres	9	1	Primary infertility (duration 2 years) 2 miscarriages Product of conception with trisomy 17
		2	37	Blastomeres	5	4	
S	45,XY,der(14;21)(q10;q10)	1	39	Blastomeres	15	5	Primary infertility (duration 6 years) 2 failed IVF attempts (1 with PGD)
T	46,XXX,der(14;15)(q10;10)	1	35	Blastomeres	5	0	Primary infertility (duration 3 years) Failed IUIs. 1 miscarriage after IVF cycle
		2	36	Blastomeres	11	3	
U	45,XY,der(14;21)(q10;q10)	1	37	Blastomeres	10	1	Primary infertility (duration 9 years) 2 failed IVF attempts 1 miscarriage

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Patient ID	Karyotype of translocation carrier	PGD Cycle No.	Maternal Age (years)	Sample type tested	No of embryos biopsied	No. of transferrable embryos	Reproductive History
<b>Robertsonian translocation - female carriers</b>							
V	45,XX,der(14;21)(q10;q10)	1	35	Blastomeres	15	1	Normal birth from previous PGD cycle 4 undiagnosed spontaneous pregnancies resulting in miscarriage
W	45,XX,der(13;14)(q10;q10)	1	41	Blastomeres	5	0	4 miscarriages

ORT =Ovarian Reserve Test,

TOPFA= Termination of pregnancy with fetal anomaly,

AMH =anti-mullerian hormone, CVS=chorionic villus sample,

IUI= Intrauterine insemination.

IVF attempts refers to cycles without PGD

\*\*The female partner was a carrier of Crouzon syndrome/OMIM:123500

## Results

### Overall summary of diagnostic data

Twenty-two PGD cycles were performed for 16 carriers of reciprocal translocations and 9 PGD cycles for 7 carriers of Robertsonian translocations using aCGH.

Table 2 Diagnostic outcome of the 31 PGD-aCGH cycles for translocation carriers

No. of embryos	Reciprocal Translocation	Robertsonian Translocation	Total
No. biopsied	214	84	298
No. transferrable	25	17	42 (14%)
No. untransferrable	189	67	256 (86%)
No. with translocation related imbalances only	46	13	59 (23%)
No. with translocation related imbalances +additional aneuploidies	56	14	70 (27%)
No. with additional aneuploidies only	28	14	42 (16%)
No. with multiple chromosome aneuploidies (>5 aneuploidies)	41	17	58 (23%)
No. with no results	18	9	27 (9%)

A total of 298 embryos was tested, 274 after biopsy of one/two cells on day 3 and 24 after TE biopsy on day 5, of which 271 (91%) gave results.

Of the embryos diagnosed 42 (14%) were either normal or balanced for the translocation with no other anomalies and were transferrable. Of the remaining 256 embryos, 170 (63%) had additional aneuploidies. Embryos were classified according to Table 2.

### **Embryos for FISH and aCGH follow up of additional aneuploidies**

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2 Of the 112 embryos with 1-5 additional aneuploidies, 63 were followed up by  
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4 FISH and 22 by aCGH, in total 85. The remaining 27 embryos were regarded  
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6 as unsuitable for follow up for various reasons.  
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### **Overall summary of follow up data**

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14 Conclusive follow up results were obtained for 83/85 embryos, 63 by FISH  
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16 and 20 by aCGH.  
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22 Meiotic aneuploidy affected 35% of embryos and 47% had mitotic events;  
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24 15% had both types. In total, meiotic and mitotic events were almost equal (60  
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26 versus 64), 97 affected whole chromosomes (58 meiotic, 39 mitotic) and 27  
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28 were segmental (2 meiotic, 25 mitotic). Of the 97 whole chromosome  
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30 aneuploidies followed up, 90 (92.7%) persisted to later stages. Whereas for  
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32 the segmental anomalies, out of the 27, only 15 (55%) were seen at later  
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34 stages.  
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### **Results from embryos with FISH follow up**

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43 Follow-up by FISH included 48 embryos from PGD for reciprocal translocation  
44  
45 cycles and 15 embryos from Robertsonian translocation cycles. In all, the 63  
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47 embryos presented with 100 additional aneuploidy events at diagnosis, 86  
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49 whole chromosomal and 14 segmental aneuploidies. Follow up was  
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51 performed for 93/100 events (93%) (Figure 1).  
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### **Classification of chromosome aneuploidy events post follow up by FISH**

