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ORIGINAL RESEARCH

Comprehensive analysis of karyotypic mosaicism between trophectoderm and inner cell mass

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ABSTRACT: Aneuploidy has been well-documented in blastocyst embryos, but prior studies have been limited in scale and/or lack mechanistic data. We previously reported preclinical validation of microarray 24-chromosome preimplantation genetic screening in a 24-h protocol. The method diagnoses chromosome copy number, structural chromosome aberrations, parental source of aneuploidy and distinguishes certain meiotic from mitotic errors. In this study, our objective was to examine aneuploidy in human blastocysts and determine correspondence of karyotypes between trophectoderm (TE) and inner cell mass (ICM). We disaggregated 51 blastocysts from 17 couples into ICM and one or two TE fractions. The average maternal age was 31. Next, we ran 24-chromosome microarray molecular karyotyping on all of the samples, and then performed a retrospective analysis of the data. The average per-chromosome confidence was 99.95%. Approximately 80% of blastocysts were euploid. The majority of aneuploid embryos were simple aneuploid, i.e. one or two whole-chromosome imbalances. Structural chromosome aberrations, which are common in cleavage stage embryos, occurred in only three blastocysts (5.8%). All TE biopsies derived from the same embryos were concordant. Forty-nine of 51 (96.1%) ICM samples were concordant with TE biopsies derived from the same embryos. Discordance between TE and ICM occurred only in the two embryos with structural chromosome aberration. We conclude that TE karyotype is an excellent predictor of ICM karyotype. Discordance between TE and ICM occurred only in embryos with structural chromosome aberrations.

Key words: trophectoderm / inner cell mass / microarray / mosaicism / aneuploidy

Introduction

Chromosome imbalances are well-documented in early stage human embryos cultured during *in vitro* fertilization (IVF) (Munné *et al.*, 1995, 2007; Wells and Delhanty, 2000; Voullaire *et al.*, 2000; Baart *et al.*, 2004, 2007; Vanneste *et al.*, 2009; Johnson *et al.*, 2010). Clinically, chromosome imbalances are diagnosed using preimplantation genetic screening (PGS) on three types of biopsies: (i) polar bodies, (ii) cleavage stage blastomeres and (iii) trophectoderm (TE) biopsies. Each type of biopsy has advantages and disadvantages.

Polar body biopsies only detect maternal meiotic chromosome imbalances and for comprehensiveness, both polar bodies I and II should be evaluated. Many embryos that extrude polar bodies do not reach blastoscyst stage, wasting effort to analyze embryos that arrest early in development.

Blastomere biopsy is generally considered the least technically challenging and is currently the most common type of biopsy for PGS. In contrast to polar body biopsies, blastomere biopsies enable the analyses of both meiotic and mitotic whole-chromosome imbalances. However, blastomere biopsy has limitations because up to 60% of embryos at cleavage stage exhibit mosaicism, where at least one cell has a different ploidy state from the other cells in the embryo (Vanneste *et al.*, 2009; Johnson *et al.*, 2010). Additionally, many cleavage stage embryos diagnosed as aneuploid with blastomere biopsy will 'self-correct' by blastocyst stage, which, from a clinical stand point, may decrease the chances of a live birth by prematurely labeling an embryo as abnormal (Baart *et al.*, 2004, 2007; Li *et al.*, 2005; Munné *et al.*, 2005; Barbash-Hazan *et al.*, 2008; Frumkin *et al.*, 2008). Although blastomere biopsies often successfully predict ploidy of the fetus, limitations such as mosaicism and self-correction complicate a correct diagnosis, even when using highly accurate PGS technologies.

