Human Reproduction, Vol.28, No.2 pp. 502–508, 2013
Advanced Access publication on November 20, 2012 doi:10.1093/humrep/des393

human reproduction

ORIGINAL ARTICLE *Reproductive genetics*

Embryos whose polar bodies contain isolated reciprocal chromosome aneuploidy are almost always euploid

E.J. Forman[1,2,*], N.R. Treff[1,2], J.M. Stevens[3], H.M. Garnsey[1], M.G. Katz-Jaffe[3], R.T. Scott Jr[1,2], and W.B. Schoolcraft[3]

[1]Reproductive Medicine Associates of New Jersey, Morristown, NJ 07960, USA [2]Department of Obstetrics, Gynecology and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08901, USA [3]Colorado Center for Reproductive Medicine, Lone Tree, CO 80124, USA

*Correspondence address. Tel: +1-973-871-1315; Fax: +1-973-871-1226; E-mail: eforman@rmanj.com

Submitted on August 9, 2012; resubmitted on October 10, 2012; accepted on October 12, 2012

STUDY QUESTION: When a chromosome aneuploidy is detected in the first polar body and a reciprocal loss or gain of the same chromosome is detected in the second polar body, is the resulting embryo usually aneuploid for that chromosome?

SUMMARY ANSWER: When reciprocal aneuploidy occurs in polar bodies, the resulting embryo is usually normal for that chromosome, indicating that premature separation of sister chromatids (PSSC)—not non-disjunction—likely occurred in meiosis I.

WHAT IS KNOWN ALREADY: Single-nucleotide polymorphism-based microarray analysis can be used to accurately determine the chromosomal status of polar bodies and embryos. Sometimes, the only abnormality found is a reciprocal gain or loss of one or two chromosomes in the two polar bodies. Prediction of the status of the resulting embryo in these cases is problematic.

STUDY DESIGN, SIZE, DURATION: Blinded microarray analysis of previously diagnosed aneuploid embryos that had reciprocal polar body aneuploidy.

MATERIALS, SETTING, METHODS: IVF cycles were performed between 2008 and 2011 in patients aged 40 ± 3 years (range 35–47 years) with an indication for polar body-based aneuploidy screening. Thirty-five aneuploid vitrified Day 3 embryos were warmed, cultured to Day 5 and biopsied for microarray analysis. Predictions were made for the ploidy status of the embryo if PSSC or non-disjunction had occurred. The signal intensity for the aneuploid chromosome in the first polar body was compared between those that resulted in euploid and aneuploid embryos.

MAIN RESULTS AND THE ROLE OF CHANCE: Among 34 embryos with evaluable results, 31 were euploid on re-analysis. Of 43 chromosomes that had reciprocal aneuploidy in the polar bodies, 41 were disomic in the embryo, indicating that PSSC was likely to have occurred 95% (95% confidence interval 85–99%) of the time. The log 2 ratio signal intensity from the chromosomes that underwent non-disjunction, resulting in unbalanced embryos, were outliers when compared with those that underwent PSSC.

LIMITATIONS, REASONS FOR CAUTION: Although most embryos with reciprocal aneuploid polar bodies were euploid, it is unknown whether they maintain equivalent reproductive potential when transferred. Further study is needed to determine whether these embryos should be re-biopsied and considered for transfer.

WIDER IMPLICATIONS OF THE FINDINGS: This study is consistent with increasing evidence that PSSC is the primary cause of meiosis I errors in embryos from women of advanced reproductive age. Clinicians should be cautious in interpreting results from polar body aneuploidy screening, especially when only the first polar body is tested.

STUDY FUNDING/COMPETING INTEREST(S): None.

Key words: aneuploidy / IVF/ICSI outcome / meiosis / PGD / chromosomal / abnormalities

Introduction

With the development of improved techniques to comprehensively assess the chromosomal status of oocytes and embryos, there has been renewed interest in the clinical application of aneuploidy screening to improve IVF outcomes and perhaps make single-embryo transfer (SET) the standard of care across age groups. There are several stages in embryonic development where genetic material can be sampled for analysis, from the unfertilized oocyte to the expanded blastocyst. Polar body-based aneuploidy screening has been advocated as a less invasive method than embryo biopsy (Handyside *et al*., 2012). Molecular analysis of polar bodies provides an indirect assessment of an individual oocyte's chromosomal status by determining whether the chromosomes correctly segregated during meiosis I and II. Since maternal meiosis is the major contributor to embryonic aneuploidy (Hassold *et al*., 2007), it has been proposed that selecting only embryos derived from euploid oocytes for future transfer could improve outcomes (Sher *et al*., 2007) and a large multi-center randomized trial has been launched to address this question (Geraedts *et al*., 2010).