1  
2 In 63 untransferred embryos, 80 whole chromosome and 13 segmental  
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4 additional aneuploidy events were re-analysed. Forty-four whole chromosome  
5  
6 additional aneuploidy events were re-analysed. Forty-four whole chromosome  
7  
8 meiotic aneuploidy events were found (Figure 1). Among these, 28/44 (64%)  
9  
10 were uniformly aneuploid meiotic events in which all the embryonic nuclei had  
11  
12 FISH signals consistent with the aneuploidy detected on diagnostic aCGH and  
13  
14 for 16/44 (36%) the embryo had over 90% nuclei with FISH signals consistent  
15  
16 with the aneuploidy detected on diagnosis and were also considered to be  
17  
18 meiotic in origin. Only one segmental meiotic event was present after follow  
19  
20 up in one embryo, this being the loss of 2q11.2-2qter in an arrested embryo  
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22 with 9 cells.  
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29 Thirty-six whole chromosome and 12 segmental mitotic aneuploidy events  
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31 were found (Figure 1). Embryos with mitotic errors were either  
32  
33 diploid/aneuploid mosaics or aneuploid mosaics. Regarding whole  
34  
35 chromosome errors, in sixteen events, the aneuploidy event present on  
36  
37 diagnosis was confirmed on follow up. In fourteen other cases, the embryo  
38  
39 contained two different cell lines showing complementary aneuploidy events  
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41 (for example: +9, -9) presumably due to mitotic non-disjunction (MND) one of  
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43 which was present on diagnostic aCGH. Additionally six whole chromosome  
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45 events in five embryos were not seen on follow up but in all these embryos  
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47 other anomalies were present.  
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1 Similarly, follow up data of the twelve segmental mitotic aneuploidies showed  
2 that three segments in three embryos were not seen on follow up. Six events  
3 with complementary findings presumably arose due to MND and the  
4 remaining three showed aneuploid cells between 11%-30% after FISH follow  
5 up.  
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### 11 **Results of embryos with aCGH follow up**

12 Validation results of eight embryonic samples showed that the BAC and the  
13 oligonucleotide-based arrays gave comparable results. A total of 22 whole  
14 embryos were subjected to WGA. Successful amplification was obtained for  
15 21/22 (95%) embryos and conclusive aCGH follow up results were obtained  
16 for 20/21 (95%). Eleven embryos were analysed by BlueGnome  
17 24Sure/24Sure+ arrays, eight by Agilent 8x60K and two were analysed by  
18 both BAC and oligo arrays (one of which was a part of the validation). Follow  
19 up by aCGH included seventeen embryos from reciprocal and five from  
20 Robertsonian translocation carriers. After aCGH follow up, the translocation  
21 status was confirmed for all 20 embryos.  
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43 The aCGH results of twenty embryos are summarised in Figure 2. Overall, of  
44 the 31 aneuploidies seen on diagnosis only 1 of 17 whole chromosome  
45 anomalies was not seen on follow up whereas only 1 of 14 segmental errors  
46 was detected. Two embryos showed evidence for MND, involving two whole  
47 chromosomes in one case and one segmental anomaly in the other. Figures 3  
48 and 4 provide examples of aCGH results of embryos followed up by BAC and  
49 oligonucleotide arrays.  
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### Combination of FISH and aCGH follow up data

Information about additional aneuploidies on diagnosis and follow up was therefore available for 83 embryos; 63 whole embryos analysed by FISH and 20 by aCGH.

Follow up data showed that the 124 aneuploid events unrelated to the translocation involved all chromosomes with the exception of chromosomes 4 and Y. Figure 5 shows chromosomes 19,16,22,15 and 2 to be frequently aneuploid. Most meiotic events were found for chromosome 22 followed by 15,16 and 19. Chromosomes 19 had the highest number of mitotic events followed by chromosomes 2, 3 and 16.

In 17 embryos, 22 aneuploid events (7 whole chromosome and 15 segmental) seen on diagnosis were not detected in the rest of the embryo on follow up. However, these 17 embryos all showed abnormalities related to the translocation and/or other additional abnormalities. The remaining 105/124 (85%) aneuploid events were either concordant with the aneuploidy seen on diagnosis or showed a complementary aneuploid event due to mitotic non-disjunction. The majority of the untransferred embryos included in follow up analysis were at various stages between the morula and blastocyst stage of embryo development.

### **Mitotic versus Meiotic errors**

1  
2 In 63 embryos with 93 aneuploid events subjected to follow up by FISH, 45  
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4 meiotic and 48 mitotic events were detected. In 20 embryos with 31 aneuploid  
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6 events subjected to follow up by aCGH, 16 mitotic and 15 meiotic aneuploid  
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8 events were detected. Therefore in a total of 83 embryos with 124 additional  
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10 aneuploid events, which were seen on diagnosis and followed up, 60 (48%)  
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12 meiotic and 64 (52%) mitotic events were found. Of the meiotic events, 58  
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14 affected whole chromosomes and 2 were segmental.  
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### **Proportion of embryos with meiotic and mitotic aneuploidy events**

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23 From the total of 85 aneuploid embryos included in follow up analysis, 30  
24  
25 (35%) had only meiotic events and 40 (47%) had only mitotic errors, 13 (15%)  
26  
27 embryos had both type of events and 2 (3%) embryos did not give results  
28  
29 (Figure 6). Whole chromosome abnormalities only were seen in 59 embryos  
30  
31 (71%) and segmental aneuploidies alone in 14 (17%). Ten embryos (12%)  
32  
33 had both whole chromosome and segmental anomalies. Excluding the 58  
34  
35 embryos with more than 5 errors, but including the 42 transferable embryos  
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37 and those with translocation errors only as well as those followed up, from our  
38  
39 diagnostic cohort, 43/184 (23%) had a meiotic error leading to an additional  
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41 aneuploidy. This is a minimal estimate since some of the multiple anomalies  
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43 not followed up may have had a meiotic origin.  
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53 Notably, in our data, 85.5% (59/69) of embryos with whole chromosome  
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55 additional aneuploidies showed an extensive number of abnormal cells; either  
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57 the aneuploidy was present throughout or in more than 50% of cells.  
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## Discussion

We have shown that additional whole chromosome aneuploidies detected by aCGH diagnosis on embryonic material from translocation carrier parents persist and are widespread in 85.5% of embryos that are followed up.

