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 55-65% embryos with additional aneuploidies with or without translocation related imbalances. The occurrence of extra abnormalities with translocations 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). In 85.5% of embryos with whole chromosome additional aneuploidies, either the aneuploidy was present throughout or in more than 50% of cells. 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, whether segmental or affecting whole chromosomes, are important findings and merit full consideration when considering the choice of embryos to transfer.

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

Introduction

According to cytogenetic studies of newborn infants carried out on 63,000 individuals from six different countries the combined incidence of balanced Robertsonian and reciprocal translocations lies between 1.5 and 1.7 per thousand (Jacobs, 1977; Maeda et al., 1991). As adults, most of these individuals are able to reproduce successfully, with the help of prenatal diagnosis where necessary. A minority will experience overt fertility problems leading them to present for investigation and to consider preimplantation genetic diagnosis (PGD). PGD was first made available in 1995 for couples at risk of chromosomally abnormal conceptions arising from the translocation carrier status of one partner (Conn et al., 1995). The approach employed the technique of fluorescence in situ hybridisation (FISH) with chromosome

probes specific to regions that had been translocated. Other publications soon followed (Conn et al., 1998; 1999; Munné et al., 1998; Scriven et al., 1998; Iwarsson et al., 2000). Later in some centres, additional probes were added to screen for common aneuploidies, but the main focus was on the translocated chromosomes. The development of techniques to allow the application of comparative genomic hybridisation (CGH) analysis to single cells paved the way to the comprehensive screening of all chromosomes (Wells et al., 2000; Voullaire et al., 2000). Initially, this approach required a high degree of skill and a lengthy period of hybridisation, so it was the advent of single cell CGH analysis via microarrays (aCGH) that opened up the field and reports of its application in translocation PGD cycles rapidly appeared (Alfarawati et al., 2011; Fiorentino et al., 2011). It quickly became apparent that additional aneuploidies were as common as translocation imbalances (Alfarawati et al., 2011; Fiorentino et al., 2011). At that time, most cases involved day 3 biopsy of blastomeres, a minority were diagnosed on trophectoderm (TE) samples. Most centres avoided the transfer of any embryo with a chromosomal anomaly but a few decided to consider only the translocation imbalances. The reasoning behind this was that the true extent of any additional aneuploidies was unknown since most were diagnosed on a single cell. There have been few reports of any follow up analyses to answer this question. In a validation study, Colls et al. (2012) re-analysed by FISH embryos diagnosed as abnormal by aCGH; of 102 embryos (not all from translocation cases) 52 were uniformly aneuploid and 45 were aneuploid mosaic or chaotic mosaic – but with all cells abnormal. Follow up analysis was also carried out by Fiorentino et al. (2011); embryos diagnosed as abnormal were re-biopsied on day 6 and

the cells subjected to analysis identical to that employed for diagnosis. In total, 57 embryos with additional aneuploidies, with or without translocation imbalance, were re-analysed and the diagnoses confirmed. Aneuploidy mosaicism was seen in 24/57 embryos but all were confirmed as abnormal despite the mosaicism; no details were given.

The aim of our study was to investigate in detail the significance of aneuploidies that occur in addition to translocation imbalances in couples undergoing PGD via aCGH and to determine whether the origin was meiotic or mitotic.

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 Ltd., now Illumina) were used for the detection of Robertsonian translocations and 24Sure+™ (BlueGnome Ltd., now Illumina) 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 ±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 0.2µl microcentrifuge tubes) for aCGH analysis. Untransferred embryos with one to five distinct additional abnormalities were followed up; embryos with multiple chromosome abnormalities (> five aneuploidies) and embryos with no diagnostic results were excluded.

Clinical and research approvals

Diagnosis and follow up was covered by the treatment licence (licence reference R0113-7-a, valid 27/11/13 to 26/11/16) from the HFEA (Human Fertilisation and Embryology Authority) held by the CRGH (Centre for Reproductive and Genetic Health). UCL Centre for PGD is an accredited laboratory (Clinical Pathology Accreditation, reference no. 2920).

Spreading of untransferred embryos and FISH procedure

Whole untransferred embryos were spread using the Tween-HCl method as described in Harper et al. (1994). The FISH protocol was performed as

previously described with slight modifications (Harper et al., 1994). FISH probes were chosen to follow up those chromosomes, unrelated to the translocation, that were identified as aneuploid on diagnosis. A combination of CEP (chromosome enumeration probes), Sub-tel (sub-telomeric probes) and LSI (locus specific identifier probes) in different fluorochromes was suitably selected. All probes were from Abbott Molecular, UK.

Microscope analysis and scoring of FISH signals

The slides were examined under an epifluorescence Olympus microscope (Olympus BX 40) equipped with suitable filters. Specific signal scoring criteria were applied to interphase nuclei for uniform evaluation of FISH signals, in accordance with Hopman et al., 1988 and Mantzouratou et al., 2007.

