Human Reproduction Update, Vol.10, No.5 pp. 401–407, 2004 doi:10.1093/humupd/dmh036

Meiotic and mitotic nondisjunction: lessons from preimplantation genetic diagnosis

Anver Kuliev[1] and Yury Verlinsky

Reproductive Genetics Institute, Chicago, IL 60657, USA

[1]To whom correspondence should be addressed at: 2825 North Halsted Street, Chicago, IL 60657, USA.
E-mail: anverkuliev@hotmail.com or rgi@flash.net

Direct testing of the outcome of the first and second meiotic divisions has become possible with the introduction of preimplantation genetic diagnosis (PGD) for aneuploidies. Testing of oocytes by fluorescent *in situ* hybridization (FISH) analysis of the first and second polar bodies showed that more than half of oocytes from the IVF patients aged 35 years and older had chromosomal abnormalities, which originated from errors in meiosis I or meiosis II, or both: 41.9% of oocytes were aneuploid after meiosis I and 37.3% aneuploid after meiosis II, with 29.1% of these oocytes having both meiosis I and meiosis II errors. As a result, one third of oocytes detected as normal after meiosis I contained the meiosis II errors, and two thirds of those with meiosis II errors were already abnormal following meiosis I. Although the rates of chromosomal abnormalities deriving from meiosis I and II were comparable, meiosis I errors predominantly resulted in extra chromosome (chromatid) material in oocytes, in contrast to a random distribution of extra and missing chromatids after meiosis II. The majority of meiosis I abnormalities were represented by chromatid errors, which seem to be the major source of chromosomal abnormalities in the resulting embryos. Approximately one third of aneuploid oocytes deriving from sequential errors in the first and second meiotic divisions resulted in a balanced karyotype, representing a possible phenomenon of "aneuploidy rescue" during the second meiotic division. However, the majority of the embryos resulting from such oocytes appeared to be abnormal for the same or different chromosome(s), or were mosaic, suggesting a possible predisposition of the resulting embryos to further mitotic errors. Although the origin of a high frequency of mosaicism at the cleavage stage is not sufficiently understood, the mosaic embryos may originate from the chromosomally abnormal oocytes, as a result of a "trisomy rescue" mechanism during the first mitotic divisions, which renders polar body FISH analysis to have important clinical value for reliable pre-selection of aneuploidy-free embryos for transfer.

Key words: advanced reproductive age/chromosomal aneuploidy/female meiosis I and II/first and second polar body/mitotic non-disjunction

Introduction

It is well known that chromosomal abnormalities originate predominantly from female meiosis. As demonstrated by DNA polymorphism studies performed in families with aneuploid spontaneous abortions or liveborn babies with trisomy syndromes, these abnormalities derive mainly from meiosis I (Sherman *et al.*, 1994; Hassold *et al.*, 1995; Peterson and Mikkelsen, 2000). It was suggested that the age-related increase of common trisomies is probably determined by the age-related reduction of meiotic recombination, resulting in premature separation of bivalents and chromosomal nondisjunction. Meiosis II errors were also postulated to derive from meiosis I, as a result of the increased meiotic recombination rate, which may lead to a separation failure of bivalents (Lamb *et al.*, 1996).

With the advent of preimplantation genetic diagnosis (PGD) for aneuploidies it has become possible to directly test the outcome of the first and second meiotic divisions, using the first and second polar bodies (PB1 and PB2) (Verlinsky *et al.*, 1995; Dyban *et al.*, 1996). PB1 is extruded following maturation of oocytes, representing a by-product of meiosis I, while PB2 is a by-product of meiosis II and is extruded following the exposure of oocytes to sperm or ICSI. This paper describes the frequency and types of chromosomal errors detected by this approach, based on direct testing of meiotic outcome using PB1 and PB2 analysis, showing a high prevalence of meiotic errors and also significant contribution of chromatid errors, reported previously in traditional studies of meiotic chromosomes in metaphase II (MII) oocytes (Angel, 1997; Nakaoka *et al.*, 1998; Pellestor

et al., 2002). The use of PB testing for predicting the chromosomal status of the resulting oocyte is based on the study of simultaneous testing of MII oocytes with their corresponding PBI, which showed that the normal chromosome pattern is represented by paired fluorescent signals for each chromosome, while the lack or addition of one or both signals in either oocyte or PB1 reflects an exactly opposite pattern in the corresponding MII oocytes or PB1, suggesting a high accuracy of PB1 testing for prediction of the oocyte genotype (Munne *et al.*, 1995; Verlinsky *et al.*, 1995; Dyban *et al.*, 1996; Cupusti *et al.*, 2003; Pujol *et al.*, 2003).