Moreover, none of the embryos with additional aneuploidies in our series were suitable for transfer; even in cases where the original aneuploidy was no longer detectable, other anomalies were present. The additional aneuploidies are therefore very significant diagnostically. We have also confirmed that although diagnosis on TE samples may be preferred, for those couples unlikely to have several blastocysts for analysis blastomere diagnosis on day 3 is still a reliable approach since anomalies seen then are real and very likely to be persistent.

It is of considerable interest that the majority of whole chromosome aneuploidies have a meiotic origin. Further work is needed to determine the parental origin of these anomalies and whether they originate in the carrier or non-carrier parent. The great majority (20/23) of couples in our study presented as infertile or sub-fertile; this may provide a causal link with the high frequency of additional meiotic errors. It is well known that males with severe sperm abnormalities show increased levels of aneuploidy (Harton et al., 2012). Less well known is the fact that female translocation carriers have a reduced reproductive lifespan, increasing the risk of additional aneuploidy at a relatively young age (Burgoyne et al., 1985; Setterfield et al., 1988; Burgoyne et al., 2009). Also, pre-meiotic or meiotic errors may be increased in

1 some individuals due to their genetic background; this may include germline  
2 mosaicism (Ghevaria et al., 2014).  
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7 Our results regarding the overall aneuploidy rate and the frequency of meiotic  
8 errors are broadly comparable to those of Fragouli et al., (2011). In this  
9 investigation the group analysed 52 good quality blastocysts donated by  
10 women undergoing routine infertility treatment. The average maternal age  
11 was 36 years, whereas for our group it was 35 years. Thirty embryos (60%)  
12 had at least some aneuploid cells compared with 63% of our total diagnostic  
13 cohort, and 30% had a meiotic error (mostly single errors) whereas 23% of  
14 ours did, but the meiotic error rate per embryo was 0.36 for the Fragouli et al.  
15 (2011) study and 0.32 for ours. So it appears that the non-translocation  
16 aneuploidy rate in our group of patients that were referred for PGD because of  
17 their translocation carrier status is very much in line with that for couples  
18 undergoing routine IVF treatment, reflecting the infertile or sub-fertile status of  
19 our patients.  
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41 As expected, post-zygotic segmental anomalies were nearly all mitotic and  
42 appeared to be subject to loss in most cases with subsequent cell division  
43 cycles. Segments will be lost unless they include a centromere or are  
44 attached to another chromosome, (Wells et al., 2000; Fragouli et al., 2013).  
45  
46 However, closer analysis revealed that half (6/12) of the mitotic segmental  
47 anomalies detected on follow up by FISH (as well as 14/36 of the whole  
48 chromosome mitotic errors) were the outcome of mitotic non-disjunction  
49 (MND) as a result of random segregation of the fragment (see Wells et al.,  
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1 2000 for a good illustration via metaphase CGH). While segmental  
2 imbalances of this nature will continue to be detected after follow up by FISH  
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4 analysis, aCGH follow up may not detect them if the gains and losses are  
5  
6 approximately equal; this is a possible reason for the much lower rate of  
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8 detection of segmental anomalies in the embryos that were followed up by  
9  
10 aCGH. So it is entirely possible that many of the segmental anomalies  
11  
12 originally detected on routine diagnosis by aCGH do persist until later stages  
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14 of development and should be taken seriously for diagnostic purposes. The  
15  
16 existence of segmental anomalies, or whole chromosome anomalies, that are  
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18 balanced by MND and are therefore not detected by initial aCGH diagnosis,  
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20 may explain why some blastocysts that are given a normal diagnostic result  
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22 fail to implant.  
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31 In conclusion, our data on the source and significance of aneuploidies  
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33 additional to those affecting the translocation chromosomes obtained by the  
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35 follow up of embryos after aCGH diagnosis show that these extra anomalies  
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37 merit full consideration when considering the choice of embryos to transfer.  
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## Figure Captions

1  
2 Figure 1 Summary of data obtained after follow up by FISH of 63

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4 untransferred embryos from translocation carriers with additional

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7 aneuploidies. MND = Mitotic Non-Disjunction

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11 Figure 2 Summary of data obtained after aCGH follow up of 20 untransferred

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13 embryos from translocation carriers with additional aneuploidies. MND =

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16 Mitotic Non-Disjunction

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21 Figure 3 aCGH follow up result of an embryo from a balanced carrier of a

22  
23 reciprocal translocation 46,XY, t(4;12)(q25;q24.31). The blue arrows indicate

24  
25 imbalances related to the translocation. The red arrow indicates additional

26  
27 aneuploidy, unrelated to the translocation. (A) Diagnostic result using

28  
29 24Sure+ (BAC) array : gain of 4p; loss of 12p ; loss of 22 (two cleavage

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31 blastomeres). (B) Follow up result using 8x60K (oligo) array: gain of 4p; loss

32  
33 of 12p; loss of 22 (rest of the embryo). The loss of 22 is a whole chromosome

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35 loss classed as meiotic as it was present on diagnosis and was detected in

36  
37 the rest of the embryo on follow up.

