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First-Meiotic-Division Nondisjunction in Human Oocytes

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Summary

Reject oocytes from in vitro–fertilization patients are currently the only practical source of human oocyte material available for meiotic studies in women. Two hundred clearly analyzable second meiotic (MII) metaphase oocytes from 116 patients were examined for evidence of first meiotic (MI) division errors. The chromosome results, in which 67% of oocytes had a normal 23,X chromosome complement but none had an extra *whole* chromosome, cast doubt on the relevance, to human oocytes, of those theories of nondisjunction that propose that both chromosomes of the bivalent fail to disjoin at MI so that both move to one pole and result in an additional whole chromosome at MII metaphase. The only class of abnormality found in the MII oocytes had single chromatids (half-chromosomes) replacing whole chromosomes. Analysis of the chromosomally abnormal oocytes revealed an extremely close correlation with data on trisomies in spontaneous abortions, with respect to chromosome distribution, frequency, and maternal age, and indicated the likelihood of the chromatid abnormalities being the MI-division nondisjunction products that lead to trisomy formation after fertilization. The most likely derivation of the abnormalities is through a form of misdivision process usually associated with univalents, in which the centromeres divide precociously at MI, instead of MII, division. In the light of recent data that show that altered recombination patterns of the affected chromosomes are a key feature of most MI-division trisomies, the oocyte data imply that the vulnerable meiotic configurations arising from altered recombination patterns are processed as functional univalents in older women. Preliminary evidence from MI-metaphase oocytes supports this view.

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Introduction

Trisomy is the most commonly occurring chromosome abnormality in the human species, arising with a frequency of ⩾4% in all human conceptions (Hassold and Jacobs 1984). Because of its close association with increased maternal age, it has always been recognized as a predominantly maternal meiotic "nondisjunction" error, although, despite much speculation, the exact nature of the chromosomal malsegregation remains unclear. Over the previous decades, studies of chromosome segregation in many species throughout the plant and animal kingdom (Darlington 1937; White 1954) have shown that meiotic factors that predispose to nondisjunction are related to disturbances of normal chromosome pairing and genetic recombination, failure to maintain pairing between homologous chromosome segments prior to first meiotic (MI) division, and disturbances of chiasma formation. In addition, disturbed functioning of the motor apparatus of the centromeres and spindles is known to prevent regular chromosome separation (Polani 1981).

Until the recent advent of in vitro fertilization (IVF) technology, human oocytes were inaccessible for meiotic studies. Consequently, indirect analysis using cytogenetic and molecular markers in the affected chromosomes of human trisomy families was applied to several of the more common trisomies: Sherman et al. (1991) showed that the origin of the meiotic error in trisomy 21 is predominantly at maternal MI division, with a small proportion arising at second meiotic (MII) division and a very small number being of paternal or mitotic origin. Yoon et al. (1996) demonstrated that the maternal age effect, known to occur in trisomy 21s of MI-division origin, is even more pronounced in those of MII-division origin. In addition, the affected chromosomes of MI-divison origin have a significant overall reduction in frequency of recombination at the proximal end of the chromosome (Sherman et al. 1994) but have a significant excess of recombination when they are of MII-division origin (Lamb et al. 1996). Chromosome 16 trisomies (Hassold et al. 1995) all arise at MI division, are maternal age dependant, and also show a reduction in recombination frequency in the pericentric chromosome region. Chromosome 15 uniparental disomies are predominantly MI-divison nondisjunction er-

rors (Robinson et al. 1993) and have reduced recombination frequencies in the affected chromosomes. Trisomy 18s are predominantly MII-division errors, but those of MI-division origin also have a proportion of nullisomic cases (Fisher et al. 1994).

In order to explain the maternal age effect in relation to the reduced recombination frequencies found in families trisomic for 16, Hassold et al. (1995) suggested that the reduced recombination patterns formed stable configurations at MI division in younger women but that, with the passage of time, they would not necessarily result in configurations that could be processed stably through meiosis. The complex situation in trisomy 21 families of MII origin that show an extreme maternal age effect and excessive pericentromeric recombination has led to the recent hypothesis (Lamb et al. 1996) that the MII error is a direct consequence of a MI error in which entanglement at MI division, leading to "true" nondisjunction, is followed by subsequent reduction division at MII.