Based on the above data, PB1 testing was applied clinically for the preselection of aneuploidy-free oocytes, which have demonstrated the practical relevance of PB1 testing for IVF patients of advanced reproductive age (Verlinsky *et al.*, 1996, 1998a, 1998b, 1999, 2000, 2001). The data also demonstrate that the genotype of the resulting zygotes could not be accurately predicted without information about the outcome of the second meiotic division, which may be inferred from PB2 testing. The present experience includes FISH analysis of more than 8000 oocytes presented below, demonstrating the accuracy of evaluation of oocyte karyotype by testing PB1 and PB2, and also providing an attractive approach for the study of the origin of human aneuploidies.

Female meiotic errors resulting in chromosomal abnormalities in the zygote

As mentioned, the direct testing of the meiotic errors has become possible with the introduction of PGD for chromosomal disorders, based on the use of PB1 and PB2 sampling, which are removed simultaneously following fertilization and fixed and analyzed by FISH on the same slide (Verlinsky and Kuliev, 2000). Because PB1 and PB2 are extruded from oocytes as a normal process of maturation and fertilization, their removal is not expected to have any biological effect on the embryo development, which is currently obvious from the outcomes of hundreds of pregnancies resulting from PGD (Verlinsky *et al.*, 2004). The biopsied and fixed PB1 and PB2 were studied using fluorescent probes specific for chromosomes 13, 16, 18, 21 and 22 (Abbott, Downers Grove, IL). The results of such studies are presently available for 8213 oocytes overall, obtained from 1551 IVF cycles performed in 1027 patients of an average age of 38.5 years (Kuliev *et al.*, 2003a).

These data further confirm that at least half of the oocytes obtained from IVF patients of advanced reproductive age are aneuploid, originating either from meiosis I, meiosis II, or from both meiosis I and II, as detected by PB1 and PB2 testing. Although some overestimate attributable to limitations of FISH technique cannot be excluded, the majority of these abnormalities in oocytes seem to represent the true errors, which is apparent from the increase of aneuploidy rate with maternal age, and also from the follow up studies of the embryos resulting from the oocytes with meiosis I and II errors (Verlinsky *et al.*, 1998a). In contrast to the traditional concept that aneuploidies mainly originate from female meiosis I, the direct testing of the outcome of meiosis I and II, presently performed by FISH analysis of 7103 PB1 and 7125 PB2, indicates comparable error rates in meiosis I (41.8%) and meiosis II (37.3%). In addition, although the aneuploidy rate is expected to be higher with the testing for additional chromosomes, available data indicate an increase of the complex abnormalities, rather than the overall aneuploidy rate (Verlinsky *et al.*, 2001). The fact that the meiotic error of one chromosome may affect the segregation of other chromosomes was demonstrated also in XO female mice (Hunt *et al.*, 1995).

The other difference of these data from the results of the traditional meiotic studies, is the observation that the majority of aneuploidies are represented by chromatid errors, as previously suggested in mouse model (Hunt *et al.*, 1995) and one of the traditional meiotic studies, which, however, failed to observe any instance of chromosomal non-disjunction (Angel, 1997). As seen from Figure 1, however, 41.8% of oocytes are abnormal following meiosis I, which includes both chromosome (2.4%) and chromatid errors (27.1%), with the majority of chromatid errors (19.8%) resulting in an extra chromatid in the MII oocyte. Even if some of these errors are attributable to technical factors, such as a possible failure of hybridization resulting in a missing signal in PB1, a $>$2-fold difference observed between missing and extra chromatids in the resulting MII oocytes may indicate the maintenance (in oocyte) rather than extrusion of the extra chromosome (chromatid) material (with PB1), when the meiosis I errors happen. This was also the case with chromosomal errors, which resulted in a ten times higher chance of an extra chromosome in the MII oocytes (2.4%) than of missing a chromosome (0.2%) (see Figure 1).

The fact that both the chromosomal and chromatid meiosis I errors lead to aneuploidy in the resulting embryos, has been also confirmed by the follow up study of the embryos resulting from these meiosis I errors, as the transfer of these embryos was avoided, and used for follow up study (Verlinsky *et al.*, 1998a). Although the differences in the effect of the chromatid and chromosomal errors on the pre- and post-implantation development cannot be excluded, this, however, has not yet been evaluated, because the embryos resulting from such oocytes were neither transferred, nor further cultured, due to their use for confirmation of PB1 diagnosis, according to the patient's consent. In contrast to meiosis I errors, there were no differences in the missing or extra chromatid error rates following meiosis II (14.9% and 14.2%, respectively) (Figure 2), suggesting different mechanisms of meiosis I and meiosis II errors.