Tubing of untransferred embryos for aCGH analysis

Whole untransferred embryos were tubed under sterile conditions. Under a dissecting microscope using 0.3μm 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 0.2μm 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., now Illumina) 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 oligonucleotides spaced at an average of 41kb intervals across the human genome (Design ID 021924). BlueGnome 24Sure/24Sure+ arrays were previously validated in our laboratory for clinical use. Validation was performed on 35 single cells from cell lines with known karyotypes (Mamas et al., 2012). The 8x60K oligonucleotide arrays were also validated in house on single blastomeres and embryos previously analysed by BAC arrays before employing them for follow-up purposes.

Array-CGH diagnosis and follow up using BlueGnome 24Sure/24Sure+ BAC arrays

Array-CGH using 24Sure (v2 and v3) or 24Sure+ arrays was performed according to the manufacturer's instructions. Briefly, test samples were

labeled with Cy3 and reference samples with Cy5 fluorophores. Labeling was carried out for 16-18 hours. Combination, ethanol precipitation and denaturation were performed on the following day and the samples were hybridized for 3-4hrs. The slides were washed and scanned using InnoScan 700 (Innopsys SA) or ScanArray Express (Perkin Elmer) or Agilent SureScan microarrays (Agilent Technologies) scanners at a resolution of 10µm and scanned images were analysed and interpreted using the BlueFuse Multi software (BlueGnome Ltd, now Illumina). The BlueFuse algorithm is based on calculating the median $\log_2$ratio of all the chromosomes for detection of gains and losses. A median $\log_2$ratio value ≥ 0.3 indicated chromosome gains whereas values ≤ –0.3 indicated chromosome losses. Gains and losses of segmental (partial) aneuploidies unrelated to the translocation were identified when a minimum of 10 consecutive BAC clones crossed the normality thresholds.

Array-CGH follow up with Agilent's 60-mer oligonucleotide microarrays

Eight embryonic samples after WGA were tested using both the high-resolution Agilent (8x60K) 60-mer oligonucleotide microarrays and BlueGnome BAC (bacterial artificial chromosome) 24Sure/24Sure+ microarrays. The manufacturer's protocol was used to perform the Agilent 8x60K oligo microarrays with certain modifications. Briefly, test samples were labeled with Cy5 and reference samples with Cy3 fluorophores. Labeling of the amplified DNA was carried out for 2.5 hours. Preparation for hybridization included combination, ethanol precipitation and denaturation, performed on the same day. Hybridisation was carried out overnight for 16 hours.

Microarray washing was performed the following day. The arrays were scanned using Agilent SureScan Microarray Scanner at 3μm and the TIFF images analysed using Agilent Genomic Workbench 7.0 (Agilent technologies). All samples were analysed by a single cell analysis method recommended by the manufacturer. The analysis method was configured with ADM-2 (Aberration Detection Method 2). This algorithm identified all aberrant intervals in the sample with consistently high or low log_2ratios based on a statistical score. The software identified the aberrations if the average log_2ratio of the sample over the reference exceeded a specific threshold; log_2ratio of ≥0.3 for gains and ≥0.55 for losses.

Classification of embryos according to their mechanism of origin of aneuploidy post FISH and aCGH follow-up analysis

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

Follow up by FISH

Embryos were classified as having a mitotic aneuploidy event when the embryos were mosaic with at least two cell lines, a diploid cell line and an aneuploid cell line or two different aneuploid cell lines. The imbalance of the

aneuploid cell line could be either concordant with that detected at clinical diagnosis (day 3/day 5) or a complementary gain or loss. The percentages of nuclei with the diploid and aneuploid cell lines for a single aneuploidy event were calculated.

Embryos were classified as having a meiotic aneuploidy event when over 90% of nuclei were scored in concordance with the aneuploidy scored at diagnosis. This is based upon our experience in following up the imbalances related to the translocation in the rest of the embryo; although it is known that the translocation related imbalances are meiotic and should be present in all of the embryonic nuclei, this is not always the case. The discrepancy rate may be up to 10%; the causes include technical difficulties with the FISH procedure and mosaicism.

Follow up by aCGH

If the aneuploidy event was seen on aCGH diagnosis and not seen on follow-up or if the aneuploidy event was seen on follow-up and not seen on biopsy, it was considered as being mitotic in origin. If the aneuploidy event was seen on diagnosis and on follow-up with no indication of mosaicism (deviation of all the clones/oligonucleotides belonging to one chromosome between the $\log_2$ ratio of zero and the normality thresholds in either direction), it was considered as being meiotic in origin.

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. A total of 298 embryos was tested, [272 after biopsy of one/two cells on day 3 and 26 after TE biopsy on day 5], of which 271 (91%) gave results. Of the embryos tested, 42 (14%) were either normal or balanced for the translocation with no other anomalies and were transferrable. Of the 256 untransferrable embryos, 170 (170/271, 63%) had been diagnosed with additional aneuploidies. Embryos were classified according to Table 2. Of the 27 embryos included in the no result group of embryos, 24 had amplification failure [24/298 (8%)] and were not processed further. Additionally, 1% embryos did not give a conclusive result (3/298).

