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ORIGINAL INVESTIGATION

The origin and impact of embryonic aneuploidy

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Abstract Despite the clinical importance of aneuploidy, surprisingly little is known concerning its impact during the earliest stages of human development. This study aimed to shed light on the genesis, progression, and survival of different types of chromosome anomaly from the fertilized oocyte through the final stage of preimplantation development (blastocyst). 2,204 oocytes and embryos were examined using comprehensive cytogenetic methodology. A diverse array of chromosome abnormalities was detected, including many forms never recorded later in development. Advancing female age was associated with dramatic increase in aneuploidy rate and complex chromosomal abnormalities. Anaphase lag and congression failure were found to be important malsegregation causing mechanisms in oogenesis and during the first few mitotic divisions. All abnormalities appeared to be tolerated until activation of the embryonic genome, after which some forms started to decline in frequency. However, many aneuploidies continued to have little impact, with affected embryos successfully reaching the blastocyst stage. Results from the direct analyses of female meiotic divisions and early embryonic stages suggest that chromosome errors present during preimplantation development have origins that are more varied than those seen in later pregnancy, raising the intriguing possibility that the source of aneuploidy might modulate impact on embryo viability. The results of this study also narrow the window of time for selection against aneuploid embryos, indicating that most survive until the blastocyst stage and, since they are not detected in clinical pregnancies, must be lost around the time of implantation or shortly thereafter.

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Introduction

Early human development is a challenging and dynamic process. The fertilized oocyte has to first facilitate the integration of the male and female genetic contributions and then begin a series of cellular divisions, producing a cleavage stage embryo. The embryo must successfully trigger activation of its genome around the 4–8 cell stage, before going on to form a morula and then a blastocyst, the latter of which displays the first morphologically visible signs of differentiation with two distinct lineages apparent; an outer trophectoderm layer, which will go on to form the placenta and other extra-embryonic tissues, and a small group of cells, the inner cell mass, from which the fetus is derived. The blastocyst must then 'hatch', emerging from the membrane (zona pellucida) that has surrounded the oocyte and embryo during the first 5 days of life, so that

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communication with the uterus can be initiated resulting in implantation. The whole chain of events leading to blastocyst formation takes approximately 5–6 days in humans.

The cytogenetics of human embryos during this critical phase of development is biologically fascinating as well as being of great clinical importance. Aneuploidy is relatively common at later developmental stages affecting at least 4–5 % of all clinical pregnancies, the vast majority of which end in miscarriage (reviewed in Hassold et al. 2007; Hassold and Hunt 2001). However, there is strong evidence that the incidence of aneuploidy is even greater at the time of conception and that a much wider range of chromosome abnormalities exists prior to implantation. It is thought that the frequency of aneuploidy in human embryos is an order of magnitude higher than seen in other mammalian species and that this may explain the relatively low pregnancy rates in natural and assisted reproductive cycles observed in our species (Delhanty et al. 1997; Wells and Delhanty 2000).

Although many cytogenetic studies of human embryos have been carried out, the exact spectrum and frequency of abnormalities, their origin, and their impact as development progresses remain poorly defined. Much of this uncertainty is a consequence of technical limitations that have, until recently, precluded analysis of all 24 types of chromosome (22 autosomes, X and Y) in individual embryos. Most previous studies examined a set of 5–12 chromosomes in each embryo [using fluorescent in situ hybridization—(FISH)]; while others succeeded in applying more comprehensive cytogenetic methodologies, who were only able to examine relatively small numbers of embryos at a single stage of preimplantation development (e.g., Munne et al. 1998, 2002; Platteau et al. 2005; Mantzouratou et al. 2007; Rubio et al. 2007; Verpoest et al. 2008; Wells and Delhanty 2000; Voullaire et al. 2000; Northrop et al. 2010; Fragouli et al. 2011a, b).

The current study involved a comprehensive assessment of aneuploidy throughout preimplantation development in a large number of samples, providing a detailed insight into cytogenetics at different preimplantation stages, from oocytes at the time of fertilization to the blastocyst stage just prior to implantation. As well as defining the frequency and types of abnormality at each stage, the results also shed light on mechanisms underlying chromosome malsegregation, on the effect of female age and on the developmental potential and ultimate fate of aneuploid conceptions.

Materials and methods

Patients and embryos

A total of 2,204 samples were cytogenetically examined. These samples were categorized into three groups, according to the stage of preimplantation development. Group 1 consisted of 420 fertilization-competent oocytes, group 2 consisted of 738 cleavage stage embryos, and group 3 consisted of 1,046 blastocysts. These samples were generated by a total of 396 couples undergoing in vitro fertilization (IVF) in combination with preimplantation genetic screening (PGS) at several IVF clinics in the UK. Most (222, 56 %) of these couples were referred for PGS due to the female partner being of advanced reproductive age. Of the remaining, 104 (27 %) had previously experienced repeated implantation failure and 70 (17 %) had had several unexplained spontaneous miscarriages. The average female age of this group was 39.6 years (age range 27–47 years). Further details about patients, oocytes, and embryos are shown in Table 1. The ovarian stimulation protocol did not differ significantly from those described previously (Fragouli et al. 2010). Participating IVF clinics had in place appropriate ethical approvals, procedures for informed patient consent, and relevant clinical treatment licenses.

Oocyte and embryo sampling and cell preparation for cytogenetic analysis

Cytogenetic assessment of oocytes involved sequential biopsy of the first and second polar bodies (PBs) after oocyte retrieval and fertilization, respectively, allowing independent analysis of both meiotic divisions. For analysis of embryos, a single blastomere was removed from those at the cleavage stage, or 5–10 cells were removed

Table 1 Patient and embryo details

Preimplantation development stage	Number of patients	Average female age (age range)	Number of embryos examined
Oocyte/zygote (Day 0)	95	40.7 years (34–47 years)	420
Cleavage (Day 3)	113	39.5 years (27–45 years)	754
Blastocyst (Day 5)	188	38.8 years (28–47 years)	1,046
Total patients		Average female age	Total embryos
396		39.6 years	2,220

from the trophectoderm at the blastocyst stage. Culture, micromanipulation, biopsy, and cell preparation for chromosome analysis took place as described previously (Fragouli et al. 2010, 2011a). All samples were analyzed with the use of a single, highly validated platform for microarray comparative genomic hybridisation (aCGH) (Fragouli et al. 2011a; Magli et al. 2011; Gutierrez-Mateo et al. 2011; Christopikou et al. 2013; Mertzanidou et al. 2013).

Microarray comparative genomic hybridisation

After cell biopsy, cytogenetic analysis was carried out using 24Sure^{TM} Cytochip V3 microarrays (BlueGnome Ltd., Cambridge, UK). The protocol employed was as reported in Fragouli et al. (2011a), with modifications according to the manufacturer (BlueGnome 24sure protocol, www.cytochip.com). In brief, the procedure included cell lysis and whole genome amplification (Sureplex, Rubicon Genomics, Ann Arbor, USA), followed by fluorescent labeling of amplified samples, and two genomic DNAs (46, XY an 46, XX), and hybridization to the microarray. The microarrays were washed, scanned (InnoScan 710, Innopsys, Carbonne, France), and the resulting images analyzed using BlueFuse software (BlueGnome Ltd, Cambridge, UK). Using this approach, it was possible to assign molecular karyotypes to the embryonic samples under investigation, allowing classification as normal or aneuploid.

Statistical analysis

Unless stated otherwise, statistical evaluations were carried out using Fisher's exact test.

Results

Oocytes

The contribution of each of the female meiotic divisions to embryonic aneuploidy was investigated in 420 oocytes. This involved the analysis of the first and second PBs. The first PB can be removed from mature oocytes on the day they are retrieved from the ovary (day 0), while the second PB is only extruded upon fertilization and is, therefore, not available until the day after oocytes are exposed to sperm (day 1). The oocytes included in the current study were derived from 95 couples of an average female age of 40.7 years (age range 34–47 years).