As a result of IVF technology, meiosis can now be studied directly in human oocytes. Our observations (Angell 1991; Angell et al. 1994) of chromosome abnormalities in human oocytes at MII metaphase have revealed an unexpected class of chromosome abnormalities, which manifest as single chromatids as opposed to whole chromosomes in MII-metaphase oocytes. The observations are incompatible with both the classic model of nondisjunction, in which whole bivalents fail to disjoin at MI division (Reiger et al. 1968), and the hypothesis of Lamb et al. (1996), outlined above. The abnormalities are assumed to have arisen through precocious division of the chromosome components of the bivalents at MI, instead of MII, division, a mechanism well known to arise in univalents in many species and hybrids throughout the plant and animal kingdom (see White 1954). On the basis of the oocyte observations, the implication for MI nondisjunction is that the meiotic configurations that are a consequence of reduced recombination patterns found in trisomies emerge from the long dictyate stage in older women, as unstable meiotic configurations that are consequently processed as univalents. Therefore, they divide precociously at MI instead of MII.

This paper is based on observations of 200 oocytes and incorporates the previous 100 observations reported by Angell et al. (1994). The results show the extremely close correlation, with respect to frequency, distribution, and maternal age, between the oocyte data and data on trisomies from the extensive cytogenetic surveys performed on spontaneous abortions and the live newborn. In addition, data are presented on bivalents and univalents in oocytes at MI metaphase, with particular emphasis on chromosome 16. These oocytes are all from women in the latter half of their reproductive life, and the bivalents show morphological differences compatible with the kind of decreased stability that, with increasing age, could lead to univalent behavior at MI division.

Material and Methods

The MII oocytes used in the study were those that had failed to fertilize after in vitro insemination and came from patients attending the Edinburgh Assisted Conception Unit for treatment of their infertility. The total culture time of the oocytes prior to fixation varied from 30 h to 72 h, according to the number of oocytes retrieved from the patients, the majority being fixed at ~70 h. Each oocyte was processed individually on a slide, according to a modification (Angell et al. 1993) of the three-stage fixation technique of Mikamo and Kamiguchi (1983).

The aim of the study was to understand the nature of chromosome abnormalities present in MII-metaphase oocytes that had arisen through MI-division nondisjunction. Our previous observations on cytogenetic abnormalities in reject oocytes from IVF patients showed that, on average, only one-third of all the oocytes studied cytogenetically qualified for this study. Grounds for exclusion have been discussed in detail elsewhere (Angell et al. 1993). They included (1) polyploid oocytes, (2) those with insufficiently clear morphology to enable classification into the basic chromosome groups A–G, (3) excessive separation of chromatids at the centromeric region of the chromosomes, and (4) chromosome or chromatid breaks in any of the chromosomes. More than 600 consecutive reject oocytes were examined cytogenetically, and the 200 discussed below are those which met the criteria for inclusion, by having a clearly analyzable set of "healthy" haploid MII chromosomes.

As the study progressed, it became increasing clear that a previously undescribed class of chromosome abnormality in human meiotic chromosomes was present. Therefore, emphasis was placed on detailed and repeated morphological examination of the abnormalities, by use of permanent preparations that had been stained with Geimsa, rather than by sacrifice of material for chromosome identification by FISH. The only abnormalities specifically identified were those of chromosome 16 origin, because of their obvious relevance to studies of trisomy formation. Methyl-green/DAPI (Sumner 1990) was selected for identification instead of FISH, because it also provided information on centromeric polymorphisms.

Preparation of MI-metaphase chromosomes have been described in detail by Angell (1995). Data presented below show the specific staining of chromosomes

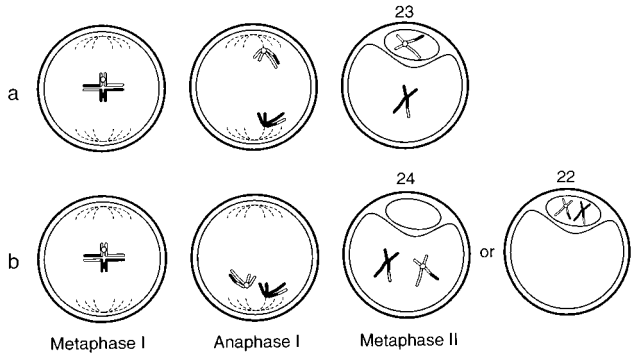

Figure 1 *a,* Normal chromosome disjunction at MI division. *b,* Nondisjunction at MI division, according to the classic model.