Complex errors and aneuloidy rescue in female meiosis

The above data show that 41.8% of oocytes are abnormal following meiosis I, based on testing of PB1 alone (Figure 1). It is of interest that 40% of these oocytes with meiosis I errors have sequential errors in meiosis II as well, as inferred from PB2 testing (Kuliev *et al.*, 2003a). On the other hand, meiosis II errors were observed in 31.9% of oocytes, detected as being normal following meiosis I, with the overall meiosis II error rate as high as 37.3% (Figure 2). Therefore, approximately one half (47.7%) of meiosis II errors originate from oocytes with meiosis I errors, and the other half (52.3%) derive from the euploid MII oocytes, confirming that the preselection of aneuploidy-free oocytes should be based on the testing of the outcome of both the first and second meiotic divisions.

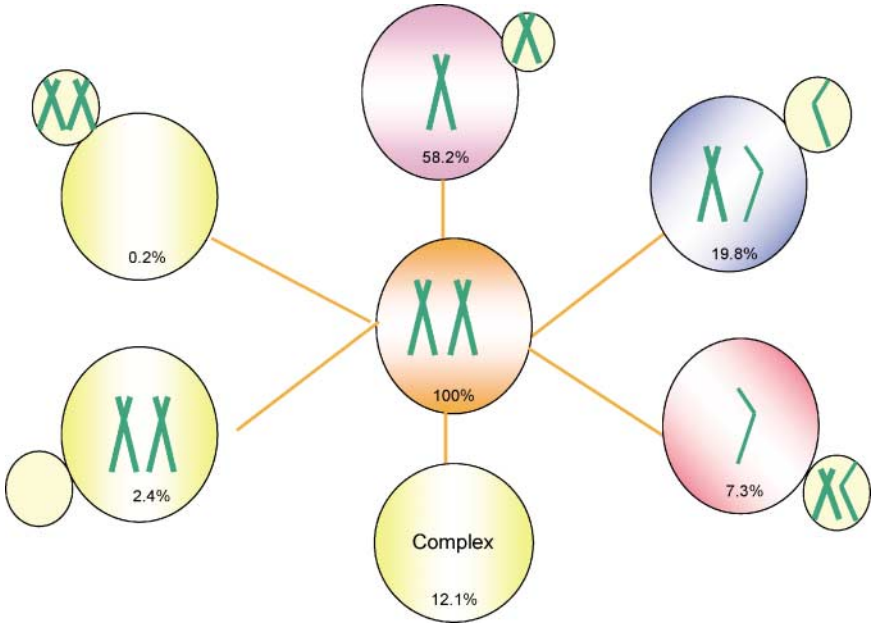

Figure 1. Meiosis I errors observed in PB1 FISH analysis (see text for explanation).

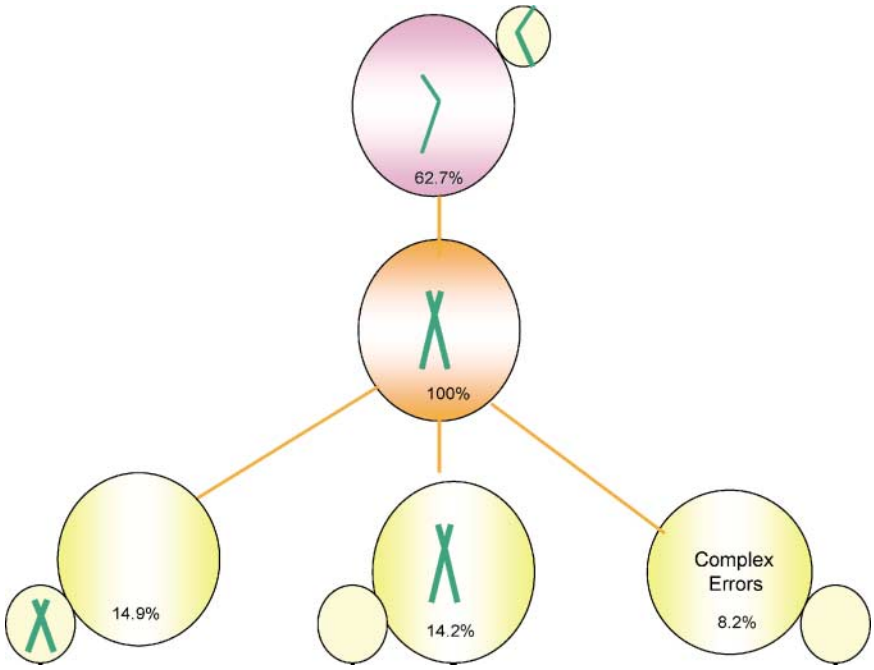

Figure 2. Meiosis II errors observed in PB2 FISH analysis (see text for explanation).