TE biopsies from Day 5 blastocysts are an increasingly attractive and prevalent alternative to polar bodies and blastomere biopsies because

TE biopsies enable detection of both mitotic and meiotic aneuploidies and are only performed on high-quality blastocysts that have had the opportunity to 'self-correct', improving laboratory efficiency. However, comparisons of aneuploidy rates with blastomere biopsies versus TE biopsies are conflicting. Whereas studies using fluorescence *in situ* hybridization (FISH) have found similar rates of aneuploidy in cleavage stage and TE measurements (Sandalinas *et al*., 2001; Bielanska *et al*., 2002, 2005; Baart *et al*., 2004, 2007; Coonen *et al*., 2004; Munné *et al*., 2005; Daphnis *et al*., 2008), studies using G-band analysis or comparative genome hybridization (CGH) found low rates of aneuploidy in blastocysts compared with cleavage stage measurements (Clouston *et al*., 1997, 2002; Voullaire *et al*., 2002; Fragouli *et al*., 2008, 2009; Schoolcraft *et al*., 2009; Sher *et al*., 2009).

Mosaicism occurs in blastocysts, but apparently at lower levels than in cleavage stage embryos. Prior work using FISH has suggested high rates of mosaicism within TE biopsies (Bielanska *et al*., 2002, 2005; Derhaag *et al*., 2003; Baart *et al*., 2006), whereas G-banding has suggested low rates of mosaicism (Clouston *et al*., 1997, 2002). Mosaicism between the TE and the inner cell mass (ICM) might lead to so-called 'confined placental mosaicism', wherein the placenta is mosaic aneuploid and the fetus is euploid (Kalousek and Dill, 1983). However, data comparing mosaicism between the TE and ICM are very limited. One small study of 10 embryos with CGH showed 100% concordance between TE and ICM (Fragouli *et al*., 2008). To date, large and comprehensive studies of concordance between TE and ICM in high-quality embryos have not been performed using 24-chromosome screening. Currently such studies are highly relevant, as reports of clinical application of PGS with TE biopsies have been increasing in number (Veeck *et al*., 2004; MacArthur *et al*., 2005; Kokkali *et al*., 2007; MacArthur *et al*., 2008; Fragouli *et al*., 2009; Schoolcraft *et al*., 2009; Sher *et al*., 2009).

In the current study, our primary goals were (i) to examine the extent and mechanism of aneuploidy in a large cohort of high-quality blastocyst stage embryos and (ii) investigate the rate of mosaicism between the TE and ICM. To this end, we obtained 51 fresh and frozen blastocysts, and disaggregated them into TE and ICM fractions. We then performed 24-chromosome microarray molecular karyotyping using a previously developed technique that makes use of parental and embryo data to diagnose whole-chromosome imbalances, structural chromosome aberrations and parental source of aneuploidy, and to determine whether certain errors resulting in additional chromosomes are mitotic or meiotic in origin (Johnson *et al*., 2010). This study was to designed to investigate whether TE biopsies are representative of chromosomal status of the ICM, a question of high relevance to their application to PGS.

Materials and Methods

Embryo culture and disaggregation

Informed consent was obtained from patients under a protocol reviewed and approved by an Institutional Review Board. Embryos were obtained from 17 couples (Supplementary Table SI). The maternal age averaged across all blastocysts in the cohort was 31. The youngest mother was 22 and the oldest was 37. Embryos were graded (1 = best; 4 = worst) according to a modification of a previously published scoring system (Schoolcraft *et al*., 1999). Embryos graded 1 had an ICM that was tightly packed with many cells, and a TE with many cells forming a cohesive epithelium; embryos graded as 2 had an ICM that was loosely grouped with many cells, and a TE with a few cells forming a loose epithelium; embryos graded as 3 had an ICM that was loosely grouped with several cells and a TE with a few cells forming a loose epithelium; and embryos graded as 4 had an ICM with very few cells and a TE with very few large cells. The average embryo grade was 1.65, and 87.8% of embryos were either hatching or expanding blastocysts.