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41 Figure 4 aCGH follow up result of an embryo from a balanced carrier of a

42  
43 reciprocal translocation 46,XY,t(1;13)(p34.3;q34). (A) Diagnostic result using

44  
45 24Sure+ BAC arrays: Balanced for the translocation chromosomes; loss of 21

46  
47 (two cleavage stage blastomeres). (B) Follow up result using 24Sure arrays:

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51 Balanced for the translocation chromosomes; loss of 21 (rest of the embryo).



1 The loss of 21 is a whole chromosome meiotic loss as it was present on  
2 diagnosis and in the rest of the embryo on follow-up.  
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7 Figure 5 Analysis of chromosomes involved in meiotic vs mitotic aneuploidy  
8 events  
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14 Figure 6 Classification of embryos after follow-up of diagnostic aneuploid  
15 events occurring in addition to any translocation related imbalances.  
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## References

- 1  
2  
3 Alfarawati, S., Fragouli, E., Colls, P. & Wells, D. 2011. First births after  
4 preimplantation genetic diagnosis of structural chromosome  
5 abnormalities using comparative genomic hybridization and microarray  
6 analysis. *Hum Reprod*, 26, 1560-74.
- 7 Burgoyne, P. S., Mahadevaiah, S. & Mittwoch, U. 1985. A Reciprocal  
8 Autosomal Translocation Which Causes Male Sterility In The Mouse  
9 Also Impairs Oogenesis. *J Reprod Fertil*, 75, 647-52.
- 10  
11 Burgoyne, P. S., Mahadevaiah, S. K. & Turner, J. M. 2009. The  
12 consequences of asynapsis for mammalian meiosis. *Nat Rev Genet*,  
13 10, 207-16.
- 14 Colls, P., Escudero, T., Fischer, J., Cekleniak, N. A., Ben-Ozer, S., Meyer, B.,  
15 Damien, M., Grifo, J. A., Hershlag, A. & Munne, S. 2012. Validation of  
16 array comparative genome hybridization for diagnosis of translocations  
17 in preimplantation human embryos. *Reprod Biomed Online*, 24, 621-9.
- 18  
19 Conn, C.M., Cozzi, J., Harper, J.C. and Delhanty, J.D.A. 1995. Segregation of  
20 chromosome 21 in oocytes and embryos from preimplantation genetic  
21 diagnosis cycles. *Am. J. Hum. Genet*, 59 (Suppl. 4), A319
- 22  
23 Conn, C. M., Harper, J. C., Winston, R. M. & Delhanty, J. D. 1998. Infertile  
24 couples with Robertsonian translocations: preimplantation genetic  
25 analysis of embryos reveals chaotic cleavage divisions. *Hum Genet*,  
26 102, 117-23.
- 27  
28 Conn, C. M., Cozzi, J., Harper, J. C., Winston, R. M. & Delhanty, J. D. 1999.  
29 Preimplantation genetic diagnosis for couples at high risk of Down  
30 syndrome pregnancy owing to parental translocation or mosaicism. *J*  
31 *Med Genet*, 36, 45-50.
- 32  
33 Fiorentino, F., Spizzichino, L., Bono, S., Biricik, A., Kokkali, G., Rienzi, L.,  
34 Ubaldi, F. M., Iammarrone, E., Gordon, A. & Pantos, K. 2011. PGD for  
35 reciprocal and Robertsonian translocations using array comparative  
36 genomic hybridization. *Hum Reprod*, 26, 1925-35.
- 37  
38 Fragouli, E., Alfarawati, S., Daphnis, D. D., Goodall, N. N., Mania, A., Griffiths,  
39 T., Gordon, A. & Wells, D. 2011. Cytogenetic analysis of human  
40 blastocysts with the use of FISH, CGH and aCGH: scientific data and  
41 technical evaluation. *Hum Reprod*, 26, 480-90.
- 42  
43 Fragouli, E., Alfarawati, S., Spath, K., Jaroudi, S., Sarasa, J., Enciso, M. &  
44 Wells, D. 2013. The origin and impact of embryonic aneuploidy. *Hum*  
45 *Genet*, 132, 1001-13.
- 46  
47 Ghevaria, H., Sengupta, S., Sarna, U., Sargeant, S., Serhal, P. & Delhanty, J.  
48 2014. The contribution of germinal mosaicism to human aneuploidy.  
49 *Cytogenet Genome Res*, 144, 264-74.
- 50  
51 Harper, J. C., Coonen, E., Ramaekers, F. C., Delhanty, J. D., Handyside, A.  
52 H., Winston, R. M. & Hopman, A. H. 1994. Identification of the sex of  
53 human preimplantation embryos in two hours using an improved  
54 spreading method and fluorescent in-situ hybridization (FISH) using  
55 directly labelled probes. *Hum Reprod*, 9, 721-4.
- 56  
57 Harton, G. L. & Tempest, H. G. 2012. Chromosomal disorders and male  
58 infertility. *Asian J Androl*, 14, 32-9.
- 59  
60 Hopman, A. H., Ramaekers, F. C., Raap, A. K., Beck, J. L., Devilee, P., Van  
61 Der Ploeg, M. & Vooijs, G. P. 1988. In situ hybridization as a tool to  
62  
63  
64  
65