Microarray CGH analysis characterized 108/420 (26 %) oocytes as normal haploid after completion of both meiotic divisions. Of the abnormal oocytes, 96 (30 %) exhibited MI errors, 133 (43 %) displayed MII errors, and the remaining 83 (27 %) had errors in both divisions. Complex aneuploidy (defined as three or more distinct aneuploidies) affected 27 % of all oocytes tested, accounting for approximately one-third (36 %) of those found to be abnormal. The other two-thirds of the abnormal oocytes had only 1–2 aneuploidies each.

A total of 914 individual chromosome abnormalities were detected. 517 of the abnormalities (56 %) involved a predicted excess of chromosomal material in the oocyte compared with 398 (44 %) losses. The difference in the incidence of predicted chromosome gains and losses in the fertilized oocyte was statistically significant ($P < 0.006$). The vast majority (886; 97 %) of aneuploidies affected entire chromosomes. However, there were 28 abnormalities that only affected part of a chromosome. These segmental aneuploidies were observed in 4 % of oocytes (18/420). In several cases, more than one segmental aneuploidy was detected in the same oocyte. Segmental abnormalities were often, although not always, accompanied by errors affecting whole chromosomes.

Both whole chromosome non-disjunction and unbalanced chromatid predivision were detected during female meiosis, as has been reported previously (Fragouli et al. 2011b; Gabriel et al. 2011; Handyside et al. 2012; Christopikou et al. 2013). Unbalanced chromatid predivision was responsible for most of the abnormalities seen in the first meiotic division (76 % chromatid errors vs. 24 % chromosome errors). There were 35 instances when a chromatid error arising during the first meiotic division was corrected by a reciprocal abnormality in meiosis II. However, in most cases, the oocytes in question remained abnormal due to the presence of additional aneuploidies, affecting other chromosomes, which were not corrected. Only five oocytes, aneuploid at the end of meiosis I, ultimately became normal at the completion of the second meiotic division. Thus, correction rescued less than 2 % of abnormal oocytes in the patients included in this study (a group with a high average female age). These results are summarized in Table 2.

Table 2 The incidence of MI chromosome and chromatid errors according to female age

Age group	Average female age	Total chromatid errors corrected during MII	Total oocytes rendered normal after correction event
Younger	36 years	8.8 % (3 of 34 errors)	12.5 % (3 of 24 oocytes)
Older	41 years	11.3 % (32 of 281 errors)	0.7 % (2 of 288 oocytes)

Every chromosome participated in aneuploidy events. However, the five with the highest abnormality rates in oocytes were of frequency: 16, 21, 22, 15, and 19. When considering only those oocytes that had a single chromosome error, together these five accounted for more than half (58 %) of all abnormalities.

In order to examine the effects of female age on oocyte aneuploidy, patients were grouped into younger and older categories. The younger group included 51 oocytes generated by 12 women of an average age of 36 years (age range 34–37 years). The older group included the remaining 369 oocytes which were generated by 83 women of an average age of 41 years (age range 38–47 years).

Among the younger patient group, 47 % of oocytes were chromosomally abnormal. The error rate was slightly higher in the first meiotic division (25 % of abnormal oocytes only contained MI errors, 20 % had mistakes derived from MII alone, and 2 % had aneuploidies arising in both divisions). There were a total of 39 individual MI errors and 17 distinct abnormalities of MII origin. Of the oocytes aneuploid after the first meiotic division, 12.5 % ultimately became chromosomally normal after the abnormality was corrected by reciprocal events in meiosis II. Only a small minority (3.9 %) of oocytes from young patients were characterized as complex abnormal. Errors affecting 1–2 chromosomes were observed in 43.1 % of oocytes. The majority of abnormalities detected (65 %) involved a predicted gain of a chromosome in the oocyte. 98 % of abnormalities involved whole chromosomes. Only 1/51 (2 %) oocytes analyzed from the younger patients had a segmental error.

The oocytes from reproductively older patients displayed a highly significant increase in the incidence of aneuploidy, with 78 % of them being affected (288/369; $P < 0.0001$). In particular, the prevalence of complex abnormalities was almost an order of magnitude higher among oocytes from the older group, observed in 31 % of those tested ($P < 0.0001$). The 288 abnormal oocytes consisted of a total of 868 distinct chromosome abnormalities.

In contrast to the results obtained from the younger women, errors of meiosis II origin predominated in oocytes from older patients. 22 % of the oocytes were only affected by MI abnormalities, whereas 34 % had MII errors alone and 23 % had a combination of aneuploidies derived from both MI and MII. While the frequency of aneuploidy in each of the two meiotic divisions rose with age, errors in MII showed the most pronounced increase (there were 378 distinct aneuploidies detected after MI compared with 490 related to errors in MII). Consequently, the relative proportion of aneuploidies attributable to MI and MII differed significantly between the younger and older patient groups ($P = 0.0002$). Although individual chromatid abnormalities derived from MI were sometimes corrected during MII, the high frequency of complex aneuploidy seen in the oocytes of older patients meant that less than 1 % became entirely normal (2/288), a correction rate significantly lower than that observed in the younger patient group ($P < 0.004$).

Errors leading to extra chromosomes in the oocyte accounted for 56 % of all aneuploidies, while the remaining 44 % of abnormalities were losses. Interestingly, partial abnormalities appeared to be more than twice as common among the oocytes of older patients, affecting 4.6 %, although the number of affected samples was too small to confirm statistical significance.

Cleavage stage

The incidence of aneuploidy at the cleavage stage was examined in a total of 754 embryos which were generated by 113 couples with an average female age of 39.5 years (age range 27–45 years). Only 130 (17 %) of all investigated embryos were characterized as euploid. The proportion of embryos with simple (1–2 abnormalities) and complex aneuploidy (three or more abnormalities) was essentially identical (314 vs. 310, respectively).

A total of 2,468 distinct chromosome imbalances were scored, evenly split between trisomy and monosomy [1,206 (49 %) chromosome gains and 1,262 (51 %) losses]. Although most aneuploidies involved whole chromosomes, segmental anomalies were also seen to occur at an appreciable frequency. A total of 173 segmental aneuploidies (representing 7 % of all abnormalities) were detected in 115 (15 %) embryos. All 24 types of chromosome contributed to cleavage stage aneuploidy, but chromosomes 22, 16, 19, 21, and 13 were the most frequently observed in the order of prevalence (together accounting for 59 % of all abnormalities in embryos that were affected by a single aneuploidy).

As far as female age was concerned, 404 individual abnormalities were scored in 191 embryos from younger women (27 patients, average age 35 years, range 27–37 years). The observed aneuploidy rate for this patient group was 73 %. In the 563 embryos derived from reproductively older women, the abnormality rate was higher still affecting 86 % (87 patients, average age 41 years, range 38–45 years). A total of 2,064 distinct abnormalities were scored in the embryos of the older group. The increase in embryo aneuploidy rate with age was highly statistically significant ($P < 0.0001$). The incidence of complex chromosome abnormalities also increased with advancing female age, affecting 29 % (55 embryos) of patients from the younger age group to 45 % (255 embryos) of those from the older patient group. The difference in the incidence of complex abnormal embryos

between the two age categories was highly significant ($P < 0.0001$). There was no significant association between the incidence of segmental abnormalities and age.

Blastocyst stage

The frequency of chromosome abnormalities during the final stage of development prior to implantation was assessed in 1,046 blastocysts, produced by a group which included 188 patients of an average female age of 38.8 years (28–47 years). According to the results obtained from comprehensive chromosome analysis, 438 (42 %) of the blastocysts were euploid, with abnormalities being scored for the remaining 608. Thus, for an average female age of approximately 39 years, the aneuploidy rate at the developmental stage just before implantation was 58 %. Complex aneuploidy affected 105 embryos (10 %).