IVF stimulation protocols were individualized based on the patient's age, determination of ovarian reserve and response to prior therapies, when available. All patients received injectable follicle stimulating hormone (FSH) or a combination of human chorionic gonadotrophin (hCG) and FSH with either a gonadotrophin releasing hormone agonist or antagonist. Oocytes were obtained by transvaginal guided follicle aspiration 36 h after administration of hCG. Fertilization was performed by either overnight co-incubation of oocytes with spermatozoa or intracytoplasmic sperm injection (~40 h after hCG), depending on clinical indications and semen parameters. Sage commercial sequential media were used for fertilization and embryo culture: Quinn's Advantage Fertilization Medium supplemented with 10% serum protein substitute (SPS), Quinn's Advantage Cleavage Medium supplemented with 10% SPS and Quinn's Advantage Blastocyst Medium supplemented with 20% SPS (CooperSurgical).

The majority of the embryos (37 of 51) were cryopreserved at Day 5 using Quinn's Advantage Blastocyst Freeze Kit (CooperSurgical). These embryos were thawed using Quinn's Advantage Blastocyst Thaw Kit (CooperSurgical) and then cultured in Sage Blastocyst Media for 18 h. Full expansion, lack of fragmentation and definable ICM were criteria for inclusion in this study (Supplementary Figs S1–III). A minority of the embryos (14 of 51) were disaggregated at Day 5 without cryopreservation. These 14 embryos were of high morphological quality, but were not cryopreserved because cleavage stage PGS for these embryos yielded no results, indicated aneuploidy or indicated a gender other than that which was desired by the patients.

Blastocysts were manually removed from zona pellucida and extraneous cells were separated from the embryo to exclude fragmented material. TE was separated from ICM using a mechanical blade (Ultra Sharp Splitting Blades, Bioniche Animal Health, WA, USA) attached to a micromanipulator (Fragouli *et al*., 2008). Visual identification of ICM was essential for inclusion in the study, even though with visual identification it is possible that TE cells would occasionally contaminate the ICM fraction. When possible, TE was mechanically separated into two fractions. Fractions were washed sequentially four times using a stripper tip in 10 μl drops of washing buffer (Johnson *et al*., 2010). Following the final washing drop, fractions were placed in a 0.2 ml microcentrifuge tube containing 5 μl of washing buffer. The tubes were then frozen on dry ice.

Lysis, amplification and genotyping of blastomeres and parental samples

We thawed all fractions at 22°C, and then added Arcturus PicoPure Lysis Buffer (Molecular Devices, Sunnyvale, CA, USA) to each of the biopsies. The tubes were incubated at 56°C for 1 h, and then heat-inactivated at 95°C for 10 min. DNA from the lysed biospsies was amplified using a commercial kit (GE Healthcare, Waukesha, WI, USA) for multiple displacement amplification (MDA). MDA reactions were incubated at 30°C for 2.5 h and then heat-inactivated at 65°C for 5 min. The amplified samples were genotyped using Illumina (San Diego, CA, USA) Infinium II genotyping microarrays (CytoSNP-12 chips) using a modified 24-h protocol, as described previously (Johnson *et al*., 2010).

Parent Buccal samples were collected using MasterAmp Buccal Swabs (Madison, WI, USA). Genomic DNA was isolated from these swabs using Epicentre DNA Extraction solution (Madison, WI, USA). For parental samples, the standard Infinium II protocol (www.illumina.com) was

used. All microarray data have been archived by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/geo/) under accession identifier GSE22864.

Determination of chromosome imbalances

Previously, we developed a genotyping microarray molecular karyotyping technology that uses parental genetic data to increase accuracy and determine mechanism and source of aneuploidy (Johnson *et al.*, 2010). The algorithm uses parental genotypes and the observed distribution of unprocessed single cell microarray channel intensities to diagnose whole-chromosome imbalances and structural chromosome aberrations (Johnson *et al.*, 2010). Because parental genotypes are available, the algorithm readily identifies parental source of whole-chromosome imbalances and structural chromosome aberrations. Additionally, the algorithm uses parental information, high-confidence disomic single cell measurements on children, and recombination probabilities (genome.ucsc.edu) to determine the phase of the parental chromosomes. The phased data are then used to determine whether certain trisomies and uniparental disomies were mitotic or meiotic in origin (Johnson *et al.*, 2010).