- study numerical chromosome aberrations in solid bladder tumors. *Histochemistry*, 89, 307-16.
- 1  
2 Iwarsson, E., Malmgren, H., Inzunza, J., Ahrlund-Richter, L., Sjoblom, P.,  
3 Rosenlund, B., Fridstrom, M., Hovatta, O., Nordenskjold, M. &  
4 Blennow, E. 2000. Highly abnormal cleavage divisions in  
5 preimplantation embryos from translocation carriers. *Prenat Diagn*, 20,  
6 1038-47.  
7
- 8 Jacobs, P. A. 1977. Structural rearrangements of the chromosomes in man.  
9 *Population Cytogenetics Studies in Humans*. Academic Press New  
10 York.  
11
- 12 Maeda, T., Ohno, M., Matsunobu, A., Yoshihara, K. & Yabe, N. 1991. A  
13 cytogenetic survey of 14,835 consecutive liveborns. *Jinrui Idengaku*  
14 *Zasshi*, 36, 117-29.  
15
- 16 Mantzouratou, A., Mania, A., Fragouli, E., Xanthopoulou, L., Tashkandi, S.,  
17 Fordham, K., Ranieri, D. M., Doshi, A., Nuttall, S., Harper, J. C.,  
18 Serhal, P. & Delhanty, J. D. 2007. Variable aneuploidy mechanisms in  
19 embryos from couples with poor reproductive histories undergoing  
20 preimplantation genetic screening. *Hum Reprod*, 22, 1844-53.  
21
- 22 Munne, S., Bahce, M., Schimmel, T., Sadowy, S. & Cohen, J. 1998. Case  
23 report: chromatid exchange and predivision of chromatids as other  
24 sources of abnormal oocytes detected by preimplantation genetic  
25 diagnosis of translocations. *Prenat Diagn*, 18, 1450-8.  
26
- 27 Scriven, P. N., Handyside, A. H. & Ogilvie, C. M. 1998. Chromosome  
28 translocations: segregation modes and strategies for preimplantation  
29 genetic diagnosis. *Prenat Diagn*, 18, 1437-49.  
30
- 31 Setterfield, L. A., Mahadevaiah, S. & Mittwoch, U. 1988. Chromosome pairing  
32 and germ cell loss in male and female mice carrying a reciprocal  
33 translocation. *J Reprod Fertil*, 82, 369-79.  
34
- 35 Voullaire, L., Slater, H., Williamson, R. & Wilton, L. 2000. Chromosome  
36 analysis of blastomeres from human embryos by using comparative  
37 genomic hybridization. *Hum Genet*, 106, 210-7.  
38
- 39 Wells, D. & Delhanty, J. D. 2000. Comprehensive chromosomal analysis of  
40 human preimplantation embryos using whole genome amplification and  
41 single cell comparative genomic hybridization. *Mol Hum Reprod*, 6,  
42 1055-62.  
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Figure 1