There is concern, however, that due to the indirect nature of polar body testing, the actual status of the oocyte is subject to interpretation. There is a substantial body of evidence that premature separation of sister chromatids (PSSC)—rather than non-disjunction of homologous chromosomes—is the primary cause of maternal meiotic errors resulting in embryonic aneuploidy in women of advanced reproductive age (Angell, 1991; Pellestor *et al*., 2003; Gabriel *et al*., 2011; Magli *et al*., 2012). When PSSC occurs in meiosis I, there is an opportunity for the error to compensate in meiosis II, though this would result in an unbalanced second polar body. When non-disjunction occurs in meiosis I, however, there is not the same opportunity for the error to be compensated in meiosis II (Fig. 1). Although the actual amount of DNA from a given chromosome present in the first polar body would differ whether PSSC or non-disjunction occurred, array-based screening technologies have not been validated to distinguish between chromatid and chromosome (sister–sister chromatids linked) losses or gains. Overall copy number losses and gains, however, can be reliably determined. Thus, when there is a gain in the first polar body and a potentially compensatory loss in the second polar body (or vice versa), the karyotype of the ensuing embryo depends on which type of error occurred. If PSSC occurred, then the resulting zygote could be disomic for that chromosome, whereas if non-disjunction occurred it will be unbalanced.

It has long been known that reciprocal aneuploidies occur often in polar bodies observed during human IVF. Using FISH-based screening, approximately one-third of meiosis I errors were observed to correct with a balanced loss or gain in the second polar body (Kuliev and Verlinsky, 2004). A study utilizing array-based comparative genomic hybridization (aCGH) found nearly 40% of meiosis I errors were balanced in the second polar body (Handyside *et al*., 2012). However, the ensuing embryos were typically diagnosed as abnormal and not recommended for transfer (Geraedts *et al*., 2011) since they were thought to carry other chromosomal errors and their reproductive competence was in question as no deliveries had been documented (Kuliev and Verlinsky, 2004; Fragouli *et al*., 2006, 2011). With comprehensive screening of both polar bodies, cases in which there are isolated reciprocal aneuploidies with normal segregation of all other chromosomes in maternal meiosis have an opportunity to produce a euploid embryo. A case report was recently published in which a euploid embryo that had a loss of chromosome 21 in the first polar body and a balancing gain in the second polar body was transferred and delivered, indicating that these embryos can possess reproductive potential (Scott *et al*., 2012).

In order to develop an evidence-based approach to polar body testing, we sought to determine how often embryos that possessed isolated reciprocal aneuploidies in their polar bodies were actually euploid, reflecting PSSC in meiosis I with compensation in meiosis II. A cohort of Day 3 vitrified embryos that had been diagnosed as abnormal due to the presence of only reciprocal aneuploid polar bodies was warmed, cultured to Day 5, biopsied and reanalyzed with the same single-nucleotide polymorphism (SNP) microarray screening technology to determine the ploidy status of the resulting embryos and the prevalence of PSSC compared with non-disjunction. The SNP microarray data were also analyzed in an attempt to determine thresholds to distinguish between PSSC and non-disjunction.

Materials and Methods

All IVF cycles were performed at the Colorado Center for Reproductive Medicine from 2008 to 2011 per practice routine. Fertilized zygotes underwent polar body biopsy prior to embryo culture and vitrification at the cleavage stage on Day 3 of development. The biopsied polar bodies were placed in lysis buffer and sent to Reproductive Medicine Associates of New Jersey for SNP microarray analysis as previously described (Treff *et al*., 2010a,b). In brief, the biopsy DNA was amplified using Sigma's WGA4 GenomePlex Whole Genome Amplification Kit (Sigma Aldrich) with subsequent hybridization to an NspI GeneChip Human Mapping 250K microarray (Affymetrix Inc., Santa Clara, CA, USA). Copy number assignment for each chromosome was made using Copy Number Analysis Tool (CNAT) version 4.0.1 (Affymetrix).