The 608 embryos characterized as chromosomally abnormal contained a total of 1,113 distinct aneuploidies, divided approximately equally between losses (53 %) and gains (47 %). Segmental aneuploidies accounted for a total of 92 (8 %) of all anomalies scored, affecting 73 (7 %) of the blastocysts tested. Although all chromosomes were seen to be affected by aneuploidy, chromosomes 22, 16, 15, 21, and 19 (in the order of frequency) were the five most often found chromosomes to be abnormal, accounting for 50.2 % of all errors in blastocysts with a single chromosome abnormality.

The relationship between advancing female age and chromosome constitution was examined at the blastocyst stage by grouping patients in the same way as described above for oocytes and cleavage stage embryos. The younger patient group consisted of 61 women (average age 35 years, range 28–37 years) who produced 420 embryos, while the older patient group consisted of the remaining 127 women (average age 41 years, range 38–47 years) with a total of 626 embryos.

Comparison of the data obtained for the two age groups clearly showed that younger women generated significantly fewer aneuploid embryos (200/420, 48 % abnormal) than older patients (408/626, 65 % abnormal) ($P < 0.0001$). It was also evident that the risk of complex aneuploidy was higher for the embryos of older women (affecting 13.7 % of all embryos) compared to the younger group (4.5 % of all embryos affected, $P = 0.0004$), although for both age categories, the majority of abnormal embryos were affected by just 1–2 aneuploidies (90.5 % of abnormal embryos for younger patients and 79 % for older).

A total of 315 distinct aneuploidies were detected in the 420 embryos of younger women, while 798 were seen in the 626 blastocysts generated by older women. For the younger age group, the incidence of gains and losses of chromosomes was almost equal (158 vs. 157). For the older age patient group, chromosome losses occurred slightly more often than gains (428 vs. 370, 54 % of all abnormalities vs. 46 %, respectively), but this difference was not statistically significant. Segmental aneuploidies were seen in 32/420 (7.6 %) of blastocysts from younger patients and 60/626 (9.5 %) from the older group.

Molecular karyotypes of a pair of 1st and 2nd PBs removed from the same oocyte, a blastomere removed from a cleavage stage embryo, and a trophectoderm sample removed from a blastocyst stage embryo, all analyzed during the course of this study, are illustrated in Fig. 1.

How the frequency and variety of chromosome errors change during progression from mature oocytes to the final stage of preimplantation development?

Overall, progression through preimplantation development was associated with a significant decline in the frequency of aneuploidy. In the patient population examined during this study, 75 % of oocytes were aneuploid after completion of both meiotic divisions, while only 58 % of blastocysts were chromosomally abnormal ($P < 0.0001$). The proportion of samples carrying multiple abnormalities also declined, from 27 % in oocytes to 10 % in blastocysts ($P < 0.0001$). However, at the cleavage stage, a transient increase in the aneuploidy rate to 83 % ($P = 0.0006$) was observed, marked by an elevated frequency of highly abnormal embryos. Complex aneuploidy affected 41 % of cleavage stage embryos, significantly higher than observed in oocytes or blastocysts ($P < 0.0001$). An increased incidence of segmental deletions and duplications was also a feature of the cleavage stage. The frequency increased threefold with respect to the incidence seen in oocytes ($P < 0.0001$) before falling by a half in blastocysts ($P < 0.0001$).

The elevated abnormality rate observed at the cleavage stage was also evident in the number of distinct chromosome errors per embryo, which increased from an average of 2.2 in oocytes to 3.3 at the cleavage stage. The increase in aneuploidy presumably reflects the introduction of additional sperm-derived aneuploidies and postzygotic abnormalities. It was also evident that as embryos moved toward the blastocyst stage, there was a significant decrease in the average number of errors per embryo (to 1.1), compared to earlier stages of development, suggesting the loss of aneuploid embryos or the abnormal cells they contain. These results are illustrated in Fig. 2.

A total of 4,495 chromosome abnormalities were scored in all three examined stages. Although a slight excess of chromosome gains compared to losses was predicted in oocytes (56 vs. 44 %), the expected preponderance of trisomies in embryos was not observed (relative frequency of trisomies and monosomies differed significantly between oocytes and embryonic stages, $P < 0.0001$). From the day