Results

Prevalence of aneuploidy is significantly lower in blastocysts than in cleavage stage embryos

We isolated 51 ICM samples and 80 matching TE samples from 51 blastocyst embryos. For 29 of the embryos (59.9%), we were able to separate the TE into two distinct fractions. We then applied 24-chromosome microarray molecular karyotyping technology to each fraction. The algorithm assigns each chromosome a confidence level, i.e. a numerical estimate of data quality. Confidence is calculated per-chromosome, not per-sample, because each chromosome is modeled independently. The average confidence in the chromosome calls was 99.95%, and 99.2% of chromosomes were called above 99% confidence. Prior work using the same microarray technology indicated that confidences are strongly correlated with accuracy (Johnson *et al.*, 2010). Thus, we conclude that the exceptionally high-confidences in the current data set indicate a particularly high-quality set of molecular karyotypes. The molecular karyotype data also enabled analysis of rates of aneuploidy, mechanism of aneuploidy (mitotic versus meiotic), parental source of aneuploidy and structural chromosome aberrations in the blastocyst fractions (Table I and Supplementary Table SII).

Overall, per-sample rates of euploidy were high, with 82.5 and 80.4% euploidy rates for the TE and ICM, respectively (Table I and Supplementary Table SII). Among aneuploid samples, the majority were simple aneuploid, i.e. only one or two non-disomic chromosomes (75.0% for TE and 70.0% for ICM). One of the two complex aneuploid embryos (4–149) had three meiotic whole-chromosome imbalances in each of the three fractions, and the second embryo (2–255) was diagnosed as maternal haploid across all three fractions. There was no relationship between reason for infertility and aneuploidy (Supplementary Table SI). The grade of aneuploid embryos was 1.7, which was no worse than euploid embryos (*t*-test, $P > 0.05$).

Next, we examined the types of aneuploidy that occurred in the blastocysts. Monosomies accounted for the majority of non-disomic chromosomes, but were not significantly more common than trisomies (χ^2, $P > 0.05$). Among monosomies, paternal source was not significantly more common than maternal source (χ^2, $P > 0.05$). Meiotic trisomies were the most common trisomies when evaluated per-sample (100% among TE and 75.0% among ICM fractions), but this trend was also not significant (χ^2, $P > 0.05$). Paternal monosomies (i.e. the maternal chromosome was missing) were significantly more common than maternal monosomies (χ^2, $P = 0.002$). There was no relationship between the reason for infertility and type of aneuploidy. In fact, egg donors suffered as much meiotic aneuploidy as women with other reasons for infertility (Supplementary Table SI).

Table I Per fraction microarray results.

	TE	ICM
Number of biopsies	80	51
Number of embryos	51	51
Euploid	66 (82.5%)	41 (80.4%)
Simple aneuploid	12 (15.0%)	7 (15.7%)
Complex aneuploid	4 (3.8%)	3 (5.9%)
Maternal monosomy	6 (7.5%)	4 (7.8%)
Paternal monosomy	3 (3.8%)	3 (5.9%)
Maternal trisomy	4 (5.0%)	2 (3.9%)
Paternal trisomy	2 (2.5%)	2 (3.9%)
Maternal meiotic trisomy	4 (5.0%)	2 (3.9%)
Paternal meiotic trisomy	2 (2.5%)	1 (1.9%)
Maternal haploid	1 (1.3%)	1 (1.9%)
Structural chromosome aberrations	2 (2.5%)	3 (5.8%)
No result	0 (0.0%)	0 (0.0%)

Aneuploidy rates classified by mechanism and parental source of aneuploidy, computed per biopsy and segregated into TE and ICM biopsies.