Embryos with reciprocal polar body aneuploidies were diagnosed as aneuploid and were not recommended for transfer. Aneuploid embryos were donated to research under an Institutional Review Board approved protocol. These embryos were warmed, cultured to Day 5 and biopsied for SNP microarray-based comprehensive chromosome screening analysis using an established protocol (Treff *et al*., 2010a,b). The biopsies were de-identified and a blinded analysis was performed. Descriptive statistics were employed.

Since polar bodies were simultaneously removed at the fertilization check, they were prospectively assigned as the first or second polar body based on morphology with the larger polar body presumed to be the first polar body since it contains more genetic material. However, a more objective assignment of cell division origin was recently established by making assignments based on overall SNP heterozygosity, rather than morphology (Treff *et al*., 2012). Since the second polar body contains individual chromatids, it will be homozygous at all loci. In contrast, the first polar body will be heterozygous at loci where crossing over occurred in prophase I. Assigning the polar body with the higher heterozygosity rate as the first polar body was shown to be more predictive than morphology in a blinded analysis. Polar bodies were thus re-designated based on their respective SNP heterozygosity rates.

Predictions were then made for the resulting embryo's karyotype assuming either non-disjunction or PSSC had occurred. If non-disjunction occurred, a gain of a chromosome in the first polar body should result in monosomy for that chromosome in the embryo; conversely, a loss in the first polar body should result in trisomy. If PSSC occurred, the embryo

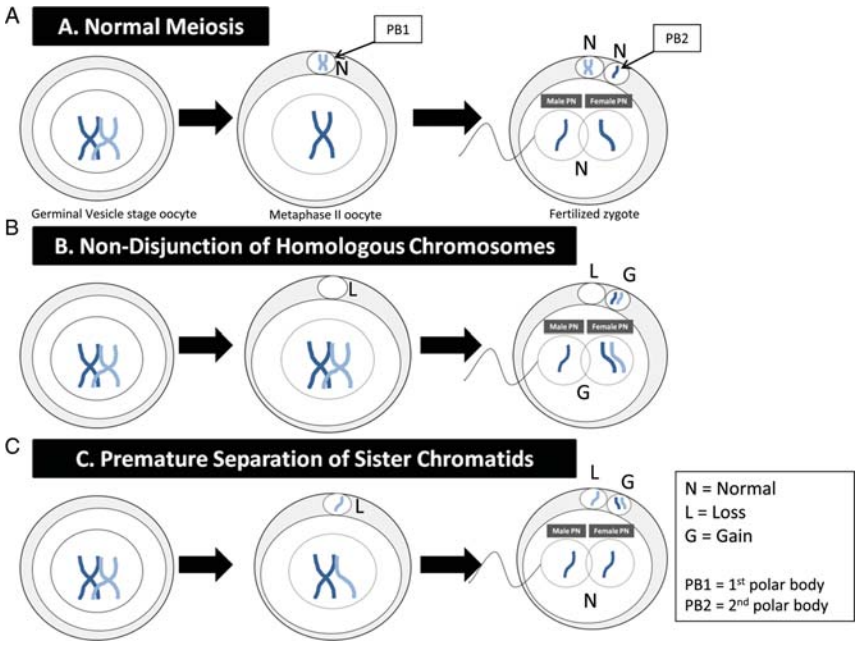

Figure 1 (A) Normal segregation (N) of homologous chromosome to first polar body (PB1) in meiosis I and then segregation of separated sister chromatids to second polar body (PB2). (B) In non-disjunction, there is a loss (L) of genetic material in PB1 with a reciprocal gain (G) in PB2, resulting in an unbalanced, trisomic zygote (G). (C) Although PSSC also results in a loss (L) in PB1 and gain (G) in PB2, the meiosis I error is compensated and results in a balanced, euploid zygote (N) after fertilization.

should be disomic for each chromosome that possessed a reciprocal abnormality in the polar bodies. The prevalence of PSSC versus non-disjunction was determined by comparing the embryo biopsy diagnosis with the predicted karyotype. The overall euploidy rate was also calculated.