Embryos for FISH and aCGH follow up of additional aneuploidies

Of the 112 embryos with 1-5 additional aneuploides, 63 were followed up by FISH and 22 by aCGH, in total 85. The remaining 27 embryos were regarded as unsuitable for follow up for various reasons.

Overall summary of follow up data

Conclusive follow up results were obtained for 83 out of 85 embryos, 63 by FISH and 20 by aCGH. Of the 85 aneuploid embryos, 30 (35.29%) had only meiotic errors and 40 (47.06%) had only mitotic errors, 13 (15.29%) embryos had both type of errors and 2 (2.35%) embryos did not give results (Figure 1).

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). Of the 97 whole chromosome aneuploidies followed up, 90 (92.8%) persisted to later stages. Whereas for the segmental anomalies, out of the 27, only 12 (44%) were seen at later stages.

However of those followed up by FISH, 10/13 were detected on follow up, whereas of those analysed by aCGH 2/14 were detected on follow up (both mitotic and meiotic events included). The difference between the two may be due to the high frequency of MND detected after FISH follow up, as discussed later.

Results from embryos with FISH follow up

Follow-up by FISH included 48 embryos from PGD for reciprocal translocation cycles and 15 embryos from Robertsonian translocation cycles. In all, the 63 embryos presented with 100 additional aneuploidy events at diagnosis, 86 whole chromosomal and 14 segmental aneuploidies. Follow up was performed for 93/100 events (93%) (Figure 2).

Classification of chromosome aneuploidy events post follow up by FISH

In 63 untransferred embryos, 80 whole chromosome and 13 segmental additional aneuploidy events were re-analysed. Forty-four whole chromosome meiotic aneuploidy events were found (Figure 2). Among these, 28/44 (64%) were uniformly aneuploid meiotic events in which all the embryonic nuclei had FISH signals consistent with the aneuploidy detected on diagnostic aCGH and for 16/44 (36%) the embryo had over 90% nuclei with FISH signals consistent with the aneuploidy detected on diagnosis and were also considered to be meiotic in origin. Only one segmental meiotic event was present after follow up in one embryo, this being the loss of 2q11.2-2qter in an arrested embryo with 9 cells.

Thirty-six whole chromosome and 12 segmental mitotic aneuploidy events were found (Figure 2). Embryos with mitotic errors were either diploid/aneuploid mosaics or aneuploid mosaics. Regarding whole chromosome errors, in sixteen events, the aneuploidy event present on diagnosis was confirmed on follow up. In fourteen other cases, the embryo contained two different cell lines showing complementary aneuploidy events (for example: +9, -9) presumably due to mitotic non-disjunction (MND) one of which was present on diagnostic aCGH. Additionally six whole chromosome events in five embryos were not seen on follow up but in all these embryos other anomalies were present.

Similarly, follow up data of the twelve segmental mitotic aneuploidies showed that three segments in three embryos were not seen on follow up. Six events with complementary findings presumably arose due to MND and the remaining three showed aneuploid cells between 11%-30% after FISH follow up.

Results of embryos with aCGH follow up

Validation results of eight embryonic samples showed that the BAC and the oligonucleotide-based arrays gave comparable results. A total of 22 whole embryos were subjected to WGA. Successful amplification was obtained for 21/22 (95%) embryos and conclusive aCGH follow up results were obtained for 20/21 (95%). Eleven embryos were analysed by BlueGnome 24Sure/24Sure+ arrays, eight by Agilent 8x60K and two were analysed by both BAC and oligonucleotide arrays (one of which was a part of the

validation). Follow up by aCGH included seventeen embryos from reciprocal and five from Robertsonian translocation carriers. After aCGH follow up, the translocation status was confirmed for all 20 embryos with conclusive results.

The aCGH results of twenty embryos are summarised in Figure 3. Overall, of the 31 aneuploidies seen on diagnosis only 1 of 17 whole chromosome anomalies was not seen on follow up whereas only 1 of 14 segmental errors was detected in the whole embryo. Two embryos showed evidence for MND, involving two whole chromosomes in one case and one segmental anomaly in the other. Figures 4 and 5 provide examples of aCGH results of embryos followed up by BAC and oligonucleotide arrays.

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 6 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. Chromosome 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 102/124 (82%) 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

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

Proportion of embryos with aneuploidy events

Whole chromosome abnormalities only were seen in 59 embryos (71%) and segmental aneuploidies alone in 14 (17%). Ten embryos (12%) had both whole chromosome and segmental anomalies. Excluding the 58 embryos with more than 5 errors, but including the 42 transferable embryos and those with translocation errors only (59) as well as those followed up, from our diagnostic cohort (83), 43/184 (23%) embryos had a meiotic error leading to an additional aneuploidy. This is a minimal estimate since some of the multiple

anomalies not followed up may have had a meiotic origin; also cases of trisomic rescue may appear to be mitotic in origin when they are truly meiotic.