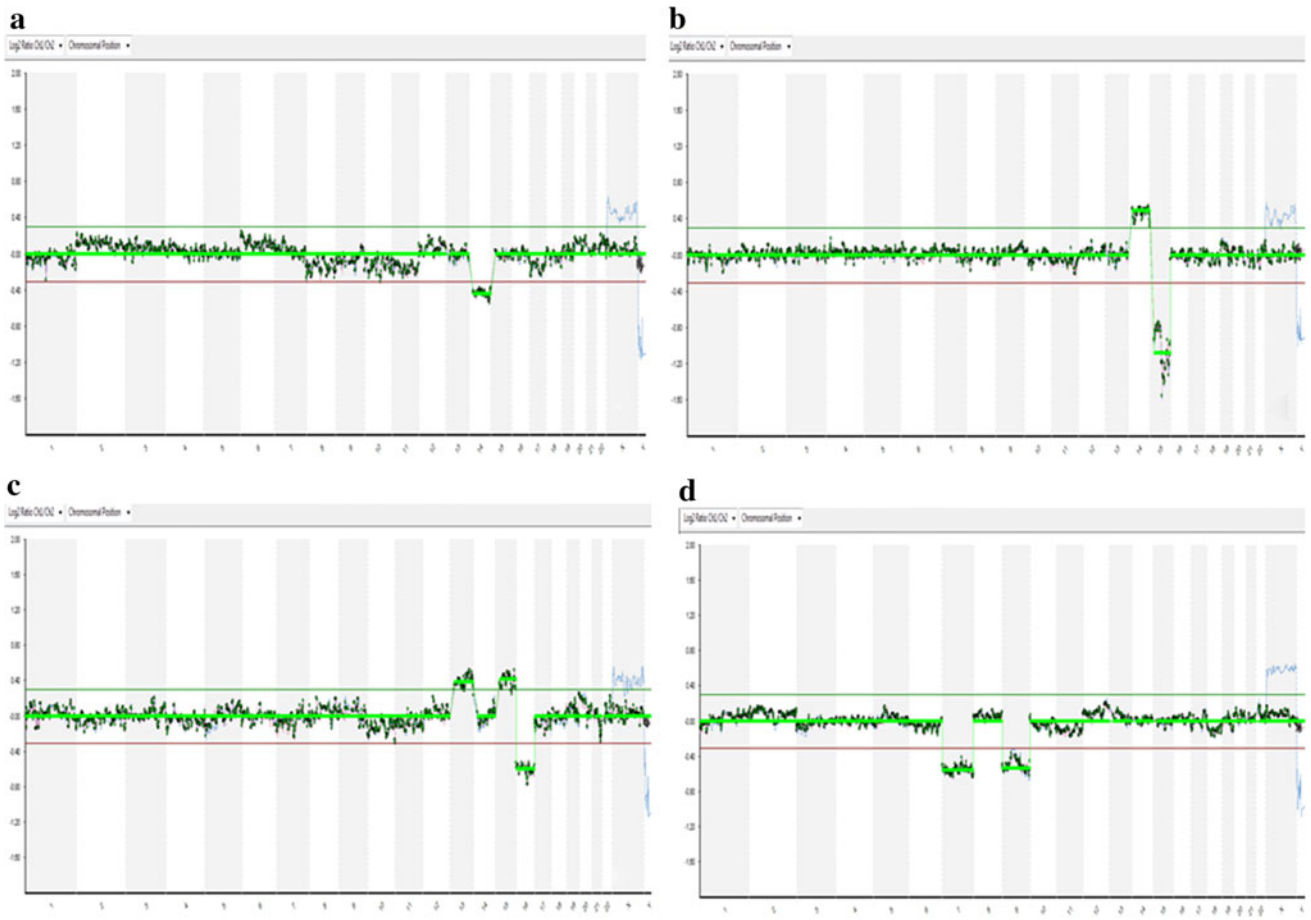

Fig. 1 Molecular karyotypes resulting after aCGH analysis. Profiles **a** and **b** were generated during the analysis of a pair of PBs removed from the same oocyte. Profile **a** represents the chromosome complement of the 1st PB. A loss of a single chromatid 14 was scored for this cell. Hence, this 1st PB was classified as 23,X,−14cht, abnormal. Profile **b** represents the chromosome complement of the 2nd PB. A gain of chromosome 14, indicating the correction of the MI error in the oocyte, along with a loss of chromosome 16 were detected in this cell. Therefore, this 2nd PB was classified as 23,X,+14,−16, abnormal; whereas the predicted karyotype of the corresponding oocyte was determined to be 24,X,+16 abnormal. Profile **c** represents the chromosome complement of a blastomere removed from a cleavage stage embryo. Three different aneuploidies were identified in this cell and these were gains of chromosomes 13 and 15 and a loss of chromosome 16. This blastomere was classified as 47,XX,+13,+15,−16, abnormal. Profile **d** represents the chromosome complement of a trophectoderm sample removed from an embryo which had become a blastocyst. Two different errors were identified in this sample in the form of losses of chromosomes 7 and 9. This trophectoderm sample was classified as 44,XX,−7,−9, abnormal

3 of development onward, the incidence of monosomy was found to be equal or perhaps slightly higher than that of trisomy (51 % losses vs. 49 % chromosome gains and 53 % losses vs. 47 % gains for cleavage and blastocyst stages, respectively). These results are shown in Fig. 3.

The survival of embryos with specific chromosomal abnormalities to the blastocyst stage

Table 3 shows the frequency of gains and losses for all 24 types of chromosome and during all three stages. In order to investigate how embryos with abnormalities affecting individual chromosomes progressed through preimplantation development, we assessed 607 embryos carrying a single aneuploidy only (124 fertilized oocytes, 158 cleavage stage embryos, 325 blastocysts). Among these, chromosomes 16, 19, 21, and 22 had the highest aneuploidy rates across all three examined stages. In general, a decrease in the frequency of total aneuploidies (gains and losses) during progression to the blastocyst was observed, with chromosome 19 displaying the most obvious decline (of samples containing a single error, 10.5 % of oocytes were aneuploid for chromosome 19 compared with 5 % of blastocysts). A steep rise in the frequency of chromosome 22 aneuploidy from 10.5 % in oocytes to 19.6 % at the cleavage stage suggests that this chromosome may be particularly prone to post-zygotic errors. These results are illustrated in Fig. 4, and summarized in Table 4. Data obtained from the cytogenetic analysis of material coming from spontaneous miscarriages are also included in Table 4 for comparison purposes.

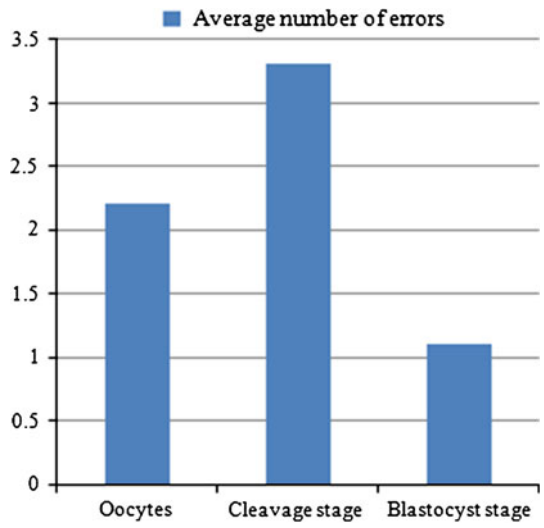

Fig. 2 The average number of errors scored per oocyte/embryo. The average number of errors increased between fertilized oocytes and the cleavage stage. This increase was reflective of the introduction of sperm and postzygotic chromosome abnormalities. Progression to the blastocyst stage was associated with a significant decrease in the average number of errors, compared to earlier developmental stages. This decrease is suggestive of the loss of aneuploid embryos and/or abnormal cell lines within embryos

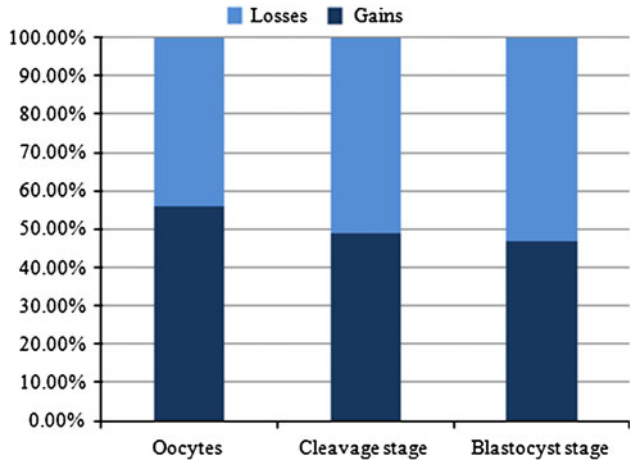

Fig. 3 The frequency of chromosome losses and gains, according to developmental stage. A small excess of gains compared to losses was predicted in the oocytes after completion of both meiotic divisions. However, the expected excess of trisomies was not observed in embryos either at the cleavage or blastocyst stage of development. Conversely, what was evident was that from day 3 of preimplantation development the incidence of monosomy was slightly more frequent compared to the incidence of trisomy

Discussion

Aneuploidy and human oocytes

In the current study, for an average female age of ~40 years, the aneuploidy rate observed in oocytes after completion of both meiotic divisions was 74 %, the great majority being a consequence of premature separation of sister chromatids. This is in agreement with previous studies examining the chromosomes of fertilized oocytes with the use of various cytogenetic methods, such as FISH, metaphase-CGH, and array-CGH (Kuliev et al. 2003; Fragouli et al. 2011a; Gabriel et al. 2011; Handyside et al. 2012). As expected, there was a strong influence of female age on the frequency of aneuploidy. Patients <38 years of age (average 36 years) exhibited abnormalities in 47 % of their oocytes, compared with 78 % for women in the older age range (average 41 years). Although the average age of the two groups of patients only differed by 5 years, the frequency of complex abnormalities was almost ten-fold higher among oocytes of the older group (3.9 vs. 31 % of oocytes affected; $P < 0.0001$).

Of the two female meiotic divisions, MII was the most error prone, contributing to chromosomal imbalance in 70 % of oocytes. It is long established in the scientific and medical literature that most aneuploidies detected in miscarriages, prenatal samples, and children born with Down syndrome are the result of errors occurring in female meiosis I (e.g., Hassold et al. 2007), and consequently, the data reported here might appear controversial. However, it is important to note that earlier studies assessing the origin of meiotic aneuploidy typically involved women in their twenties or early thirties. Our data agrees that for younger women (<36), the most error prone division is MI. However, the fidelity of the second meiotic division is more dramatically affected by advancing age than that of MI. Consequently, MII chromosome segregation errors equal those of MI in the late thirties and become the dominant source of aneuploidy thereafter. Since industrialized countries are witnessing a dramatic demographic shift toward delayed childbearing, chromosome malsegregation during MII is likely to become of increasing clinical importance.