Low prevalence of mosaicism between TE and ICM

We next examined rates of mosaicism between ICM and TE, as well as between TE fractions, when such comparisons were possible (Tables II and III; Supplementary Table SII). Only two embryos (3.9%) were mosaic between ICM and TE. None of the 30 matched TE fractions were discordant. Prior work using the same DNA microarray technology indicated a 57.7% rate of mosaicism in a cohort of cleavage stage embryos, significantly higher than the rate of mosaicism between the TE and ICM in the current cohort of blastocysts (χ^2, $P < 0.01$; Johnson *et al.*, 2010).

We also examined whether parental source of aneuploidy or meiotic/mitotic whole-chromosome imbalances were associated with mosaicism. We did not observe mosaicism in any of the three embryos with putative whole-chromosome meiotic errors. The maternal haploid embryo was also not mosaic. None of the maternal or paternal whole-chromosome monosomies were mosaic. The one embryo that was mosaic for a whole-chromosome imbalance (7–249) was discordant between the TE and ICM for a paternal mitotic trisomy on Chromosome 13. Although our sample size is small, we speculate that mosaicism may result from mitotic whole-chromosome imbalances rather than meiotic whole-chromosome imbalances.

Table II Mosaicism in aneuploid embryos.

Embryo	Maternal age	Family	ICM	TE-1	TE-2	Mosaic
2–252	37	252	45XY, −16(mat)(struct)(mei)	45XY, −16(mat)	45XY, −16(mat)	Yes
4–253	29	253	45XX, −14(mat)	45XX, −14(mat)	45XX, −14(mat)	No
6–149	28	149	45XY, −16(mat)	45XY, −16(mat)		No
9–249	31	249	45XY, −12(mat)(struct)	45XY, −12(mat)(struct)		No
2–148	22	148	45X, −X(pat)	45X, −X(pat)		No
2–255	34	255	23X, −1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, sex(pat)	23X, −1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, sex(pat)	23X, −1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, sex(pat)	No
7–249	31	249	46XY, −12(pat)(struct), +13(pat)mit	45XY, −12(pat)(struct)		Yes
3–252	37	252	47XXY, +sex(pat)mei	47XXY, +sex(pat)mei	47XXY, +sex(pat)mei	No
4–149	28	149	49XX, +3(mat)mei, +6(mat)mei, +19(mat)mei	49XX, +3(mat)mei, +6(mat)mei, +19(mat)mei	49XX, +3(mat)mei, +6(mat)mei, +19(mat)mei	No
4–249	31	249	47XY, +15(mat)mei	47XY, +15(mat)mei	47XY, +15(mat)mei	No

Concordance between ICM and TE fractions for the 10 aneuploid embryos. Karyotypes are indicated with standard nomenclature, except 'mat' indicates maternal source, 'pat' indicates paternal source, 'mei' indicates meiotic mechanism, 'mit' indicates mitotic mechanism and 'struct' indicates a structural chromosome aberration. The 'Mosaic' column indicates whether any one chromosome differed between any pair of biopsies from the same embryo.

Table III Structural chromosome aberrations.

Embryo	Maternal age	Family	ICM	TE-1	TE-2	Mosaic?
2–252	37	252	−16:p11.2-qter (mat), +16:p11.2-pter (mat)(mei)	ND	ND	Yes
7–249	31	253	−12:q14.2-qter (mat)	−12:q14.2-qter (mat)	NA	No
9–249	31	149	−12:q14.2-qter (pat)	−12:q14.2-qter (pat)	NA	No

Summary of specific karyotype for the three embryos that suffered structural chromosome aberrations. In the karyotype annotation, 'mat' indicates maternal source, 'pat' indicates paternal source and 'mei' indicates meiotic mechanism. 'ND' indicates that no structural chromosome aberration was detected and 'NA' indicates that no measurement was made. The 'Mosaic' column indicates whether any one chromosome differed between any pair of biopsies from the same embryo.