Notably, in our data, 85.5% (59/69) of embryos with whole chromosome additional aneuploidies showed an extensive number of abnormal cells; either the aneuploidy was present throughout or in more than 50% of cells.

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. We found that overall meiotic and mitotic events were almost equal. For the whole chromosome aneuploidies meiotic errors predominated whereas for segmental anomalies, almost all were mitotic.

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 important 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 men 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 some individuals due to their genetic background; this may include germline mosaicism (Ghevaria et al., 2014).

Our results regarding the overall aneuploidy rate and the frequency of meiotic errors are broadly comparable to those of Fragouli et al., (2011). In this investigation the group analysed 52 good quality blastocysts donated by women undergoing routine infertility treatment. The average maternal age was 36 years, whereas for our group it was 35 years. Thirty embryos (60%) had at least some aneuploid cells compared with 63% of our total diagnostic cohort, and 30% had a meiotic error (mostly single errors) whereas 23% of ours did, but the meiotic error rate per embryo was 0.36 (19/52) for the Fragouli et al. (2011) study and 0.32 (60/184) for ours. So it appears that the non-translocation aneuploidy rate in our group of patients that were referred for PGD because of their translocation carrier status is very much in line with that for couples undergoing routine IVF treatment, reflecting the infertile or sub-fertile status of our patients.

Regarding the segmental anomalies, in comparison to our study, in the study published by Fragouli et al., (2013), the authors analysed a large number of embryos at different stages of preimplantation development in patients undergoing PGS. They found that segmental anomalies occur at a reduced frequency in oocytes (4%) and then are found at an appreciable level at 15% of cleavage stage embryos and then reducing to 8% at the blastocyst stage. Hence the segmental anomalies found in the present study are comparable to their study in the incidence of segmental aneuploidies at cleavage stage compared with blastocyst stage.

As expected, in our data, the post-zygotic segmental anomalies were nearly all mitotic and appeared to be subject to loss in most cases with subsequent cell division cycles. Segments will be lost unless they include a centromere or are attached to another chromosome, (Wells et al., 2000; Fragouli et al., 2013). However, closer analysis revealed that half (6/12) of the mitotic segmental anomalies detected on follow up by FISH (as well as 14/36 of the whole chromosome mitotic errors) were the outcome of mitotic non-disjunction (MND) as a result of random segregation of the fragment (see Wells et al., 2000 for a good illustration via metaphase CGH). While segmental imbalances of this nature will continue to be detected after follow up by FISH analysis, aCGH follow up may not detect them if the gains and losses are approximately equal; this is a possible reason for the much lower rate of detection of segmental anomalies in the embryos that were followed up by aCGH. So it is entirely possible that many of the segmental anomalies originally detected on routine diagnosis by aCGH do persist until later stages

of development and should be taken seriously for diagnostic purposes. The existence of segmental anomalies, or whole chromosome anomalies, that are balanced by MND and are therefore not detected by initial aCGH diagnosis, may explain why some blastocysts that are given a normal diagnostic result fail to implant.

In conclusion, our data on the source and significance of aneuploidies additional to those affecting the translocation chromosomes obtained by the follow up of embryos after aCGH diagnosis show that these extra anomalies, both whole chromosome and segmental, merit full consideration when considering the choice of embryos to transfer.

Figure Captions

Figure 1 Classification of embryos after follow up of diagnostic aneuploid events occurring in addition to any translocation related imbalances.

Figure 2 Summary of data obtained after follow up by FISH of 63 untransferred embryos from translocation carriers with additional aneuploidies. MND = Mitotic Non-Disjunction

Figure 3 Summary of data obtained after aCGH follow up of 20 untransferred embryos from translocation carriers with additional aneuploidies. MND = Mitotic Non-Disjunction

Figure 4 aCGH follow up result of an embryo from a balanced carrier of a reciprocal translocation 46,XY, t(4;12)(q25;q24.31). The blue arrows indicate imbalances related to the translocation. The red arrow indicates additional aneuploidy, unrelated to the translocation. (A) Diagnostic result using 24Sure+ (BAC) array : gain of 4p; loss of 12p ; loss of 22 (two cleavage blastomeres). (B) Follow up result using 8x60K (oligo) array: gain of 4p; loss of 12p; loss of 22 (rest of the embryo). The loss of 22 is a whole chromosome loss classed as meiotic as it was present on diagnosis and was detected in the rest of the embryo on follow up.

Figure 5 aCGH follow up result of an embryo from a balanced carrier of a reciprocal translocation 46,XY,t(1;13)(p34.3;q34). (A) Diagnostic result using 24Sure+ BAC arrays: Balanced for the translocation chromosomes; loss of 21

(two cleavage stage blastomeres). (B) Follow up result using 24Sure arrays: Balanced for the translocation chromosomes; loss of 21 (rest of the embryo). The loss of 21 is a whole chromosome meiotic loss as it was present on diagnosis and in the rest of the embryo on follow-up.