Chromosome abnormality at the cleavage stage

After progression to the cleavage stage, a significant rise in the aneuploidy rate was observed (83 % of embryos abnormal; $P < 0.0006$), with complex chromosome abnormalities displaying particularly large increases in frequency. This can be attributed to the introduction of sperm-derived (male meiotic) errors, in addition to those abnormalities already present in the oocyte and to post-zygotic (mitotic) chromosome malsegregation. Since aneuploidy occurs relatively infrequently in spermatozoa, estimated to affect fewer than 5 % (Carrell et al. 2003; Petit et al. 2005), the increase in chromosomal abnormality rates seen at the cleavage stage is likely to be primarily attributable to mitotic errors.

Table 3 The relative contribution to aneuploidy of specific chromosome abnormalities in oocytes, cleavage stage embryos, and blastocysts

Chromosome	Oocyte gains (%)	Oocyte losses (%)	Oocyte totals (%)	Cleavage gains (%)	Cleavage losses (%)	Cleavage totals (%)	Blastocyst gains (%)	Blastocyst losses (%)	Blastocysts totals (%)
1	1.2	1.53	2.73	2.15	2.1	4.25	1.88	0.98	2.86
2	1.42	1.86	3.28	1.94	2.3	4.24	0.89	2.5	3.39
3	1.31	0.55	1.86	1.78	1.46	3.24	0.72	1.6	2.32
4	1.75	0.87	2.62	1	1.66	2.66	1.6	2.7	4.3
5	1.42	1.64	3.06	1.5	1.7	3.2	0.72	1.34	2.06
6	0.76	1.2	1.96	1.46	1.33	2.79	1.17	0.72	1.89
7	1.64	1.2	2.84	1.95	1.7	3.65	1.8	1.6	3.4
8	0.77	1.2	1.97	1.42	2	3.42	1.8	1.5	3.3
9	2.62	1.31	3.93	1.9	2.1	4	1.35	1.44	2.79
10	2	0.87	2.87	2	1.86	3.86	1.44	1.07	2.51
11	1.75	1.75	3.5	1.66	1.66	3.32	1.35	2.42	3.77
12	1.86	1.1	2.96	2.19	1.74	3.93	1.17	0.98	2.15
13	1.75	2.3	4.05	2.4	2	4.4	1.5	3	4.5
14	1.64	1.86	3.5	2	2.26	4.26	1.08	1.5	2.58
15	4.8	3.17	7.97	3	3.12	6.12	4.6	3.14	7.74
16	5	4	9	3	3.44	6.44	4.76	5	9.76
17	0.87	2	2.87	2.1	1.78	3.88	2.06	1.17	3.23
18	2.84	1.1	3.94	1.54	2.5	4.04	1.6	2.87	4.47
19	2.4	3.8	6.2	3.7	2.19	5.89	2.51	1.26	3.77
20	5.13	1.96	7.09	1.82	2.15	3.97	1.44	1.88	3.32
21	5.9	3.6	9.5	2.63	2.76	5.39	2.78	4.5	7.28
22	4.9	3.27	8.17	4	3.8	7.8	7.27	4.3	11.57
X	2.73	1.2	3.93	1.17	2.8	3.97	1.35	4.85	6.2
Y				0.36	0.4	0.76	0.54	0.09	0.63

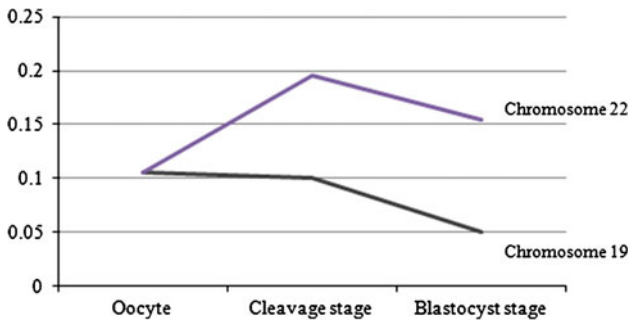

Fig. 4 Chromosomes 19 and 22 were among the ones mostly affected by aneuploidy events throughout preimplantation development. A decline in the rate of chromosome 19 abnormalities was evident from just after fertilization to just before implantation. The incidence of chromosome 22 errors, however, increased from just after fertilization to the cleavage stage, indicating that this chromosome could be particularly prone to postzygotic malsegregation

Advancing female age was associated with significantly higher aneuploidy rates for cleavage stage embryos ($P < 0.0001$), attributable to the increased risk of oocyte-derived errors. For patients over 37 years of age (mean 41 years), only 14 % of cleavage stage embryos were chromosomally normal. This extraordinary rate of aneuploidy likely explains the low success rates of IVF treatment for women in their late thirties and forties. For women aged 41–42 years, only 9.6 % of embryos transferred to the uterus during IVF cycles succeed in implanting (Centers for Disease Control and Prevention 2013). The high aneuploidy rates are also a likely explanation for the reduced fecundity and elevated miscarriage rates seen in natural reproductive cycles with advancing female age.

Aneuploidy at the blastocyst stage

Transition to the blastocyst stage was associated with a decrease in the aneuploidy rate to 58 %, significantly lower than seen in oocytes and an even more dramatic decline with respect to the cleavage stage ($P < 0.0001$ in both cases). A decrease in the average number of chromosome abnormalities per embryo was also seen, reflective of a great reduction in the proportion of embryos affected by complex aneuploidy (on average, each blastocyst contained 1.1 aneuploid chromosomes, compared with 3.3 at the cleavage stage and 2.2 in oocytes). The decrease in aneuploidy is presumably due to impaired viability, leading

Table 4 The progress of single chromosome errors from the oocyte to the blastocyst stage and after implantation

Chromosome	Oocyte			Cleavage			Blastocyst			Spontaneous abortions* Trisomies (%)
	Gains (%)	Losses (%)	Totals (%)	Gains (%)	Losses (%)	Totals (%)	Gains (%)	Losses (%)	Totals (%)	
1	1.61	1.61	3.22	1.26	0.63	1.89	1.23	0.62	1.85	0
2	0	1.61	1.61	1.26	0.63	1.89	0.92	1.23	2.15	2
3	0	0	0	0	0	0	0	0.6	0.6	2
4	2.4	0	2.4	0	1.26	1.26	1.23	1.77	3	2.4
5	0	0.8	0.8	0	0	0	0	0.6	0.6	0.2
6	0.8	0.8	1.6	0.63	0.63	1.26	1.55	0.6	2.15	1.6
7	1.6	0	1.6	0.63	0	0.63	0.32	0.6	0/92	3
8	0.8	0.8	1.6	1.26	3.14	4.4	1.77	1.77	3.54	2
9	0.8	1.6	2.4	0.63	2.47	3.1	1.77	0.32	2.09	2.2
10	2.4	0	2.4	1.9	1.26	3.16	0.92	0.6	1.52	1.3
11	0	0	0	0.63	2.47	3.1	0.92	0.92	1.84	0.9
12	0.8	1.6	2.4	0.63	0	0.63	1.23	1.55	2.78	1.8
13	3.22	3.22	6.44	5	3.8	8.8	1.77	2.23	4	7.3
14	0.8	1.6	2.4	0.63	1.26	1.89	0.6	1.63	2.23	3.3
15	8.9	1.6	10.5	1.9	3.8	5.7	6.15	3.69	9.84	5.6
16	11.3	3.22	14.52	5.7	5.7	11.4	7	6.23	13.23	25.3
17	1.6	0	1.6	1.26	0.63	1.89	2.23	0.32	2.55	0.2
18	1.6	0	1.6	0.63	1.26	1.89	2.23	2.77	5	6.2
19	3.2	7.3	10.5	4.4	5.6	10	2.77	2.23	5	0
20	5.6	0	5.6	1.26	2.47	3.73	1.23	1.55	2.78	3.6
21	9.7	2.3	12	3.8	5	8.8	2.46	4.31	6.77	11.6
22	4.8	5.7	10.5	10	9.6	19.6	9.53	5.87	15.4	7.3
X	3.2	0.8	4	0	0	0	3.23	1.77	5	
Y				0	0	0	0.92	0	0.92	
X0				4.4		4.4	4.3		4.3	

Considering only oocytes and embryos that contained a single aneuploid chromosome

* Data from Lebedev (2011)

some abnormal embryos to undergo developmental arrest before reaching the blastocyst stage. Individual types of aneuploidy appeared to have differing impacts on developmental capacity, discussed in more detail in the following sections. As with cleavage stage embryos, significantly elevated abnormality rates were observed in blastocysts from older women (48 % in patients averaging 35 years and 65 % in patients averaging 41 years; $P < 0.0001$), in line with the increased frequency of oocyte-derived errors.