There are various ways to analyze data obtained from SNP microarrays. This study assessed differences in both the number of SNP calls made and the relative intensity of those calls. With each type of meiosis I error (PSSC or non-disjunction), there should be a different relative amount of the mal-segregated chromosome in the first polar body. For example, normally segregated chromosomes will extrude one homolog with both intact sister chromatids to the first polar body. In the case of non-disjunction predisposing to trisomy, both homologs remain in the oocyte and the first polar body receives zero copies of that chromosome. Alternatively in non-disjunction predisposing to monosomy, both homologs are extruded to the first polar body with zero copies remaining in the oocyte. With PSSC, there is a relative loss (or gain) of DNA from the mal-segregated chromosome in the first polar body as there is one chromatid (or three) from that chromosome, rather than the two chromatids normally extruded in meiosis I.

Using the Affymetrix Genechip Genotyping Analysis Software (GTYPE) Version 4.1, the SNP call rate for each chromosome (number of SNPs with genotype reading divided by total number of SNPs for that chromosome) was calculated to determine whether non-disjunction results in lower SNP call rates than PSSC when there is a loss in the first polar body. A normalized SNP call rate was calculated by dividing the SNP call rate from the aneuploid chromosome by the SNP call rate from the euploid chromosomes in that same polar body. At each SNP locus, a fluorescent signal intensity is generated that can be transformed into a log 2 ratio. For each chromosome, the mean log 2 ratio across all SNPs was calculated. The mean log 2 ratio for the chromosome that underwent abnormal segregation to the first polar body was calculated. The mean log 2 ratios were compared amongst the chromosomes that had gains or losses of genetic material. An outlier analysis was performed using box-and-whisker plots and inter-quartile ranges (IQR). Values that fell more than 3 IQR from the nearest quartile were considered outliers.

Results

A total of 1024 oocytes were screened by simultaneous polar body biopsy at the time of fertilization check, with 754 (73.6%) diagnosed as aneuploid. In 91 oocytes (8.9%), a diagnosis could be made for only one of the two polar bodies, and these were excluded. There were 42 oocytes from 40 individual patients in which the two polar bodies had isolated reciprocal abnormalities, that is normal segregation patterns of all autosomes with the exception of one or two chromosomes that had a loss in one polar body followed by its gain in the second polar body or vice versa. Thirty-five of these embryos from 33 individual patients had been donated for research purposes and were available to warm and biopsy.

Patients with an isolated reciprocal polar body aneuploidy had a mean maternal age of 40 $\pm$ 3 years (range 35–47 years). Consistent with prior studies, the morphology-based polar body assignment was contradictory with the heterozygosity-based assignment in 15 of 35 (43%) of cases and the polar bodies were reassigned accordingly. Most cases had a single chromosome abnormality, though 10 out of 35 (29%) had two chromosomes with reciprocal losses and gains. The microarray diagnosis for the polar body pairs and the embryo biopsy are listed in Table I.

Table I SNP microarray-based diagnosis for the first polar body (PB1), second polar body (PB2) and embryo biopsy for each embryo tested.

Patient #	Age	Polar body 1	Polar body 2	Prediction based on PB1 signal intensity	Day 5 morphology	Embryo diagnosis	Prediction based on embryo diagnosis
1	37	+21	−21	ND	Morula	Aneuploid male, +17, −21	ND[a]
2	41	−4, −7	+4, +7	ND	Early Blastocyst	Male, +4 (indeterminate), +7 (indeterminate)	Undetermined
3	43	+21, −22	−21, +22	PSSC (21), ND (22)	Morula	Aneuploid male, −18 (indeterminate), +22	PSSC (21), ND (22)[a]
4	36	−11	+11	PSSC	Morula	Euploid female	PSSC
5	40	−12	+12	PSSC	Morula	Euploid male	PSSC
6	40	−13	+13	PSSC	Morula	Euploid female	PSSC
7	37	−13	+13	PSSC	Morula	Euploid female	PSSC
8	38	−14	+14	PSSC	Morula	Euploid female	PSSC
9	42	−15	+15	PSSC	Morula	Aneuploid male, −8, −12, −14, −20, −22	PSSC[a]
10	43	−16	+16	PSSC	Morula	Euploid female	PSSC
11	35	−21	+21	PSSC	Morula	Euploid female	PSSC
12	45	+11	−11	PSSC	Morula	Euploid female	PSSC
13	38	+15	−15	PSSC	Morula	Euploid female	PSSC
14	40	+21	−21	PSSC	Morula	Euploid female	PSSC
15	37	+21	−21	PSSC	Morula	Euploid male	PSSC
16	47	+22	−22	PSSC	Morula	Euploid male	PSSC
17	40	−1, +14	+1, −14	PSSC	Morula	Euploid female	PSSC
18	41	−19	+19	PSSC	Early blastocyst	Euploid female	PSSC
19	40	+4	−4	PSSC	Early blastocyst	Euploid female	PSSC
19	40	+19, +22	−19, −22	PSSC	Early blastocyst	Euploid female	PSSC
20	40	−8	+8	PSSC	Expanded blastocyst	Euploid female	PSSC
21	40	−10	+10	PSSC	Expanded blastocyst	Euploid male	PSSC
22	37	−19	+19	PSSC	Expanded blastocyst	Euploid male	PSSC
23	39	−21	+21	PSSC	Expanded blastocyst	Euploid male	PSSC
24	44	−22	+22	PSSC	Expanded blastocyst	Euploid female	PSSC
25	36	+3	−3	PSSC	Expanded blastocyst	Euploid male	PSSC
26	37	+13	−13	PSSC	Expanded blastocyst	Euploid female	PSSC
27	38	+16	−16	PSSC	Expanded blastocyst	Euploid male	PSSC
28	42	+21	−21	PSSC	Expanded blastocyst	Euploid female	PSSC
29	39	+8, +22	−8, −22	PSSC	Expanded blastocyst	Euploid male	PSSC
30	45	+14, +18	−14, −18	PSSC	Expanded blastocyst	Euploid female	PSSC
17	40	+15, +21	−15, −21	PSSC	Expanded blastocyst	Euploid female	PSSC
31	40	−4, +21	+4, −21	PSSC	Expanded blastocyst	Euploid male	PSSC