Structural chromosome aberrations occur at blastocyst stage

Next, we examined structural chromosome aberrations among the blastocyst fractions (Supplementary Table SII). Only three embryos and three chromosomes were diagnosed with structural chromosome aberrations. Two embryos from one of the families (7–249 and 9–249) revealed structural chromosome aberrations on two separate embryos on Chromosome 12. The diagnosis was consistent between TE and ICM fractions in each of these two embryos. One of the embryos was missing 12:q14.2-qter from the maternal copy and the other embryo was missing 12:q14.2-qter from the paternal copy. Additionally, both of these structural chromosome aberrations are associated with mitotic malsegregation of other chromosomes in the same embryo. This patient was undergoing IVF due to endometriosis. The third embryo with a structural chromosome imbalance (2–252) showed deletion of maternal 16:p11.2-qter combined with a meiotic duplication of maternal 16:p11.2-pter, but only in the ICM fraction. Chromosome 16 was missing the full maternal copy in both TE fractions from this embryo. This patient was infertile due to tubal disease.

Discussion

We have performed the largest-ever reported study of mosaicism between the ICM and TE in blastocyst stage human IVF embryos using 24-chromosome microarray screening. We found significantly higher rates of euploidy in blastocysts than we have seen previously in cleavage stage embryos (Johnson *et al.*, 2010). Several large studies of blastocysts using FISH, G-band analysis or CGH reported aneuploidy rates between 32 and 77.4% (Evsikov and Verlinsky, 1998; Magli *et al.*, 2000; Clouston *et al.*, 2002; Voullaire *et al.*, 2002; Derhaag *et al.*, 2003; Fragouli *et al.*, 2008, 2009; Schoolcraft *et al.*, 2009; Sher *et al.*, 2009). Our cohort of embryos had an average maternal age of 31, younger than prior studies (Evsikov and Verlinsky, 1998; Clouston *et al.*, 2002; Voullaire *et al.*, 2002; Derhaag *et al.*, 2003; Fragouli *et al.*, 2008, 2009; Schoolcraft *et al.*, 2009), which probably accounted for the relatively higher rate of euploidy. Presumably, the lower rate of aneuploidy by blastocyst stage is due to selective arrest by Day 5 of embryos diagnosed as aneuploid at cleavage stage (Fragouli *et al.*, 2008).

Generally, because aneuploidy rates were so low in this cohort of blastocysts, we were not able to find any statistically significant

trends with regards to the prevailing mechanisms of aneupoloidy at the blastocyst stage. However, we saw a low rate of complex aneuploidy by the blastocyst stage, suggesting that most embryos that suffer from severe complex aneuploidy at cleavage stage will not form blastula. Although the trend was not statistically significant, 9 of 10 reported trisomies were meiotic in origin, suggesting that meiotic mechanisms predominate among aneuploid blastocysts. Chromosome 16 aneuploidy occurred in 3 of 10 of the aneuploid embryos, and was thus the most frequently aneuploid chromosome. The propensity of Chromosome 16 to suffer whole-chromosome imbalance has been previously reported in cleavage stage embryos (Munné *et al*., 1995; Munné *et al*., 2007), blastocysts (Clouston *et al*., 1997, 2002) and pregnancy wastage (Simpson 2007). One of the embryos completely lacked the paternal genetic complement. This phenomenon has been reported once previously in a series of 91 blastocysts (Evsikov and Verlinsky, 1998), but the parental source of haploidy could not be identified in that report, because FISH technology was used.