Complex chromosome abnormality and mosaicism

Young women typically produce very few oocytes affected by multiple aneuploidies, yet the incidence of complex chromosomal abnormality was high in their cleavage stage embryos. This suggests that this phenomenon is largely a consequence of mitotic errors originating in the first few cell divisions following fertilization. The fidelity of mitotic chromosome segregation seems to be unaffected by maternal age. Taking into account the frequency of complex aneuploidy in the oocytes of women of different ages, it can be calculated that 20–25 % of cleavage stage embryos in the current study became highly abnormal as a consequence of mitotic errors, regardless of maternal age.

Previous research has shown that cleavage stage embryos affected by complex aneuploidy are usually highly mosaic, composed of several abnormal cell lines. This is consistent with a mitotic origin for the aneuploidies detected. Such embryos often display chaotic patterns of chromosome segregation and rarely contain any euploid cells (e.g., Delhanty et al. 1997; Wells and Delhanty 2000; Gutierrez-Mateo et al. 2011). The aberrant gene expression caused by the presence of multiple aneuploidies is likely to lead to a lethal collapse in cellular pathways. Even mosaic embryos containing a subpopulation of euploid cells may suffer reduced viability, as the presence of aneuploid cells may disrupt critical cell–cell interactions, leading to a

failure to achieve the reorganization and differentiation necessary for blastocyst formation. Studies of blastocysts and miscarriages have produced data supporting the notion that mosaic embryos have reduced survival rates (Santos et al. 2010; Fragouli et al. 2011a; Lebedev 2011).

Although mosaicism is most prevalent at the cleavage stage, it can still be detected in some blastocyst stage embryos. However, chaotic patterns of chromosome segregation are much less common and the proportion of abnormal cells in mosaic diploid-aneuploid embryos is generally reduced (Santos et al. 2010; Northrop et al. 2010; Fragouli et al. 2011a). This suggests that during the latter stages of preimplantation development, euploid cells have preferential survival rates or undergo mitosis more rapidly, thus coming to dominate the embryo (Sandalinas et al. 2001; Li et al. 2005; Rubio et al. 2007; Fragouli et al. 2011b; Santos et al. 2010). It may be that complex abnormal cells (or the embryos that contain a high proportion of such cells) cease dividing altogether or undergo apoptosis, but this remains to be conclusively demonstrated.

Survival of specific aneuploidies and unique cytogenetic aspects of specific developmental stages

Comparison of the three developmental stages revealed that cytogenetic results from oocytes and blastocysts resembled each other closely, whereas the intermediate, cleavage stage had many peculiarities, distinguishing it from earlier and later stages. Not only did the cleavage stage have a significantly higher incidence of aneuploidy and complex chromosome abnormality than the preceding and following stages, but the spectrum of aneuploidies observed also displayed some unique characteristics.

One distinctive feature of the cleavage stage was a significant increase in the frequency of segmental chromosome abnormalities, in which breakage of DNA strands results in loss or gain of chromosomal fragments. Abnormalities of this type were only seen in 4 % of oocytes, but occurred in three times as many cleavage stage embryos (15 %) ($P < 0.0001$). Segmental abnormalities have previously been reported in cleavage stage embryos (Wells and Delhanty 2000; Voullaire et al. 2000; Vanneste et al. 2009), but relatively little is known about their incidence in oocytes and blastocysts or their ultimate fate. The large increase in the frequency of segmental aneuploidy observed at the cleavage stage during this study coupled with the fact that such abnormalities usually exist in a mosaic form, indicating that most originate during the first few mitotic divisions. It has been proposed that such segmental abnormalities could be forming via a mechanism of chromosome catastrophe or chromothripsis (Liu et al. 2011). This phenomenon was first described during the cytogenetic investigation of cancer cells (Stephens et al. 2011). It was thought to occur via a single event replicative mechanism and could reflect the state of DNA metabolism (Liu et al. 2011). The increase in partial chromosome errors observed at the cleavage stage may be a consequence of depletion of oocyte-derived nucleotide precursors or other resources, during a phase where the embryo has relatively little metabolic capacity, as it has just started expressing its own genome. The frequency of segmental aneuploidy halved at the blastocyst stage suggested that the affected cells/embryos experience reduced viability.

The similarity between the types of aneuploidy recorded in oocytes and blastocysts indicates that mitotic chromosome abnormalities, arising at the cleavage stage, are usually lost before formation of a clinical pregnancy, only rarely contributing to aneuploidies seen later in development. This possibility is concordant with cytogenetic studies examining products of conception (i.e., miscarriages), which have concluded that the vast majority of aneuploidies detected after embryo implantation originate from errors taking place during female meiosis (reviewed in Hassold and Hunt 2001).

All chromosomes were seen to participate in aneuploidy events, although not to the same extent. As previously reported, larger chromosomes tended to be less often affected by malsegregation (Voullaire et al. 2000; Wells and Delhanty 2000; Pellestor et al. 2003; Clouston et al. 2002; Fragouli et al. 2011a, b). In general, the incidence of abnormality for individual chromosomes increased slightly at the cleavage stage, before undergoing a small decline at the blastocyst stage. However, a few types of aneuploidy displayed more dramatic fluctuations in frequency as preimplantation development progressed. Abnormalities involving chromosome 22 and the sex chromosomes demonstrated a more significant increase at the cleavage stage than other chromosomes ($P < 0.05$ and $P < 0.02$, respectively). An increase in sex chromosome abnormalities following fertilization was not unexpected, as unlike most other aneuploidies, a significant proportion of those detected in established pregnancies are known to be of male origin ($\sim$50 %) (Hassold et al. 1992). The reason for the disproportionate increase in aneuploidy involving chromosome 22 at the cleavage stage is not clear. Most instances of trisomy 22 detected during pregnancy or in miscarriages are of maternal origin. This suggests one of two possibilities: (1) The extra chromosome 22 abnormalities are introduced by the sperm, but trisomy 22 is more lethal when of male origin and consequently, pregnancies affected by trisomy 22 tend to be derived from errors occurring in female meiosis and (2) Sperm contribute relatively few chromosome 22 aneuploidies, but the chromosome has an unusually high-risk of mitotic error during the early cleavage divisions.

During progression to the blastocyst stage, aneuploidies involving chromosome 19 declined at a disproportionately rapid rate compared with other chromosomes, indicating that such abnormalities are particularly detrimental even at early developmental stages. This may be related to the large number of genes on chromosome 19 (although small in size, chromosome 19's gene content is almost equal to that of chromosome 1; reviewed in Cremer and Cremer 2001). It may also be that genes important for preimplantation development and/or blastocyst formation are located on that chromosome. Aneuploidy for chromosome 19 is not observed in established pregnancies (Lebedev 2011).