Continued

Table I *Continued*

Patient #	Age	Polar body 1	Polar body 2	Prediction based on PB1 signal intensity	Day 5 morphology	Embryo diagnosis	Prediction based on embryo diagnosis
32	41	−15, +22	+15, −22	PSSC	Expanded blastocyst	Euploid female	PSSC
33	42	−16, +19	+16, −19	PSSC	Expanded blastocyst	Euploid female	PSSC

The patient's age, the embryo morphology at the time of biopsy and the type of meiotic error, non-disjunction (ND) or PSSC, as predicted by the signal intensity in PB1 and the embryo karyotype, are also included.
[a]These samples also possessed aneuploidy derived from either paternal meiosis or post-zygotic mitotic errors.
ND, non-disjunction; PSSC, premature separation of sister chromatids.

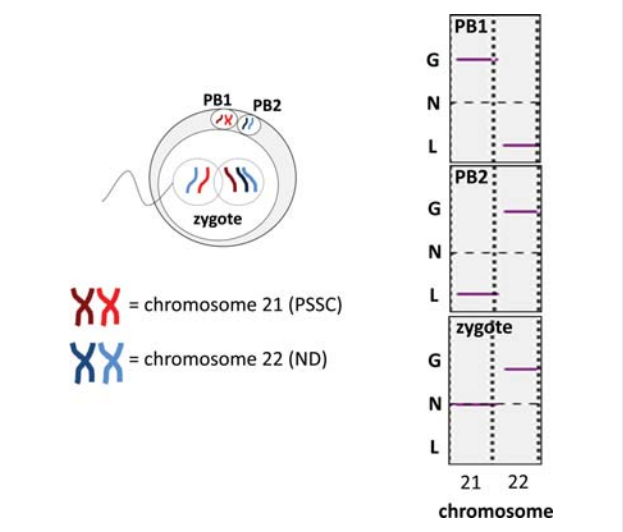

Figure 2 Polar bodies demonstrating a SNP microarray pattern consistent with PSSC for chromosome 21 and non-disjunction (ND) of chromosome 22, resulting in a trisomy 22 embryo. For chromosome 21, there is a relative gain in the first polar body, loss in the second polar body and the embryo is balanced. This can be explained by PSSC in meiosis I with compensation in meiosis II. For chromosome 22, there is a relative loss in the first polar body, gain in the second polar body, and the embryo is trisomic, consistent with ND of homologous chromosomes in meiosis I.