Figure 6 Analysis of chromosomes involved in meiotic vs mitotic aneuploidy events

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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
Reciprocal translocation - male carriers							
A	46,XY,t(1;13)(p34.3;q34) **	1	23	Blastomeres	14 (2)	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)	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 No previous pregnancies
		2	34	Trophectoderm cells	8	0	
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 (1)	2	
E	46,XY,t(9;15)(q33.2;q11.2)	1	35	Blastomeres	5 (1)	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

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
							previous PGD cycle
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 woman 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)
Reciprocal translocation - female carriers							
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 woman with polycystic ovaries.
O	46,XX,t(11;19)(q14.2;q13.3)	1	39	Blastomeres	3	0	1 miscarriage (8/40),

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
							1 TOPFA with balanced karyotype but with cloacal abnormality, Suboptimal ORT
P	46,XX,t(11;22)(q23.3;q11.2)	1	39	Blastomeres	9 (3)	0	3 miscarriages 1 TOPFA after abnormal CVS result with an unbalanced form of translocation
		2	39	Blastomeres	13 (1)	2	
Robertsonian translocation - male carriers							
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)	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 (1)	5	Primary infertility (duration 6 years) 2 failed IVF attempts (1 with PGD)
T	46,XYY,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 (1)	3	

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
U	45,XY,der(14;21)(q10;q10)	1	37	Blastomeres	10	1	Primary infertility (duration 9 years) 2 failed IVF attempts 1 miscarriage
Robertsonian translocation - female carriers							
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
§The number in brackets indicates those 12 embryos which did not give a conclusive result on day 3 but gave a result after TE biopsy (day 5/6).

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	42/298 (14%)
No. untransferrable	189	67	256/298 (86%)
No. with translocation related imbalances only	46	13	59/256 (23%)
No. with translocation related imbalances with 1 – 5 additional aneuploidies	56	14	70/256 (27%)
No. with 1 – 5 additional aneuploidies only	28	14	42/256(16%)
No. with multiple chromosome aneuploidies (>5 aneuploidies)	41	17	58/(256) (23%)
No. with no results or amplification failure	18	9	27/298 (9%)

Figure 1

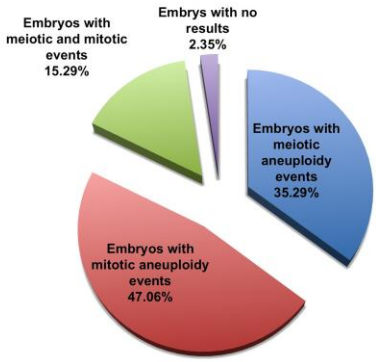

Embryos with meiotic and mitotic events 15.29%
Embrys with no results 2.35%
Embryos with meiotic aneuploidy events 35.29%
Embryos with mitotic aneuploidy events 47.06%