Data from the cytogenetic analysis of products of conception reveal that at least 4–5 % of all clinically recognized pregnancies and ~50 % of all spontaneous abortions carry an autosomal trisomy, whereas monosomies have rarely been observed (Lestou and Kalousek 1998; Hassold and Hunt 2001; Menasha et al. 2005). This situation differs greatly from that seen in the oocytes and embryos assessed during this study, in which approximately half of all aneuploidies involved chromosome loss. The rate of monosomy remained equal to that of trisomy during progression to the blastocyst stage, indicating that embryos are able to tolerate this type of abnormality during the first few days of life. The reason that so many unfamiliar types of aneuploidy, varieties never seen in miscarriages or prenatal samples, can be observed during preimplantation development probably reflects the fact that, during the first 2–3 days of life, the embryonic genome is inactive and cellular processes are still supported by proteins and mRNA transcripts supplied by the oocyte (Braude et al. 1988; Fragouli et al. 2012). However, by the time the blastocyst stage is reached, the embryo has been expressing its own genome for 2–3 days, and there is evidence that some forms of abnormality are beginning to have a negative impact on development. While the frequency of monosomic embryos is unchanged in blastocysts, those affected by chromosome loss tend to achieve poorer morphological scores (a visual measure of developmental competence assigned by embryologists in IVF clinics) than normal or trisomic embryos (Alfarawati et al. 2011). As discussed above, complex aneuploid embryos show reduced survival to the final stage of preimplantation development and those that do succeed in forming blastocysts have an increased likelihood of suboptimal morphology.

Mechanisms leading to aneuploidy

Approximately, 4,500 individual chromosome abnormalities were scored during the course of this study. After completion of both meiotic divisions, a small but significant excess (56 %) of chromosome gains compared to losses (44 %) was predicted in oocytes ($P < 0.006$), leading to a greater risk of trisomy than monosomy in the resulting embryo. During the first meiotic division, abnormalities caused by nondisjunction of homologous chromosomes or premature separation of sister chromatids are expected to lead to an equal frequency of chromosome losses and gains. The excess of gains seen in this study suggests that mechanisms leading to retention of chromosomes in the oocyte, such as anaphase lag and/or congression failure (unsuccessful migration and attachment of chromosomes on the spindle) also occur and contribute significantly to aneuploidies arising during human oogenesis.

Although the cytogenetic analysis of oocytes predicted an excess of trisomic embryos, examination of the cleavage and blastocyst stages actually revealed a slight overrepresentation of monosomy (47 vs. 53 % at the blastocyst stage). This suggests that mitotic chromosome loss, most likely a consequence of anaphase lag (Coonen et al. 2004; Daphnis et al. 2005), is common during the first few cell divisions as well as during female meiosis. Lagging chromosomes, which fail to migrate to one of the spindle poles along with the other chromosomes, tend to be left behind forming separate micronuclei during the following interphase before ultimately being lost altogether. Micronuclei are often observed in human embryos at the cleavage stage, providing further evidence for an appreciable frequency of anaphase lag during early embryogenesis (Munne et al. 1998, 2002; Colls et al. 2007).

Since the first few mitotic divisions rely on mRNA transcripts and proteins provided by the oocyte, it is conceivable that oocyte deficiencies could also have effects during early mitoses. Analyses of embryonic stages provided some evidence in support of this idea, indicating that the effects of advancing female age may not be limited to meiotic fidelity, but might also extend to the mitotic divisions following fertilization. In embryos derived from younger women, there were an equal proportion of chromosome gains and losses; whereas the embryos of older women displayed more losses. The difference was not statistically significant, but warrants further investigation.

The genomic instability observed at the cleavage stage for all patient age groups, including significant levels of chromosome breakage and mitotic chromosome malsegregation, bears a close resemblance to cytogenetic anomalies often described in tumor cells. As with tumors, one of the principal underlying causes is likely to be a deficiency in cell cycle-regulatory mechanisms, which usually act to monitor and maintain accurate chromosome segregation and other aspects of genomic integrity (e.g., cell cycle checkpoints). The significant reduction in aneuploidy seen in blastocysts suggests that after activation of the embryonic genome at the cleavage stage, these mechanisms

become active, reducing the risk of further errors occurring and beginning a process of clearing abnormal cells, especially those affected by multiple aneuploidies.

Conclusions

In conclusion, this investigation demonstrates that chromosome abnormalities are present at a high frequency throughout preimplantation development, a consequence of the accumulated errors occurring during gametogenesis and early mitotic divisions. Advancing female age was associated with increasing aneuploidy rates in oocytes and embryos and was particularly closely linked to the presence of complex chromosomal abnormalities. The excess of chromosome losses seen, especially during female meiosis, suggests that anaphase lag and/or failure of chromosomes to be captured by the microtubules of the spindle (congression failure) are important aneuploidy causing mechanisms during oogenesis and continue to have a significant impact during the first few mitotic divisions. Progression to the final stage of preimplantation development, the blastocyst stage was associated with a decrease in aneuploidy rate, although overall chromosome abnormality rates remained high. Some forms of abnormality (e.g., complex aneuploidy and imbalance affecting chromosome 19) showed particularly large declines in frequency as development progressed, but most aneuploidies had relatively little impact up until the time of blastocyst formation.

Molecular genetic analyses of chromosomal abnormalities occurring in miscarriages have revealed that most of the aneuploidies arise during female meiosis, usually as a consequence of nondisjunction in the first meiotic division (Hassold and Hunt 2001). However, direct observation of female meiotic divisions (via polar body analysis) and early embryonic stages during the current study clearly show that, prior to implantation, a wide range of aneuploidies are present and their origins are much more varied than those seen in later pregnancy. Premature separation of sister chromatids during both female meiotic divisions, aneuploidies arising due to mitotic chromosome malsegregation, and, to a lesser extent, male-meiotic errors are common. The fact that aneuploidies with these origins are rarely seen later in pregnancy raises the possibility that the origin of aneuploidy somehow influences the impact on embryo viability.

Of the many types of aneuploidy recorded during this investigation, including at the final stage of preimplantation development (the blastocyst stage), only a handful have ever been reported in clinical pregnancies. This narrows the potential window of time during which selection against aneuploid embryos occurs, demonstrating that the vast majority of aneuploid human embryos survive preimplantation development and are lost at the time of implantation or shortly thereafter.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Alfarawati S, Fragouli E, Colls P, Stevens J, Gutiérrez-Mateo C, Schoolcraft WB, Katz-Jaffe MG, Wells D (2011) The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. Fertil Steril 95:520–524

Braude P, Bolton V, Moore S (1988) Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature 332:459–461

Carrell DT, Wilcox AL, Lowy L, Peterson CM, Jones KP, Erickson L, Campbell B, Branch DW, Hatasaka HH (2003) Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. Obstet Gynecol 101:1229–1235

Centers for Disease Control and Prevention (2013) 2010 Assisted Reproductive Technology National Summary Report

Christopikou D, Tsorva E, Economou K, Shelley P, Davies S, Mastrominas M, Handyside AH (2013) Polar body analysis by array comparative genomic hybridization accurately predicts aneuploidies of maternal meiotic origin in cleavage stage embryos of women of advanced maternal age. Hum Reprod. [Epub ahead of print] PMID: 23477909 [PubMed—as supplied by publisher]

Clouston HJ, Herbert M, Fenwick J, Murdoch AP, Wolstenholme J (2002) Cytogenetic analysis of human blastocysts. Prenat Diagn 22:1143–1152

Colls P, Escudero T, Cekleniak N, Sadowy S, Cohen J, Munné S (2007) Increased efficiency of preimplantation genetic diagnosis for infertility using "no result rescue". Fertil Steril 88:53–61

Coonen E, Derhaag JG, Dumoulin JC, van Wissen LC, Bras M, Janssen M, Evers JL, Geraedts JP (2004) Anaphase lagging mainly explains chromosomal mosaicism in human preimplantation embryos. Hum Reprod 19:316–324

Cremer T, Cremer C (2001) Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2:292–301

Daphnis DD, Delhanty JD, Jerkovic S, Geyer J, Craft I, Harper JC (2005) Detailed FISH analysis of day 5 human embryos reveals the mechanisms leading to mosaic aneuploidy. Hum Reprod 20:129–137