One embryo biopsy yielded an indeterminate karyotype as SNP concordance fell below previously established thresholds (Treff *et al*., 2010a,b). Among the other 34 embryos with evaluable microarray results, 31 [91%; 95% confidence interval (CI) 78–98%] were euploid for all chromosomes. One embryo had trisomy 22, consistent with the non-disjunction prediction. That same embryo had another reciprocal abnormality of chromosome 21, which was balanced in the embryo (Fig. 2) consistent with the PSSC prediction. Another embryo had monosomy 21, consistent with the non-disjunction prediction. Interestingly, that same embryo was trisomic for chromosome 17, indicating a paternal meiotic or post-fertilization mitotic error had occurred. Finally, one embryo demonstrated correction of

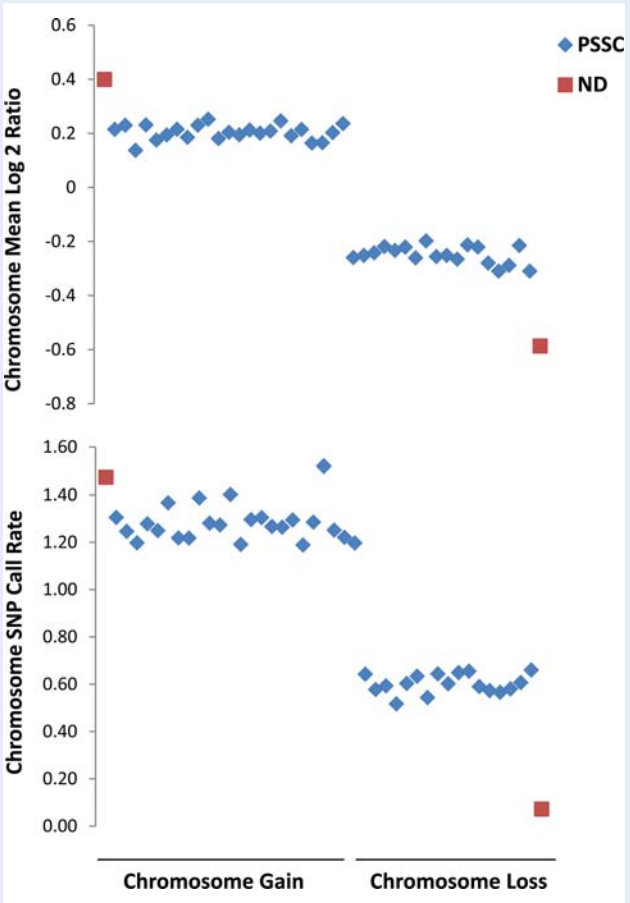

Figure 3 Normalized SNP call rate and mean log 2 ratios are plotted from the first polar body (PB1) of the individual chromosome that had a reciprocal aneuploidy in the second polar body. The results from cases with PB1 chromosome gains are plotted on the left and chromosome losses are on the right. The SNP call rate and mean log 2 ratio from the chromosomes that were balanced in the embryo, consistent with PSSC, are shown in blue diamonds. The SNP call rate and mean log 2 ratio from the chromosomes that were unbalanced in the embryo, consistent with non-disjunction, are shown in red squares.

chromosome 15, which had the reciprocal polar body aneuploidy, but exhibited multiple monosomies due to errors presumably occurring after maternal meiosis.

Of 43 chromosomes that demonstrated reciprocal aneuploidies in the 34 embryos, 41 were balanced for that chromosome in the embryo, indicating PSSC occurred 95% (95% CI 85–99%) of the time. One embryo yielded indeterminate results due to two chromosomes that fell slightly beneath the SNP concordance for trisomy. Even assuming these were due to non-disjunction, PSSC would still account for 91% (95% CI 80–97%) of the meiosis I errors.

When analyzing the normalized SNP call rates and signal intensities (log 2 ratio) for the chromosomes that underwent abnormal segregation patterns to the first polar body, outliers were derived only from the embryos that were aneuploid (consistent with the non-disjunction prediction). In the case of a loss in the first polar body, there was one outlier that had a normalized SNP call rate and mean log 2 ratio that fell more than 3 IQR from the lowest quartile. These values were derived from the one embryo that exhibited the non-disjunction predicted trisomy (Fig. 3). Interestingly, the embryo with two chromosomes that were non-concordant for the predicted trisomy in the indeterminate case also would have been an outlier based on its polar body SNP call rate and log 2 ratio for the abnormal chromosomes. In the case of a gain in the first polar body, there was one outlier (>3 IQR) for the mean log 2 ratio, and this was the embryo that had the non-disjunction predicted monosomy. In contrast, when analyzing the SNP call rate per chromosome, there were no outliers. The embryo with the non-disjunction predicted monosomy, had a normalized SNP call rate between 1.5 and 3 IQR, but there was another euploid embryo that also had a SNP call rate falling in this range.