As seen from Figures 1 and 2, there was a comparable proportion of complex errors in meiosis I (12.1%) and meiosis II (8.2%), which includes oocytes with different chromosomes involved in the errors, and different type of errors involved (Kuliev *et al.*, 2003a). This may be due to the age-related effects on the recombination frequency (Sherman *et al.*, 1993; Lamb *et al.*, 1996, 1997), or spindle formation errors, also reported to increase with age (Battaglia *et al.*, 1996; Eichenlaub-Ritter *et al.*, 2003). The recent data on the molecular mechanisms of cohesion of sister chromatids in meiosis may also be of relevance for understanding the nature of the age-related increase of meiotic errors, resulting in complex abnormalities (Nashmyth *et al.*, 2000; Yuan *et al.*, 2002). Whatever the cause of the observed aneuploidy rates, approximately one third of chromosomal abnormalities in the resulting zygotes, overall, were represented by complex errors, suggesting that the testing for only five chromosomes would probably detect the majority of chromosomal abnormalities resulting from meiosis I and II. This is not only because the abnormalities of these five chromosomes are most common, but also because the application of additional chromosome-specific probes will probably detect the proportional increase of the complex error rate, rather than

Table I. Origin of non-disjunction in aneuploidies by PB FISH analysis of chromosomes 13, 16, 18, 21 and 22

Chromosome	# Oocytes studied	# Abnormal	MI errors	MII errors	MI & MII errors
13	5907	354 (6.0%)	165 (46.6%)	128 (36.2%)	61 (17.2%)
18	6648	455 (6.8%)	274 (60.2%)	135 (29.7%)	46 (10.1%)
21	6648	725 (10.9%)	314 (43.3%)	269 (37.1%)	142 (19.6%)
16	4583	294 (6.49%)	77 (26.2%)	154 (52.4%)	63 (21.4%)
22	4583	539 (11.8%)	166 (30.8%)	274 (50.8%)	99 (18.4%)

a significant overall increase of chromosomally abnormal oocytes.

The follow up of the outcome of meiosis I errors through meiosis II showed that almost one third (32.5%) of meiosis I errors appeared to result in an apparently euploid zygote, following a sequential error in meiosis II. Although the mechanism of the observed formation of such balanced zygotes is not yet understood, being different from that which could have been expected according to current knowledge, it may be similar to the well-known phenomenon of 'trisomy rescue' in postzygotic embryo development, which may result in uniparental disomy and imprinting disorders. As will be described below, the observed aneuploidy rescue mechanism in female meiosis cannot ensure the chromosomal normalcy of the resulting embryos to be useful for the embryo transfer.

Analysis of the chromosome-specific patterns of meiosis errors showed that, as expected, chromosome 21 and 22 errors were most prevalent, 10.9% and 11.8%, respectively, with the rates of 6%, 6.4% and 6.8% observed for chromosomes 13, 16 and 18 errors, respectively (Table I). As previously demonstrated, the chromosome-specific patterns of errors in meiosis I and II were not identical (Kuliev *et al.*, 2003a). In contrast to the previous reports on the predominant origin of chromosome 16 and 21 errors in meiosis I (Sherman *et al.*, 1994; Hassold *et al.*, 1995; Peterson and Mikkelsen, 2000), and chromosome 18 errors in meiosis II (Fisher *et al.*, 1996), the direct data showed no significant difference in the origin of the chromosome 21 errors, and the opposite tendencies for the chromosome 16 and 18 errors (Table I). The chromosome 13 error pattern was similar to that of chromosome 21, originating with a comparable frequency from meiosis I and II, and the chromosome 22 specific pattern was similar to the chromosome 16 pattern, originating predominantly in meiosis II (50.8% meiosis II errors vs 30.8% meiosis I errors), in contrast to chromosome 18 errors, deriving more frequently from meiosis I (60.2% from meiosis I vs 29.7% from meiosis II). The comparison of these direct data to the data derived from DNA polymorphism studies, obtained from live-born babies and spontaneous abortions with similar aneuploidies, will be of relevance for understanding the possible differences in viability of aneuploid embryos of different origin.