1, 9, and 16 in the bivalents in two preparations (donor 91/39 [age 28.2 years] and donor 91/34 [age 31.4 years) selected at random from the population described by Angell (1995). Informed consent from the patients who donated their oocytes, as well as ethical permission from the local ethical committee, was obtained in all cases.

Results

Chromosome Analysis

As expected, the majority of the 200 oocytes had a normal 23,X MII-metaphase complement, presumably having undergone normal disjunction at MI metaphase (fig. 1*a*). Since the mean age of the patients seeking treatment for their infertility was 33.7 years, considerably older than the mean age of women conceiving in the general population (26.2 years; Scottish Health Service, 1992, personal communication), it was expected that $\geqslant 8\%$ of the oocytes would have undergone MI-division nondisjunction and, according to the classic model of nondisjunction, would have 24 or 22 chromosomes at MII (fig. 1*b*). However, none were found to have 24 whole chromosomes, and five oocytes with 22 chromosomes were presumed to have suffered chromosome loss during fixation. Sixty-one of the oocytes had an abnormal and previously unrecognized class of chromosome abnormality in which a half-chromosome (or single chromatid) replaced a whole chromosome. The analysis (table 1) revealed three subgroups:

1. $23+{}^1/_2$: Twenty-three whole chromosomes plus one extra chromatid (half chromosome) (see fig. 2).
2. $22+{}^1/_2$: Twenty-two whole chromosomes plus one single chromatid (see fig. 3).
3. $22+{}^1/_2+{}^1/_2$: Twenty-two whole chromosomes plus two single chromatids (see figs. 4 and 5).

The last subgroup ($22+{}^1/_2+{}^1/_2$) was present in 30 of the oocytes, whereas there were only 5 $23+{}^1/_2$ oocytes and 6 $22+{}^1/_2$ oocytes. In addition, 11 oocytes had as many as four chromosomes expressing one of the three types of abnormality, and 9 oocytes had more than four such half-chromosomes; analysis was not attempted in the latter 9 oocytes. Figures 2–5 show the designated descriptions (Mittelman 1995) of the meiotic abnormalities.

Eleven of the abnormal oocytes had sibling oocytes with a normal 23,X chromosome complement. One remarkable patient 32.9 years of age had all four analyzable oocytes with abnormalities. The six other patients with two or more chromosomally abnormal oocytes were all >34.9 years of age.

Chromosome Frequency

The outstanding feature of the distribution of chromosome abnormalities was the excess of oocytes with E group (chromosomes 16–18) chromatid abnormalities ($n = 20$). Of 10 examined with methyl-green/DAPI, all but 1 (fig. 2) were identified as chromosome 16. Next most frequent was the G group (chromosomes 21 and 22) ($n = 10$), followed by C group (chromosomes X and 6–12) ($n = 5$), D group (chromosomes 13–15) ($n = 5$), and then F group (chromosomes 19 and 20) ($n = 1$). There were no abnormalitites from the A group (chromosomes 1–3) or B group (chromosomes 4 and 5). Oocytes with two or more chromosomes represented by chromatids had a different distribution, those in the G group being most frequent ($n = 10$), followed by the D group ($n = 5$) and E group ($n = 5$), C group ($n = 4$), and F group ($n = 3$).

Patient Age

The age of the patients in relation to their chromosome constitution is shown in table 1. Despite the fact that the mean age of women receiving IVF treatment for their infertility was 33.7 years, there were clear significant differences in relation to chromosome complement. The mean age of the 81 patients with 23,X oocytes was 31.8 (SD 3.0) years, and that of the 41 patients with only one abnormal chromosome was 33.1 (SD 3.6) years; these ages were significantly different ($P = .04$). If this latter group was subdivided, then the mean age of the 19 patients with a presumed chromosome 16 abnormality—32.0 (SD 3.9) years—was not significantly different from that of those with chromosomally normal oocytes, but it was significantly different ($P = .05$) from that of the other 22 patients, who had non–chromosome 16 abnormalities, whose mean age was 34.1 (SD 4.1) years. The mean age, 38.4 (SD 3.4) years, of the 11 patients bearing oocytes with more than one abnormal chromosome was highly significantly greater ($P > .00001$) than that of those with normal oocytes. The mean age of all 51 patients with oocytes with chromatid abnormalities was 34.2 (SD 4.1)