Discussion

Using a validated method of comprehensive chromosome analysis, this study has demonstrated that—contrary to previous reports—oocytes from women >35 years old who have isolated reciprocal aneuploidies in their polar bodies most often produce chromosomally balanced embryos. PSSC in meiosis I is the most biologically plausible mechanism to explain this outcome and fits with the growing body of evidence that chromatid cohesion dysfunction is the primary contributor to age-related maternal meiotic errors. Uniparental disomy (UPD) is a theoretically possible explanation for 'trisomy rescue' (Kuliev and Verlinsky, 2004), but this is a very rare phenomenon and there was no evidence of UPD when analyzing 'loss of heterozygosity' plots from the euploid embryos as previously described (Northrop *et al.*, 2010). Interestingly, the incidence of embryonic aneuploidies in other chromosomes was very low, again in contradistinction to prior reports (Kuliev and Verlinsky, 2004) and arguing against a fundamental spindle abnormality that might predispose to other meiotic aneuploidies (Magli *et al.*, 2012). It is also reassuring that the microarray signal data fit with the results obtained from assessing the embryos directly. The cases with the largest differences in signal intensity and call rates were derived from those oocytes that resulted in unbalanced embryos, as would be predicted if non-disjunction occurred.

Although it has been documented that an embryo that underwent abnormal segregation of chromosomes in meiosis was able to implant and deliver a healthy newborn, the present study cannot demonstrate whether these embryos have equivalent developmental and reproductive potential. Nevertheless, there is now sufficient evidence that embryos with isolated reciprocal polar body aneuploidies following PSSC have a high likelihood of euploidy.

Future studies are indicated to determine whether these embryos should be re-biopsied or whether the signal intensity data are reliable enough to distinguish between chromatid and whole chromosome losses and gains in order to make accurate predictions of which type of meiotic error occurred and of the embryo's resulting ploidy status. A previous study using aCGH on polar bodies also found that single chromatid errors were much more common than whole chromosome errors (92 versus 8%); however, the thresholds used were not validated by comparing to the outcome in the embryo (Gabriel *et al.*, 2011). In the current study, in contrast, the mean log 2 ratio data and SNP call rates were directly compared with the actual outcome of meiosis by directly analyzing DNA from the embryo. Based on preliminary data from this study, it is likely that SNP microarray can be employed to accurately classify first polar body losses and gains as non-disjunction or PSSC. It is important that the designation of the first polar body is accurate, either by sequential biopsy or by SNP heterozygosity, since polar body morphology is not adequately reliable (Treff *et al.*, 2012). The data from this study demonstrate that when non-disjunction results in a loss of DNA in the first polar body, there is significantly diminished signal and decreased SNP call rates from that chromosome to distinguish it from the loss that occurs in PSSC. When analyzing gains in the first polar body due to PSSC or non-disjunction, the SNP call rate could not adequately distinguish non-disjunction from PSSC cases. In those cases, the log 2 ratio data appeared more reliable. Still, there was only one case of predicted non-disjunction that had a loss or a gain, so further validation is required. These thresholds will be tested prospectively by reanalyzing cases in which polar body reciprocal aneuploidies occurred (even if they were not isolated) and making blinded predictions of the chromosomal status of the embryo. Such work is currently underway in our laboratories. It will also be interesting to learn whether euploid embryos that underwent PSSC with compensation in meiosis II maintain equivalent reproductive potential or whether the fact that they were derived from oocytes that underwent abnormal meiosis imparts a diminished implantation potential.

Authors' roles

E.J.F.: Designed project, performed microarray, analyzed data, wrote and edited manuscript. M.G.K.-J.: Designed project and edited manuscript. J.M.S.: Performed polar body and blastocyst biopsy. H.M.G.: Performed microarray, assisted in data analysis, edited manuscript. N.R.T.: Designed project, wrote and edited manuscript. R.T.S.: Designed project, wrote and edited manuscript. W.B.S.: Designed project and edited manuscript.

Funding

Funding to pay the Open Access publication charges for this article was provided by Reproductive Medicine Associates of New Jersey.

Conflict of interest

None declared.