Mitotic errors in relation to meiotic errors in cleaving embryos

As shown above, approximately half of meiosis II errors are observed in the oocytes with prior errors in meiosis I. As a result of such sequential errors, almost one third of the resulting zygotes may have been considered normal (euploid), provided that the preceding errors in meiosis I and II have no effect on the further preimplantation developments of the corresponding embryos. To investigate if these meiosis errors could affect the sequential mitotic divisions in the resulting zygotes, and if these apparently euploid zygotes may develop into the chromosomally normal embryos acceptable for embryo transfer in PGD cycles, the follow up testing of these embryos was carried out at the cleavage stage. As seen from Table II, of 100 embryos tested overall, only 18%, deriving from the apparently balanced zygotes, were euploid for all the five chromosomes analyzed, while the remaining majority had chromosomal abnormalities (Kuliev *et al.*, 2003b,c).

All of the chromosomally normal (euploid for five chromosomes tested) embryos appeared to result from zygotes with only one chromosomal error rescue, with none resulting from the zygotes balanced for two chromosomes. The fact that only a few resulting embryos (11%) were abnormal for the same chromosome, for which sequential meiosis I and II led to the balanced set, may suggest that the observed sequential errors in female meiosis may be attributable to the meiotic apparatus abnormality overall, rather that to a single chromosome segregation defect, which may further lead to a general defect of mitotic apparatus of the resulting embryos. This seems to be also in agreement

Table II. Chromosomes 13, 16, 18, 21 and 22 testing in day 3 embryos originating from oocytes with meiosis I and II errors resulting in balanced chromosome set

Balanced oocytes	#	Resulting embryos	#
1 chromosome	70	Abnormal for the same chromosome	8
		Abnormal for 1 different chromosome	9
		Complex abnormality	35
		Normal for 5 chromosomes	18
2 chromosomes	10	Complex abnormality	10
1 Balanced & 1 Unbalanced	20	Complex abnormality	20
Total	100	Total normal (%)	18

Table III. Chromosome-specific aneuploidy rates in oocytes and cleavage-stage embryos* (chromosomes 13, 16, 18, 21 and 22)

Chromosome	# Oocytes Studied	# Abnormal	Embryos Studied	Abnormal
13	5907	354 (6.0%)	882	21 (2.4%)
18	6648	455 (6.8)	999	17 (1.7%)
21	6648	725 (10.9%)	882	38 (4.3%)
16	4583	294 (6.4%)	520	27 (5.2%)
22	4583	539 (11.8%)	302	17 (5.6%)

*Data for Cleavage Stage embryos are taken from Munne, 2002.

with the observed types of aneuploidies detected in the resulting embryos, which in 79.3% of cases were represented by complex errors, including mosaicism, known to be highly prevalent at the cleavage stage (Gianaroli *et al.*, 2001; Munne, 2002).

As the average reproductive age of the patients from whom the oocytes were obtained was ~38.5 years, the observed genomic instability in mitotic divisions of the apparently balanced zygotes following meiosis II rescue may be age related. Although the mechanism by which the age factor may lead to these changes is not known, the underlying mechanisms of the aging process involve increasing errors in the mitotic machinery of dividing cells and chromosomal abnormalities (Ly *et al.*, 2000). It was also suggested that deviations in the cytoplasmic organization, such as mitochondrial distribution, may reduce meiotic competence of oocytes and predispose the embryos to common cleavage abnormalities (Kim *et al.*, 1998; Barrit *et al.*, 1999; Perez *et al.*, 2000; VanBlercom *et al.*, 2000). The relationship between these cytoplasmic changes and the nuclear organization during maturation and fertilization of oocytes may determine an abnormal development and mitotic errors at the cleavage stage, as suggested in prospective analysis of pronuclear zygote morphology in relation to chromosomal abnormalities detected in PGD for poor prognosis IVF patients (Kahraman *et al.*, 2002; Gianaroli *et al.*, 2003).

According to the data on PGD for aneuploidies performed at the cleavage stage, at least 60% of the embryos tested had chromosomal abnormalities (Gianaroli *et al.*, 2001; Munne, 2002; Munne *et al.*, 2003). Although the reported types of aneuploidies may differ in different studies, there seems to be no doubt that approximately half of these abnormalities are represented by mosaicism. As there was no information about the initial chromosomal set of the zygotes from which the mosaic embryos originated in any of these studies, the nature of mosaicism in preimplantation embryos is not known, despite its high prevalence and the potential clinical relevance. There were, however, some indirect observations suggesting that the observed mosaicism at the cleavage stage may be of a different nature, with some mosaic types increasing with maternal age (Munne *et al.*, 2002), and therefore, probably stemming from the female meiosis errors; and the others possibly attributable to an immaturity of centrosome structures in sperm, expected to be active from the first mitotic divisions of zygote, suggested for the cases of TESE patients (Silber *et al.*, 2002). It may also be suggested that a significant proportion of mosaic embryos originate from the oocytes that are aneuploid from the onset, through a process of trisomy 'rescue'. A possible high rate of further mitotic errors in cleaving embryos, deriving from the oocytes with the complex aneuploidies, may also explain the phenomenon of chaotic embryos, which makes up almost half of the embryos with mosaicism. A comparable prevalence of aneuploidies in oocytes and embryos, with the differences of the types of chromosomal anomalies mainly attributable to a high frequency of mosaicism at the cleavage stage embryos, may also support a prezygotic origin of the majority of the embryo chromosome abnormalities, including mosacism.