Figure 2

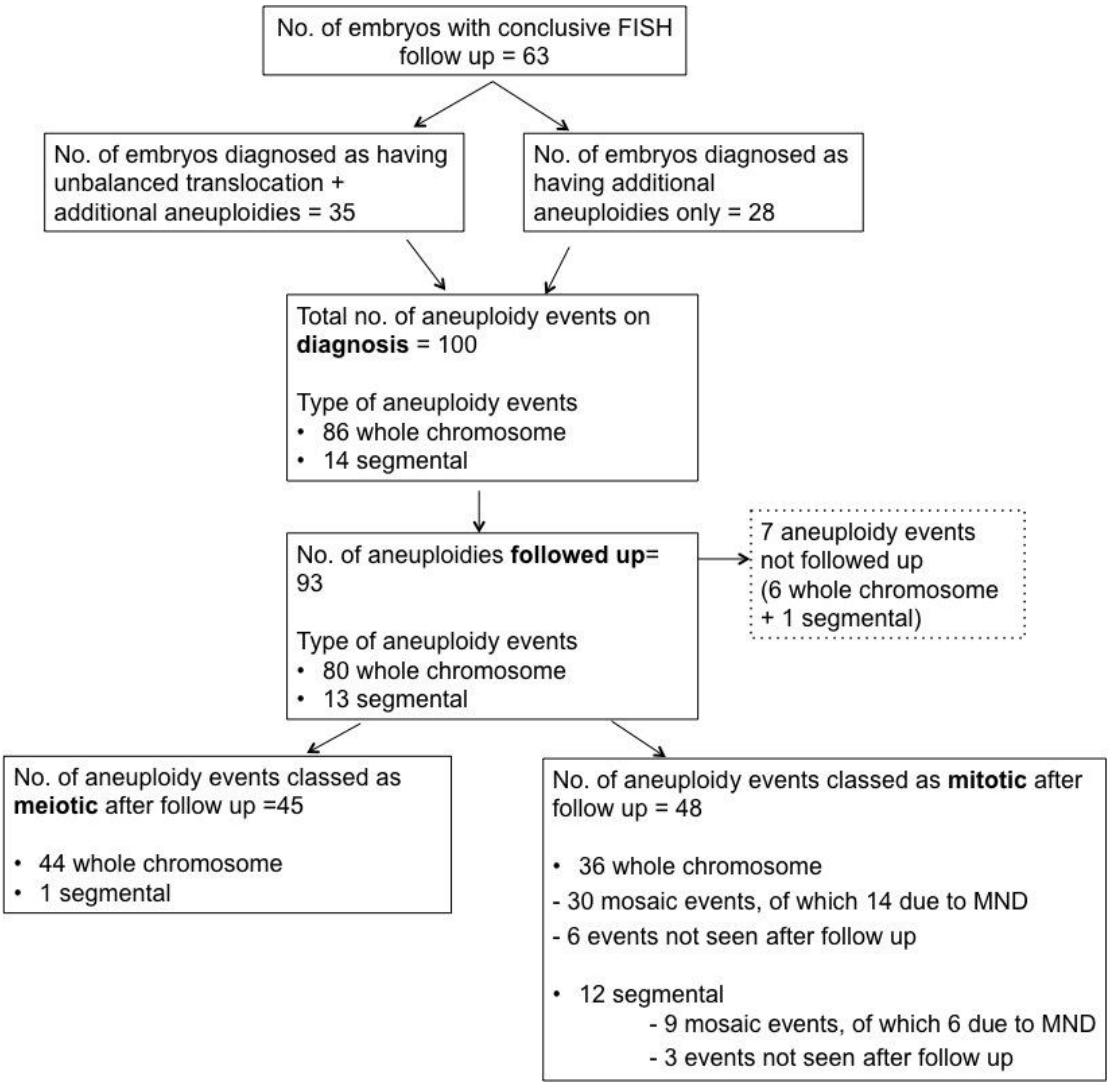

No. of embryos with conclusive FISH follow up = 63
No. of embryos diagnosed as having unbalanced translocation + additional aneuploidies = 35
No. of embryos diagnosed as having additional aneuploidies only = 28
Total no. of aneuploidy events on **diagnosis** = 100
Type of aneuploidy events
• 86 whole chromosome
• 14 segmental
No. of aneuploidies **followed up**= 93
Type of aneuploidy events
• 80 whole chromosome
• 13 segmental
7 aneuploidy events not followed up (6 whole chromosome + 1 segmental)
No. of aneuploidy events classed as **meiotic** after follow up =45
• 44 whole chromosome
• 1 segmental
No. of aneuploidy events classed as **mitotic** after follow up = 48
• 36 whole chromosome
- 30 mosaic events, of which 14 due to MND
- 6 events not seen after follow up
• 12 segmental
- 9 mosaic events, of which 6 due to MND
- 3 events not seen after follow up