References

Angell RR. Predivision in human oocytes at meiosis I: a mechanism for trisomy formation in man. *Hum Genet* 1991;**86**:383–387.

Fragouli E, Wells D, Thornhill A, Serhal P, Faed MJ, Harper JC, Delhanty JD. Comparative genomic hybridization analysis of human oocytes and polar bodies. *Hum Reprod* 2006;**21**:2319–2328.

Fragouli E, Alfarawati S, Goodall NN, Sanchez-Garcia JF, Colls P, Wells D. The cytogenetics of polar bodies: insights into female meiosis and the diagnosis of aneuploidy. *Mol Hum Reprod* 2011;**17**:286–295.

Gabriel AS, Thornhill AR, Ottolini CS, Gordon A, Brown AP, Taylor J, Bennett K, Handyside A, Griffin DK. Array comparative genomic hybridisation on first polar bodies suggests that non-disjunction is not the predominant mechanism leading to aneuploidy in humans. *J Med Genet* 2011;**48**:433–437.

Geraedts J, Collins J, Gianaroli L, Goossens V, Handyside A, Harper J, Montag M, Repping S, Schmutzler A. What next for preimplantation genetic screening? A polar body approach! *Hum Reprod* 2010; **25**:575–577.

Geraedts J, Montag M, Magli MC, Repping S, Handyside A, Staessen C, Harper J, Schmutzler A, Collins J, Goossens V *et al.* Polar body array CGH for prediction of the status of the corresponding oocyte. Part I: clinical results. *Hum Reprod* 2011;**26**:3173–3180.

Handyside AH, Montag M, Magli MC, Repping S, Harper J, Schmutzler A, Vesela K, Gianaroli L, Geraedts J. Multiple meiotic errors caused by predivision of chromatids in women of advanced maternal age undergoing in vitro fertilisation. *Eur J Hum Genet* 2012;**20**:742–747.

Hassold T, Hall H, Hunt P. The origin of human aneuploidy: where we have been, where we are going. *Hum Mol Genet* 2007;**16**:R203–R208.

Kuliev A, Verlinsky Y. Meiotic and mitotic nondisjunction: lessons from preimplantation genetic diagnosis. *Hum Reprod Update* 2004; **10**:401–407.

Magli MC, Grugnetti C, Castelletti E, Paviglianiti B, Ferraretti AP, Geraedts J, Gianaroli L. Five chromosome segregation in polar bodies and the corresponding oocyte. *Reprod Biomed Online* 2012; **24**:331–338.

Northrop LE, Treff NR, Levy B, Scott RT Jr. SNP microarray-based 24 chromosome aneuploidy screening demonstrates that cleavage-stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts. *Mol Hum Reprod* 2010;**16**:590–600.

Pellestor F, Andreo B, Arnal F, Humeau C, Demaille J. Maternal aging and chromosomal abnormalities: new data drawn from in vitro unfertilized human oocytes. *Hum Genet* 2003;**112**:195–203.

Scott RT Jr, Treff NR, Stevens J, Forman EJ, Hong KH, Katz-Jaffe MG, Schoolcraft WB. Delivery of a chromosomally normal child from an oocyte with reciprocal aneuploid polar bodies. *J Assist Reprod Genet* 2012;**29**:533–537.

Sher G, Keskintepe L, Keskintepe M, Ginsburg M, Maassarani G, Yakut T, Baltaci V, Kotze D, Unsal E. Oocyte karyotyping by comparative genomic hybrydization provides a highly reliable method for selecting 'competent' embryos, markedly improving in vitro fertilization outcome: a multiphase study. *Fertil Steril* 2007;**87**:1033–1040.

Treff NR, Su J, Kasabwala N, Tao X, Miller KA, Scott RT Jr. Robust embryo identification using first polar body single nucleotide polymorphism microarray-based DNA fingerprinting. *Fertil Steril* 2010a;**93**:2453–2455.

Treff NR, Su J, Tao X, Levy B, Scott RT Jr. Accurate single cell 24 chromosome aneuploidy screening using whole genome amplification and single nucleotide polymorphism microarrays. *Fertil Steril* 2010b; **94**:2017–2021.

Treff NR, Scott RT Jr, Su J, Campos J, Stevens J, Schoolcraft W, Katz-Jaffe M. Polar body morphology is not predictive of its cell division origin. *J Assist Reprod Genet* 2012;**29**:137–139.