The comparison of the chromosome-specific aneuploidy rates in oocytes and embryos may be also be of relevance to understanding the relationship between oocyte and embryo abnormalities (Table III). As can be seen from these data, there is an almost 2-fold higher rate for each chromosome error in oocytes compared to embryos, which may indicate a possible correction of some of the aneuploidies through the mechanism of trisomy rescue; probably resulting in a certain proportion of mosaic embryos following the first three cleavage divisions. In fact, the exact data on the mosaicism rate in preimplantation development is not known, because only a limited number of the preimplantation embryos were fully studied, with the majority available from PGD for aneuploidies performed through a single biopsied blastomere, which may not be representative of the whole embryo. Although the possibility of postzygotic mitotic errors in cleavage-stage embryos euploid from the onset cannot be excluded, the proportion of the aneuploidy and mosaicism stemming from these errors is not known, as well as the impact of these postzygotic errors on the pre- and post-implantation embryo development.

Based on the above data, it may be suggested that the most accurate pre-selection of embryos for transfer in PGD for aneuploidies may be performed by a sequential testing of meiosis I, meiosis II and mitotic errors through sequential PB1, PB2 and blastomere sampling. This may allow an avoidance of transfer of embryos with prezygotic chromosomal errors, which seem to be the major source of chromosomal abnormalities in the embryo, and also detection of possible mitotic errors in embryos resulting from the euploid zygotes, the proportion of which cannot be evaluated at the present time. The accumulated data on such sequential sampling will help to evaluate possible differences in viability of the embryos with chromosomal abnormalities of meiotic and mitotic origin.

The introduction of meiosis error testing as a possible integral component of IVF may also be useful in avoiding some of the imprinting disorders which have recently been reported to be associated with IVF procedures (DeBaun *et al.*, 2003; Gicquel *et al.*, 2003; Maher *et al.*, 2003). The fact that more than half of IVF patients are 35 years and older, and that more than half of their oocytes may have aneuploidies, avoiding the transfer of the embryos resulting from these oocytes through PGD for aneuploidies should be clinically useful, in addition to potentially improving implantation and pregnancy rates, and avoiding the transfer of embryos with uniparental disomies, as possible contributors to the imprinting disorders.

Conclusion

New information on meiotic and mitotic errors has become available with the advent of PGD for aneuploidies, presently performed for at least 5000 poor prognosis IVF patients. The practical relevance of PGD for such patients is obvious from the fact that more than half of the tested oocytes or embryos had aneuploidies, which may clearly affect the developmental competence and the embryo's potential to implant, if not removed from transfer. In contrast to the data obtained in traditional meiotic studies, the direct testing of the meiotic outcomes in patients of advanced reproductive age showed that chromosomal abnormalities originate comparably from meiosis I and meiosis II, and are predominantly of chromatid origin. Although isolated errors in meiosis I and II were also observed (39% and 31.9%, respectively), 42.7% of oocytes with meiosis I errors, overall, also had sequential meiosis II errors, resulting in apparently

balanced zygotes in 32.5% of cases, which may represent a phenomenon of aneuploidy rescue in female meiosis. However, the resulting embryos from such balanced zygotes were predominantly aneuploid, suggesting the inherent predisposition of these zygotes to postzygotic chromosomal errors, following sequential errors in meiosis I and II. The chromosome-specific patterns of errors in meiosis I and II were different for each chromosome tested, and these patterns were not in agreement with the previously reported data based on DNA polymorphism studies in liveborn trisomies or spontaneous abortions. Comparison of the types of chromosomal aneuploidies, and the prevalence of each chromosome-specific error in oocytes and embryos, allows the suggestion that the majority of chromosomal aneuploidies in embryos originate from female meiosis, predisposing to further sequential postzygotic errors, which may explain the high rate of mosaicism in preimplantation embryos. This may also indicate the requirement for both oocyte and embryo testing in PGD for aneuploidies, to exclude the possibility of transferring embryos with aneuploidies originating from meiosis and mitotic errors.