Prior studies using FISH and G-banding have indicated that between 1.4 and 60.7% of blastocysts contain a degree of mosaicism between cells of the same tissue (Clouston *et al*., 1997, 2002; Evsikov and Verlinsky, 1998; Magli *et al*., 2000; Derhaag *et al*., 2003; Baart *et al*., 2004; Coonen *et al*., 2004; Munné *et al*., 2005; Daphnis *et al*., 2008). Unlike these prior studies, we made microarray measurements on embryo fractions comprised of at least several cells. Mosaicism may have occurred within these fractions. Mosaic embryos may appear euploid if a mitotic error results in trisomy in a fraction of the embryo and monosomy in the other fraction. However, we would expect that confidences would be lower in mosaic fractions. Given that only 3 chromosomes out of 3013 were reported below 90% confidence, and no chromosomes were reported below 85% confidence, we find it unlikely that mosaicism was common in these biopsies. However, in light of reports of mosaicism in the literature, we cannot exclude the possibility that mosaicism occurred occasionally within the blastocyst fractions that we tested.

We found only two embryos that were discordant between TE and ICM. This statistic is more or less in line with prior reports that used CGH technology to compare TE and ICM (Clouston *et al*., 1997; Fragouli *et al*., 2008). Both of the mosaic embryos in the current study suffered from structural chromosome aberrations. Accordingly, the only embryo that was mosaic between ICM and TE in one study (Clouston *et al*., 1997) also suffered a structural chromosome aberration. Furthermore, structural chromosome aberrations have been previously associated with mosaic or chaotic karyotypes in blastocysts, indicating a mitotic mechanism (Fragouli *et al*., 2008). Prior reports have also indicated that mosaic tetraploidy is common in both cleavage stage embryos and blastocysts (Clouston *et al*., 1997, 2002; Evsikov and Verlinsky, 1998; Veiga *et al*., 1999; Ruangvutilert *et al*., 2000; Sandalinas *et al*., 2001). Our current study would not have detected mosaic mitotic tetraploidy in the TE or ICM, because mitotically tetraploid cells are indistinguishable from two diploid cells using microarray molecular karyotyping. Regardless, the clinical importance of the diagnosis of tetraploidy is not clear (Clouston *et al*., 2002; Krieg *et al*., 2009).

One of the families had two embryos that suffered from 12:q14.2-qter deletions in all of the fractions tested. One of the embryos had the paternal copy of 12:q14.2-qter missing, whereas the other embryo had the maternal copy of 12:q14.2-qter missing. A karyotype is not available for this couple, but one would expect that it would be exceptionally unlikely that familial balanced translocations or inversions would only co-occur in both parents and on the same chromosome. This suggests that certain chromosomes have a higher propensity to suffer from *de novo* breakage, as proposed in prior studies (Wells and Delhanty, 2000; Fragouli *et al*., 2008).

Contemporary technologies, such as CGH, are being applied in a clinical setting to obtain a molecular karyotype of TE biopsies (MacArthur *et al*., 2008; Fragouli *et al*., 2009; Schoolcraft *et al*., 2009; Sher *et al*., 2009). One study has reported doubling of pregnancy rates when using 24-chromosome screening (Sher *et al*., 2009) and another study reported a 50% improvement in implantation rates when using 24-chromosome screening (Schoolcraft *et al*., 2009). Given the promising results of these prior studies, the microarray technology described here is now being applied to TE biopsies in a clinical setting at several IVF clinics in the USA and Europe.

Authors' roles

D.S.J. conceived the study, performed data analysis and prepared the manuscript. C.C., R.R. and A.F. performed embryology. G.G., M.H., A.R. and M.R. assisted with data analysis. D.S. provided samples. M.J.M. helped design the study, provided samples, consented patients and edited the manuscript.

Supplementary data

Supplementary data are available at http://molehr.oxfordjournals.org/.

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Conflict of interest: M.H., C.C., A.R., M.R., G.G. own stock and receive salaries as compensation for work related to the aneuploidy screening technology described in this article. D.J., R.R. own stock as compensation for work related to the aneuploidy screening technology described in this article.

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