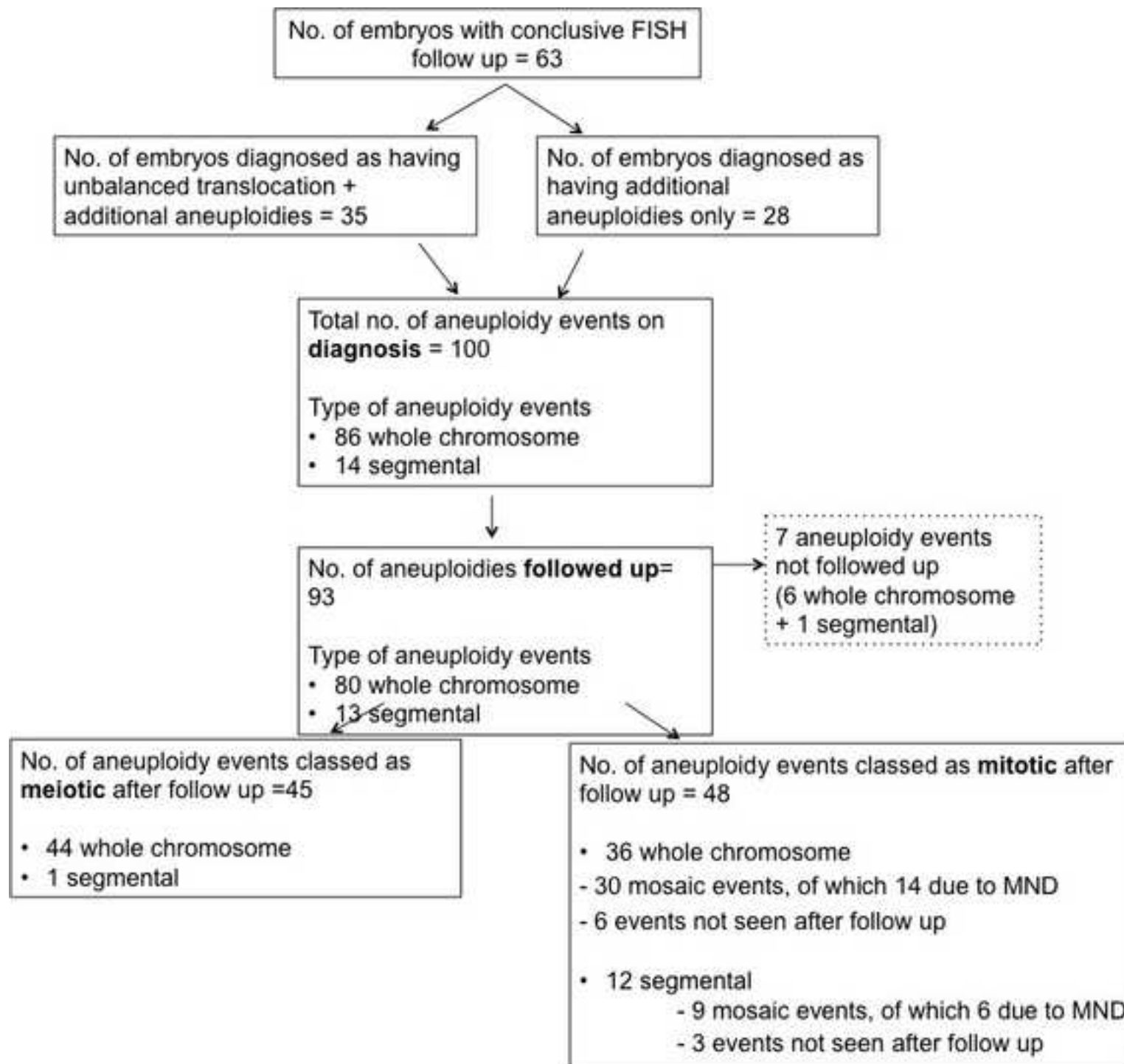


Figure 2

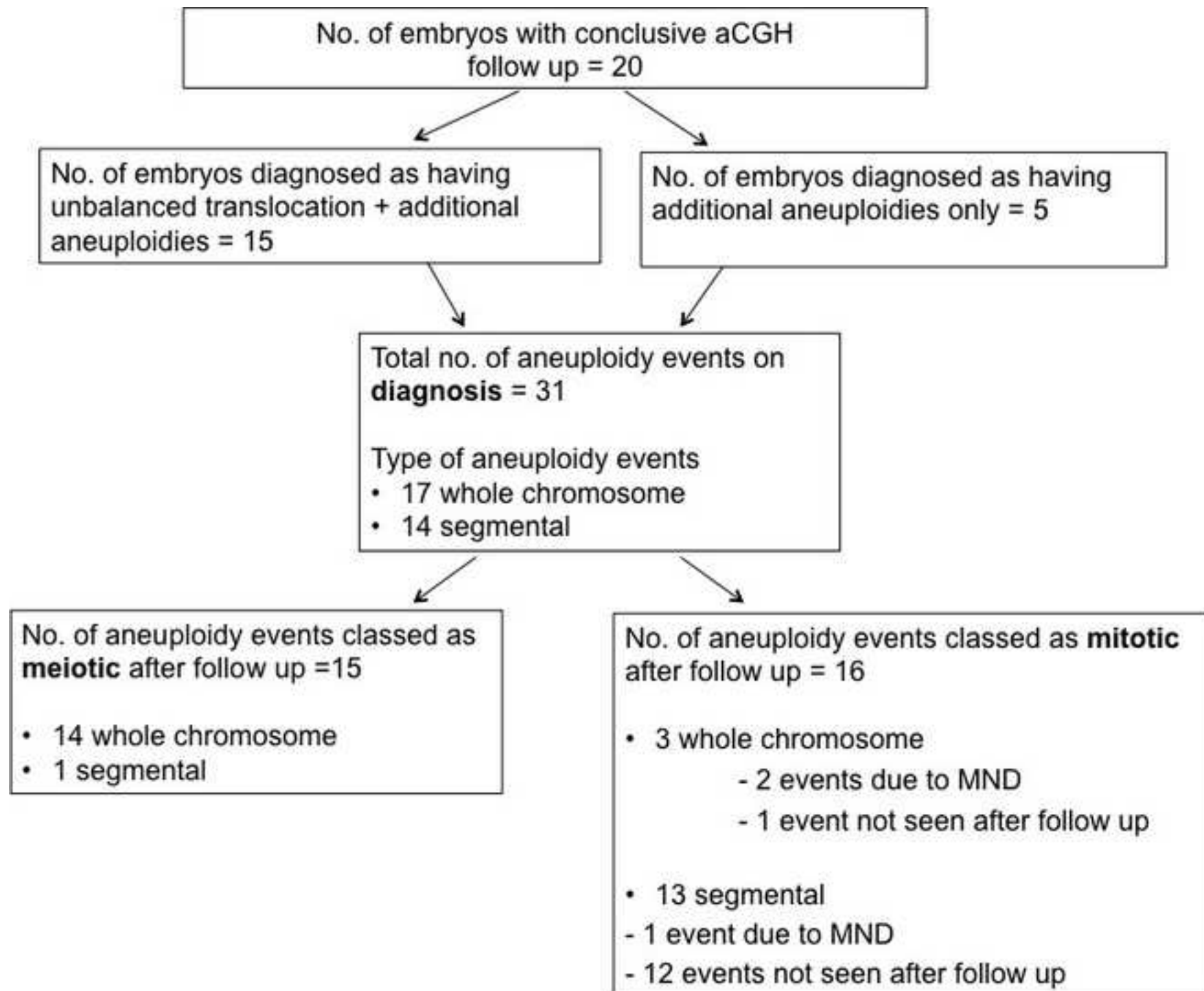


Figure 3