References

Angel R (1997) First meiotic division nondisjunction in human oocytes. Am J Hum Genet 65,23–32.

Barrit J, Brenner C, Cohen J and Matt D (1999) Mitochondrial DNA rearrangement in human oocytes and embryos. Mol Hum Reprod 5, 927–933.

Battaglia DE, Goodwin P, Klein NA and Soules MR (1996) Influence of maternal age on meiotic spindle assembly in oocytes from naturally cycling women. Hum Reprod 11,2217–2222.

Cupisti S, Conn CM, Fragouli E et al. (2003) Sequential FISH analysis of oocytes and polar bodies reveals aneuploidy mechanisms. Prenat Diagn 23,663–668.

DeBaun MR, Niemitz EL and Feinberg AP (2003) Association of in vitro fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. Am J Hum Genet 72,156–160.

Dyban A, Fredine M, Severova E et al. (1996) Detection of aneuploidy in human oocytes and corresponding first polar bodies by FISH. J Assist Reprod Genet 13,72–77.

Eichenlaub-Ritter U, Vogt E, Yiu H and Gosden R (2003) Spindles, mitochondria and redox potential in ageing oocytes. Reprod BioMed Online 7,117–127.

Fisher JM, Harvey JF, Morton NE and Jacobs PA (1996) Trisomy 18: studies of the parent and cell division of origin and effect of aberrant recombination on nondisjunction. Am J Hum Genet 56,669–675.

Gianaroli L, Magli MC and Ferraretti AP (2001) The in vivo and in vitro efficiency and efficacy of PGD for aneuploidy. Molec Cell Endocrin 183,S13–S18.

Gianaroli L, Magli MC, Ferraretti AP et al. (2003) Pronuclear morphology and chromosomal abnormalities as scoring criteria for embryo selection. Fertil Steril 80,837–844.

Gicquel C, Gaston V, Maldenbaum J et al. (2003) In Vitro Fertilization May Increase the Risk of Beckwith–Videmann Syndrome Related to the Abnormal Imprinting of the KCNQ1OT Gene. Am J Hum Genet 72, 1338–1341.

Hassold T, Merril M, Adkins K, Freemen S and Sherman S (1995) Recombination and maternal age-dependent nondisjunction: molecular studies of trisomy 16. Am J Hum Genet 57,867–874.

Hunt P, LeMaraire R, Embury P, Sheean L and Mroz K (1995) Analysis of chromosome behaviour in intact mammalian oocytes: monitoring the segregation of a univalent chromosome during female meiosis. Hum Mol Genet 4,2007–2012.

Kahraman S, Kumpete Y, Sertyel S et al. (2002) Pronuclear scoring and chromosomal status of embryos in severe male infertility. Hum Reprod 17,3193–3200.

Kim NH, Chung HM, Cha KY and Chung KS (1998) Microtubule and microfilament organization in maturing human oocytes. Hum Reprod 13, 2217–2222.

Kuliev A, Cieslak J, Illkevitch Y and Verlinsky Y (2003a) Chromosomal Abnormalities in a Series of 6733 human oocytes in preimplantation diagnosis for age-related aneuploidies. Reprod Bio Med Online 6,54–59.

Kuliev A, Cieslak J, Zlatopolsky Z, Ilkevitch Y, Kirilova I and Verlinsky Y (2003b) Origin of aneuploidies in preimplantation embryos. In 2003 Fifth International Symposium on Preimplantation Genetics, 5-7 June Antalya, Turkey. pp 16–17.

Kuliev A, Cieslak J, Zlatopolsky Z, Ilkevitch Y, Kirilova I and Verlinsky Y (2003c) Aneuploidy rescue after female meiosis I and follow up analysis of its outcome in resulting preimplantation embryos. Am J Hum Genet 73 (Suppl),189.

Lamb NE, Freeman S, Savage-Austin A et al. (1996) Susceptible chiasmate configurations of chromosome 21 predispose to nondisjunction in both maternal meiosis I, and meiosis II. Nat Genet 14,400–405.

Lamb NE, Feingold E, Savage-Austin A et al. (1997) Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. Hum Mol Genet 6,1391–1401.

Ly HD, Lockhart D, Lerner RA and Schultz DG (2000) Mitotic Misregulation and Human Aging. Science 287,2486–2491.

Maher ER, Brueton LA, Bowdin SC et al. (2003) Beckwith–Wiedemann syndrome and assisted reproduction technology (ART). J Med Genet 40, 62–64.