Figure 3

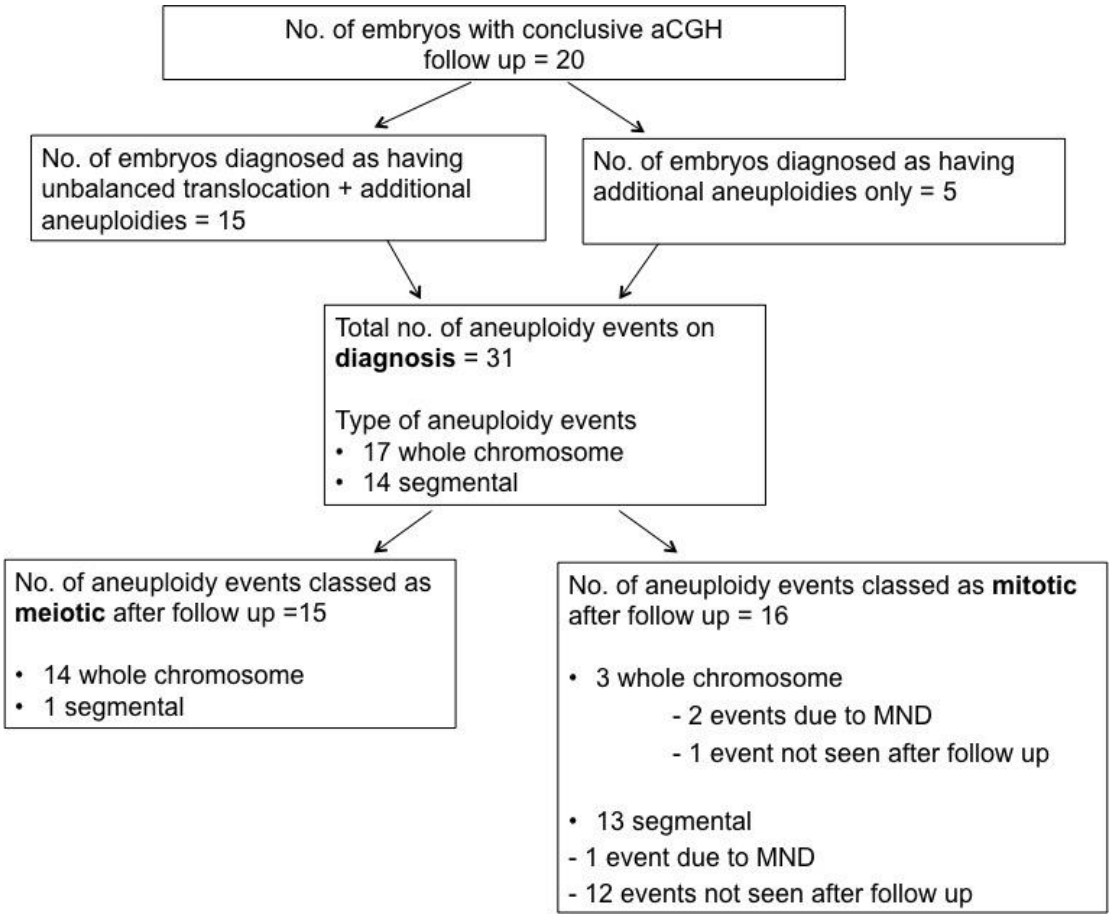

No. of embryos with conclusive aCGH follow up = 20
No. of embryos diagnosed as having unbalanced translocation + additional aneuploidies = 15
No. of embryos diagnosed as having additional aneuploidies only = 5
Total no. of aneuploidy events on **diagnosis** = 31
Type of aneuploidy events
• 17 whole chromosome
• 14 segmental
No. of aneuploidy events classed as **meiotic** after follow up =15
• 14 whole chromosome
• 1 segmental
No. of aneuploidy events classed as **mitotic** after follow up = 16
• 3 whole chromosome
- 2 events due to MND
- 1 event not seen after follow up
• 13 segmental
- 1 event due to MND
- 12 events not seen after follow up

Figure 4

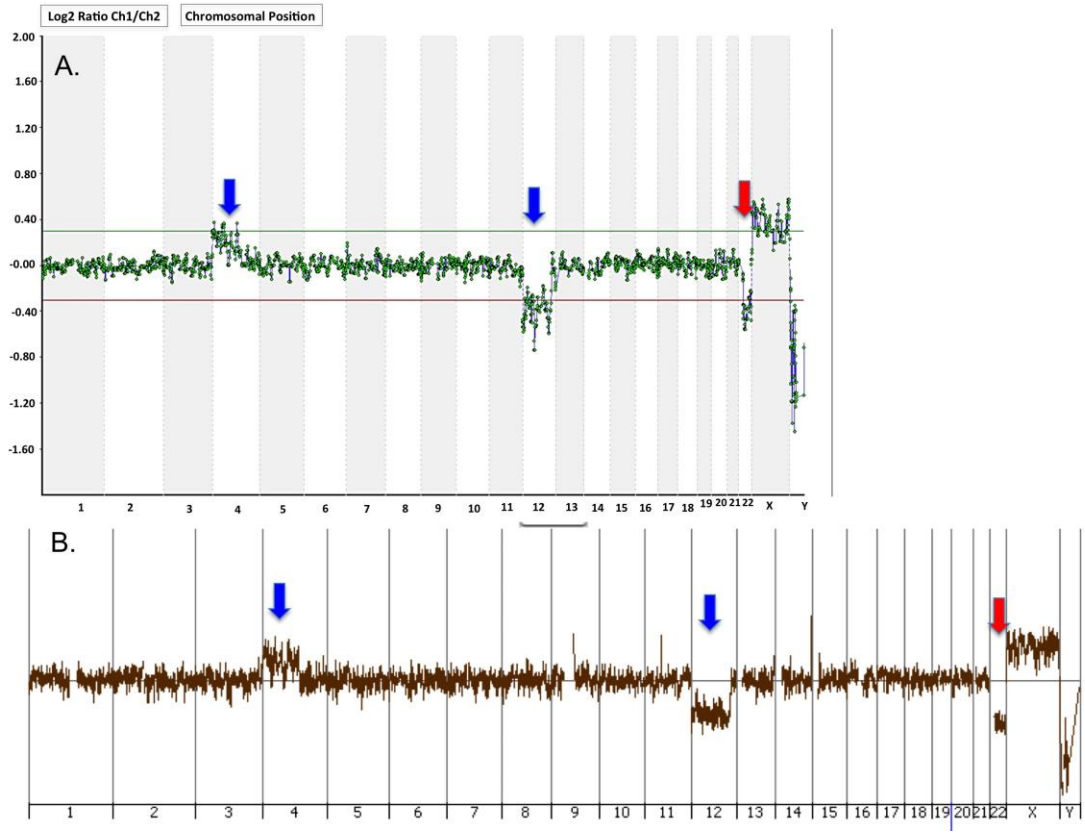

Log2 Ratio Ch1/Ch2
Chromosomal Position
A.
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
B.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y