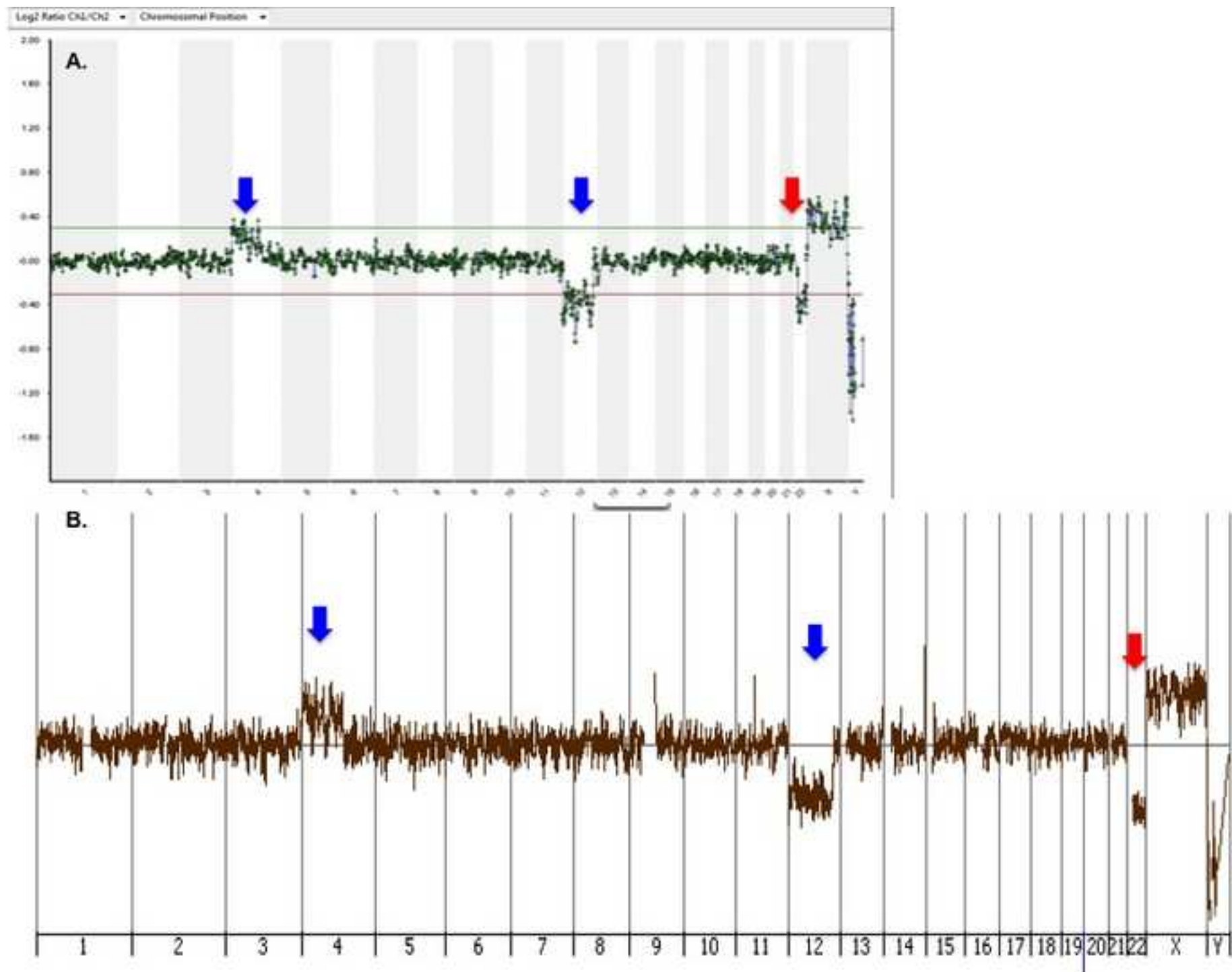


Figure 4

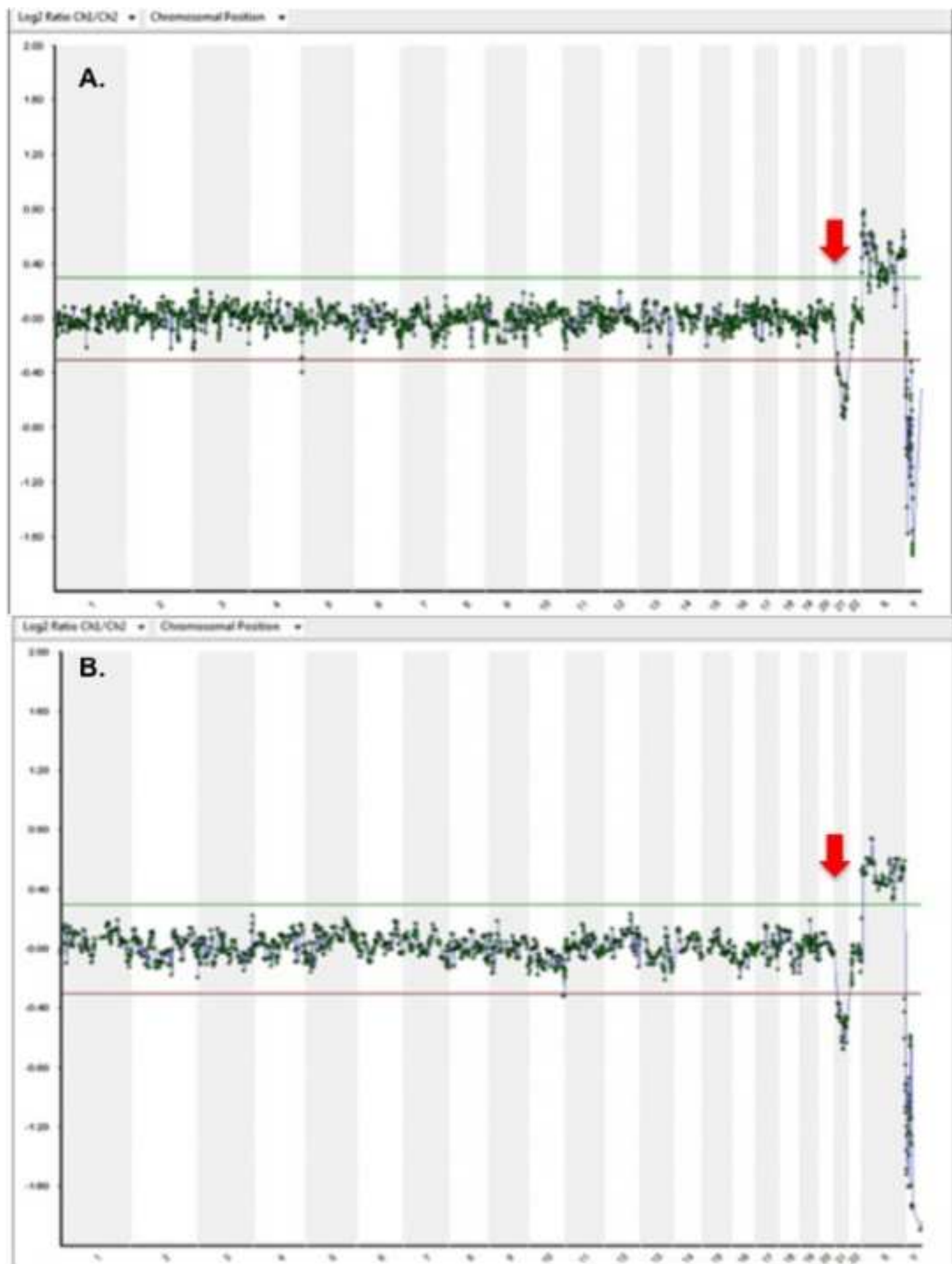