Munne S (2002) Preimplantation Genetic Diagnosis of Numerical and Structural Chromosome Abnormalities. Reprod BioMed Online 4,183–196.

Munne S, Daily T, Sultan KM, Grifo J and Cohen J (1995) The use of first polar bodies for preimplantation diagnosis of aneuploidy. Hum Reprod 10,1014–1120.

Munne S, Sandalinas M, Escudero T et al. (2002) Some Mosaic Types Increase with Maternal Age. Reprod BioMed Online 4,223–232.

Munne S, Bahce M and Sandalinas M (2004) Differences in chromosome susceptibility to aneuploidy and survival to first trimester. Reprod BioMed Online 8,81–90.

Nakaoka Y, Okamoto E, Miharu N and Ohama K (1998) Chromosomal analysis in human oocytes remaining unfertilized after in vitro insemination: effect of maternal age and fertilization rate. Hum Reprod 113, 419–424.

Nashmyth K, Peters JM and Uhlman F (2000) Splitting the chromosome: cutting the ties that bind sister chromatids. Science 288,1379–1384.

Pellestor F, Andreo B, Armal F, Humeau C and Demaille J (2002) Mechanisms of non-disjunction in human female meiosis: the co-existence of two modes of malsegregation evidenced by the karyotyping of 1397 in-vitro unfertilized oocytes. Hum Reprod 17,2134–2145.

Perez G, Flaherty S, Barry M and Matthews C (1997) Preliminary observations of polar body extrusion and pronuclear formation in human oocytes using timeplapse video cinematography. Hum Reprod 12,532–541.

Peterson MB and Mikkelsen M (2000) Nondisjunction in trisomy 21: Origin and mechanisms. Cytogenet Cell Genet 91,199–203.

Pujol A, Boiso I, Benet J et al. (2003) Analysis of nine chromosome probes in first polar bodies and metaphase II oocytes for the detection of aneuploidies. Eur J Hum Genet 11,325–336.

Sherman SL, Peterson MB, Freeman SB et al. (1994) Nondisjunction of chromosome 21 in maternal meiosis I: evidence for a maternal age-dependent mechanism involving reduced recombination. Hum Mol Genet 3,1529–1535.

Silber S, Sadowy S, Lehahan K, Kilani Z, Gianaroli L and Munne S (2002) High Rate of Chromosome Mosaicism but not Aneuploidy in Embryos from Karyotypically Normal Men Requiring TESE. Reprod BioMed Online 4 (Suppl 2),20.

Van Blercom J, Davis P and Alexander S (2000) Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres: relationship to microtubular organization, ATP content and competence. Hum Reprod 15, 2621–2633.

Verlinsky Y and Kuliev A (2000) Atlas of Preimplantation Genetic Diagnosis. Parthenon, New York.

Verlinsky Y, Cieslak J, Freidin M et al. (1995) Pregnancies following preconception diagnosis of common aneuploidies by FISH. Hum Reprod 10,1923–1927.

Verlinsky Y, Cieslak J, Ivakhnenko V et al. (1996) Birth of healthy children after preimplantation diagnosis of common aneuploidies by polar body FISH analysis. Fertil Steril 66,126–129.

Verlinsky Y, Cieslak J, Ivakhnenko V et al. (1998a) Preimplantation diagnosis of common aneuploidies by the first and second polar body FISH analysis. J Assist Reprod Genet 15,285–289.

Verlinsky Y, Cieslak J, Ivakhnenko V et al. (1998b) Prepregnancy genetic testing for common age-related aneuploidies by polar body analysis. Genet Testing 1,231–235.

Verlinsky Y, Cieslak J, Ivakhnenko V et al. (1999) Prevention of age-related aneuploidies by polar body testing of oocytes. J Assist Reprod Genet 16,165–169.

Verlinsky Y, Cieslak J, Ivakhnenko V, Wolf G, Strom C and Kuliev A (2000) Polar body preimplantation diagnosis in aging IVF patients. Ref Gynec Obstet 7,191–194.

Verlinsky Y, Cieslak J, Ivakhnenko V et al. (2001) Chromosomal abnormalities in the first and second polar body. Mol Cell Endocrinol 183, S47–S49.

Verlinsky Y, Munne S, Cohen J et al. (2004) Over a decade of preimplantation genetic diagnosis experience—A multi-center report. Fertil Steril 82,292–294.

Yuan L, Liu J, Hoja M, Wilbertz J, Nordqvist K and Hoog C (2002) Female germ cell aneuploidy and embryo death in mice lacking the meiosis-specific protein SCP3. Science 296,1115–1118.

Submitted on April 19, 2004; revised on June 11, 2004; accepted on July 12, 2004