Delhanty JD, Harper JC, Ao A, Handyside AH, Winston RM (1997) Multicolour FISH detects frequent chromosomal mosaicism and chaotic division in normal preimplantation embryos from fertile patients. Hum Genet 99:755–760

Fragouli E, Katz-Jaffe M, Alfarawati S, Stevens J, Colls P, Goodall NN, Tormasi S, Gutierrez-Mateo C, Prates R, Schoolcraft WB, Munne S, Wells D (2010) Comprehensive chromosome screening of polar bodies and blastocysts from couples experiencing repeated implantation failure. Fertil Steril 94:875–887

Fragouli E, Alfarawati S, Daphnis DD, Goodall NN, Mania A, Griffiths T, Gordon A, Wells D (2011a) Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. Hum Reprod 26:480–490

Fragouli E, Alfarawati S, Goodall NN, Sanchez–Garcıa JF, Colls P, Wells D (2011b) The cytogenetics of polar bodies: insights into female meiosis and the diagnosis of aneuploidy. Mol Hum Reprod 5:286–295

Fragouli E, Wells D, Iager AE, Kayisli UA, Patrizio P (2012) Alteration of gene expression in human cumulus cells as a potential indicator of oocyte aneuploidy. Hum Reprod 27:2559–2568

Gabriel AS, Thornhill AR, Ottolini CS, Gordon A, Brown AP, Taylor J, Bennett K, Handyside A, Griffin DK (2011) 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 48:433–437

Gutierrez-Mateo C, Colls P, Sanchez-Garcıa J, Escudero T, Prates R, Ketterson K, Wells D, Munne S (2011) Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. Fertil Steril 95:953–958

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

Hassold T, Hunt P (2001) To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2:280–291

Hassold T, Pettay D, Robinson A, Uchida I (1992) Molecular studies of parental origin and mosaicism in 45, X conceptuses. Hum Genet 89:647–652

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

Kuliev A, Cieslak J, Ilkevitch Y, Verlinsky Y (2003) Chromosomal abnormalities in a series of 6,733 human oocytes in preimplantation diagnosis for age-related aneuploidies. RBM Online 6:54–59

Lebedev I (2011) Mosaic aneuploidy in early fetal losses. Cytogenet Genome Res 133:169–183

Lestou VS, Kalousek DK (1998) Confined placental mosaicism and intrauterine fetal growth. Arch Dis Child Fetal Neonatal Ed 79:223–226

Li M, DeUgarte CM, Surrey M, Danzer H, DeCherney A, Hill DL (2005) Fluorescence in situ hybridization reanalysis of day–6 human blastocysts diagnosed with aneuploidy on day 3. Fertil Steril 84:1395–1400

Liu P, Erez A, Nagamani SCS, Dhar SU, Kołodziejska KE, Dharmadhikari AV, Cooper ML, Wiszniewska J, Zhang F, Withers MA, Bacino CA, Campos-Acevedo LD, Delgado MR, Freedenberg D, Garnica A, Grebe TA, Hernandez-Almaguer D, Immken LD, Lalani SR, McLean SD, Northrup H, Scaglia F, Strathearn L, Trapane P, Kang SHL, Patel A, Cheung SW, Hastings PJ, Stankiewicz P, Lupski JR, Bi W (2011) Chromosome catastrophes involve replication mechanisms generating complex genomic rearrangements. Cell 146:889–903

Magli MC, Montag M, Köster M, Muzi L, Geraedts J, Collins J, Goossens V, Handyside AH, Harper J, Repping S, Schmutzler A, Vesela K, Gianaroli L (2011) Polar body array CGH for prediction of the status of the corresponding oocyte. Part II: technical aspects. Hum Reprod 26:3181–3185

Mantzouratou A, Mania A, Fragouli E, Xanthopoulou L, Tashkandi S, Fordham K, Ranieri DM, Doshi A, Nuttall S, Harper JC, Serhal P, Delhanty JD (2007) Variable aneuploidy mechanisms in embryos from couples with poor reproductive histories undergoing preimplantation genetic screening. Hum Reprod 22:1844–1853

Menasha J, Levy B, Hirschhorn K, Kardon NB (2005) Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. Genet Med 7:251–263

Mertzanidou A, Spits C, Nguyen HT, Van de Velde H, Sermon K (2013) Evolution of aneuploidy up to Day 4 of human preimplantation development. Hum Reprod. [Epub ahead of print] PMID: 23526301 [PubMed—as supplied by publisher]

Munne S, Marquez C, Magli C, Morton P, Morrison L (1998) Scoring criteria for preimplantation genetic diagnosis of numerical abnormalities for chromosomes X, Y, 13, 16, 18 and 21. Mol Hum Reprod 4:863–870

Munné S, Sandalinas M, Escudero T, Márquez C, Cohen J (2002) Chromosome mosaicism in cleavage-stage human embryos: evidence of a maternal age effect. RBM Online 4:223–232

Northrop LE, Treff NR, Levy B, Scott RT (2010) 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 16:590–600

Pellestor F, Andreo B, Arnal F, Humaeu C, Demaille J (2003) Maternal ageing and chromosomal abnormalities: new data drawn from in vitro unfertilized human oocytes. Hum Genet 112:195–203

Petit FM, Frydman N, Benkhalifa M, Le Du A, Aboura A, Fanchin R, Frydman R, Tachdjian G (2005) Could sperm aneuploidy rate determination be used as a predictive test before intracytoplasmic sperm injection? J Androl 26:235–241

Platteau P, Staessen C, Michiels A, Van Steirteghem A, Liebaers I, Devroey P (2005) Preimplantation genetic diagnosis for aneuploidy screening in women older than 37 years. Fertil Steril 84:319–324

Rubio C, Rodrigo L, Mercader A, Mateu E, Buendía P, Pehlivan T, Viloria T, De los Santos MJ, Simón C, Remohí J, Pellicer A (2007) Impact of chromosomal abnormalities on preimplantation embryo development. Prenat Diagn 27:748–756

Sandalinas M, Sadowy S, Alikani M, Calderon G, Cohen J, Munne S (2001) Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage. Hum Reprod 16:1954–1958

Santos MA, Teklenburg G, Macklon NS, Van Opstal D, Schuring-Blom GH, Krijtenburg PJ, de Vreeden-Elbertse J, Fauser BC, Baart EB (2010) The fate of the mosaic embryo: chromosomal constitution and development of Day 4, 5 and 8 human embryos. Hum Reprod 25:1916–1926

Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, Pleasance ED, Lau KW, Beare D, Stebbings LA, McLaren S, Lin ML, McBride DJ, Varela I, Nik-Zainal S, Leroy C, Jia M, Menzies A, Butler AP, Teague JW, Quail MA, Burton J, Swerdlow H, Carter NP, Morsberger LA, Iacobuzio-Donahue C, Follows GA, Green AR, Flanagan AM, Stratton MR, Futreal PA, Campbell PJ (2011) Massive genomic rearrangement acquired in a single catastrophic event during cancer development. Cell 144:27–40

Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F, Fryns JP, Verbeke G, D'Hooghe T, Moreau Y, Vermeesch JR (2009) Chromosome instability is common in human cleavage-stage embryos. Nat Med 15:577–583

Verpoest W, Fauser BC, Papanikolaou E, Staessen C, Van Landuyt L, Donoso P, Tournaye H, Liebaers I, Devroey P (2008) Chromosomal aneuploidy in embryos conceived with unstimulated cycle IVF. Hum Reprod 10:2369–2371

Voullaire L, Slater H, Williamson R, Wilton L (2000) Chromosome analysis of blastomeres from human embryos by using comparative genomic hybridization. Hum Genet 106:210–217

Wells D, Delhanty JD (2000) Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. Mol Hum Reprod 6:1055–1062