Figure 5

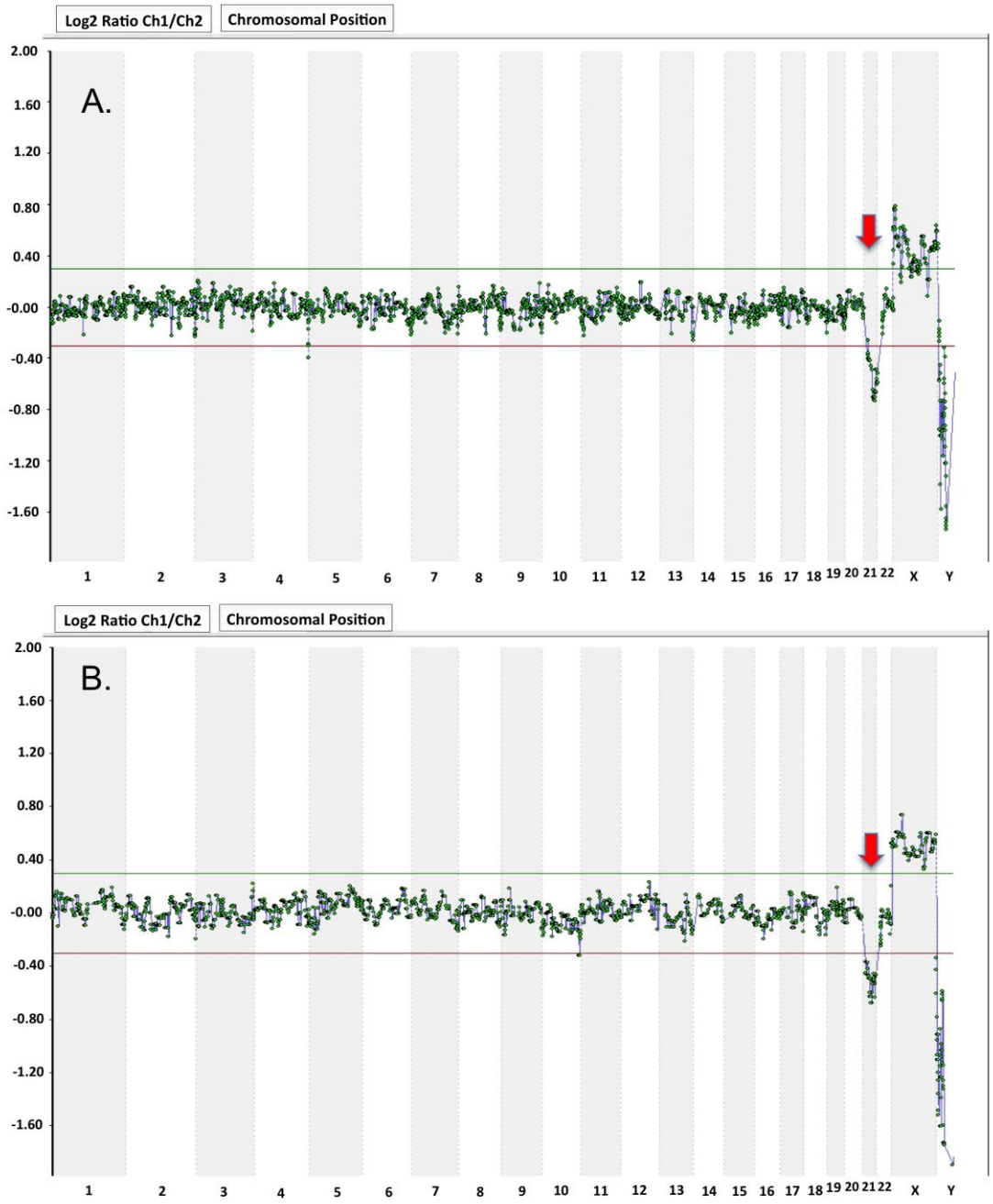
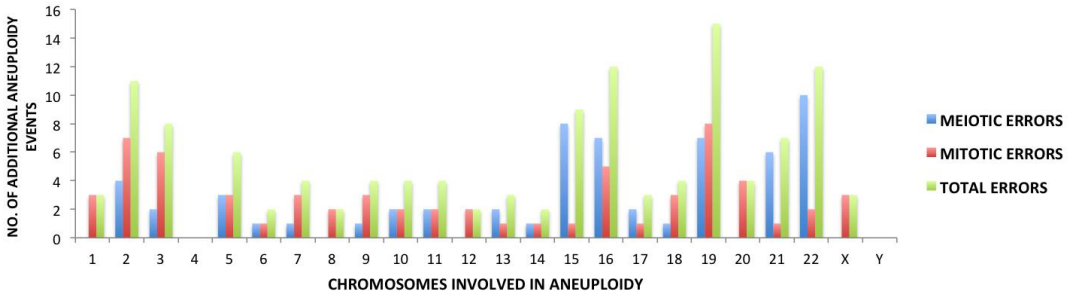

Log2 Ratio Ch1/Ch2
Chromosomal Position
A.
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Log2 Ratio Ch1/Ch2
Chromosomal Position
B.
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y

Figure 6


NO. OF ADDITIONAL ANEUPLOIDY EVENTS
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
CHROMOSOMES INVOLVED IN ANEUPLOIDY
MEIOTIC ERRORS
MITOTIC ERRORS
TOTAL ERRORS