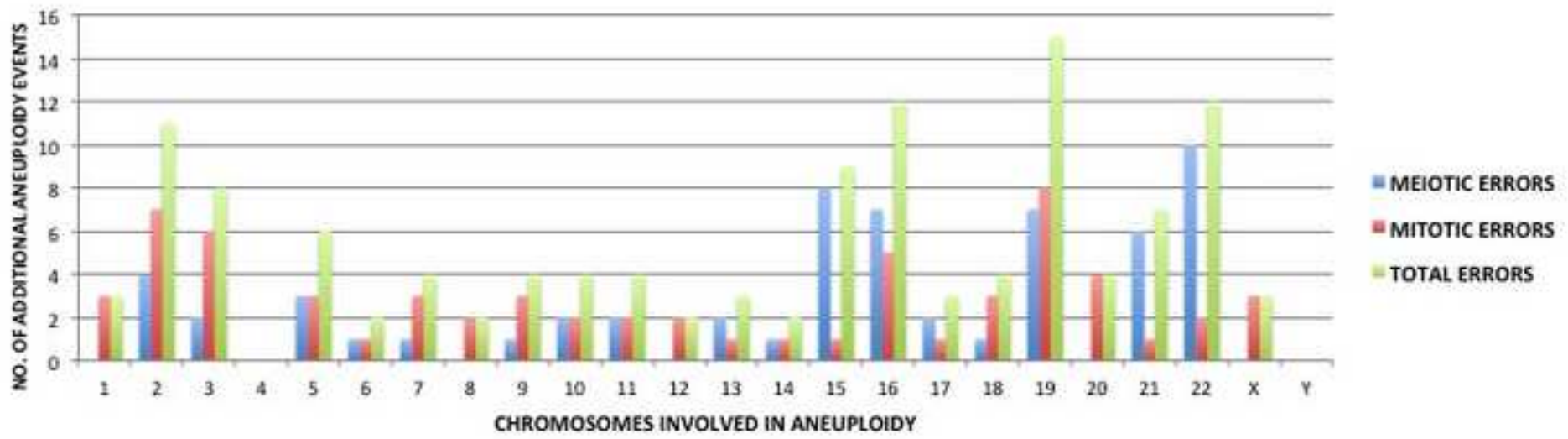


Figure 5





## Proportion of embryos with meiotic and mitotic aneuploidy events