Table 1

Chromatid Abnormalities in MII-Metaphase Oocytes

Chromatid Class and Patient	Chromatid Constitution[a]	Maternal Age (years)
23+½:		
2698	23,X,+Dcht	34.9
2498	23,X,+Echt	31.6
3120	23,X,+Echt (17 or 18)	32.9
4454	23,X,+Echt	33.9
2566	23,X,+Gcht	33.0
22+½:		
4903	22,X,+Echt	25.5
3474	22,X,+Echt	29.7
4808	22,X,+Echt (16)	32.7
2698	22,X,+Echt	34.9
4394	22,X,+Gcht	33.9
2162	22,X,+Gcht	34.1
22+½+½:		
3673	22,+Ccht,+Ccht	28.0
3120	22,+Ccht,+Ccht	32.9
3954	22,+Ccht,+Ccht	34.8
2878	22,+Ccht,+Ccht	36.2
4504	22,+Ccht,+Ccht	38.3
3330	22,X,+Dcht,+Dcht	32.3
4589	22,X,+Dcht,+Dcht	35.6
4854	22,X,+Dcht,+Dcht	36.6
4544	22,X,+Dcht,+Dcht	39.5
4205	22,X,+Echt,+Echt	25.0
6430	22,X,+Echt,+Echt (16)	26.8
5435	22,X,+Echt,+Echt (16)	28.2
3026	22,X,+Echt,+Echt	29.1
1673	22,X,+Echt,+Echt (16)	31.0
2932	22,X,+Echt,+Echt (16)	31.5
2755	22,X,+Echt,+Echt (16)	32.0
4285	22,X,+Echt,+Echt (16)	33.1
3515	22,X,+Echt,+Echt (16)	35.2
2660	22,X,+Echt,+Echt	36.3
6000	22,X,+Echt,+Echt (16)	36.4
4483	22,X,+Echt,+Echt	37.5
6624	22,X,+Echt,+Echt	37.7
3954	22,X,+Fcht,+Fcht	34.4
3296	22,X,+Gcht,+Gcht	28.7
1686	22,X,+Gcht,+Gcht	32.0
4709	22,X,+Gcht,+Gcht	32.2
4630	22,X,+Gcht,+Gcht	32.2
6431	22,X,+Gcht,+Gcht	33.5
4255	22,X,+Gcht,+Gcht	34.5
4872	22,X,+Gcht,+Gcht	41.1
Multiples ≤4:		
4166	22,X,+3Cchts	40.4
3120	21,X,+2Echts,+2Gchts	32.9
3120	21,+2Cchts,+Gcht	32.9
4680	21,X,+4Gchts	33.0
4695	21,X,+2Echts,+2Dchts	37.1
4579	21,X,+2Gchts,+2Dchts	39.4
5480	21,X,+4Gchts	39.6
2660	20,+2Gchts,+2Echts,+2Cchts	36.3
4166	20,X,+2Gchts,+2Fchts,+2Dchts	40.4
4608	20,X,+2Fchts,+2Dchts,+2Echts	41.8
4579	19,X,+2Gchts,+2Fchts,+2Echts,+2Dchts	39.4

(*continued*)

Table 1 (continued)

Chromatid Class and Patient	Chromatid Constitution[a]	Maternal Age (years)
Multiples >4:		
4608	16,chroms+chts	41.8
4608	14,chroms+chts	41.8
3284	18,chroms+chts	38.3
9611	23,chroms+5chts	34.5
4411	16,chroms+chts	43.9
4411	12,chroms+chts	43.9
4411	10,chroms+chts	43.9
4411	5,chroms+chts	43.9
4411	5,chroms+chts	43.9

[a] cht = chromatid; and chroms = chromosomes.

years, also significantly different ($P = .0001$) from that of the 23,X group.

MI-Metaphase Oocytes

Figure 6 shows the bivalent appearance of chromosomes 16, 9, and 1 at MI metaphase in oocytes retrieved from patients 28.2 and 31.4 years of age. The large bivalents—for example, chromosome 1—have interlocking chiasma, in contrast to some of the smaller chromosomes, including chromosome 16, which show secondary association of the bivalent components, with no clear evidence of chiasma.

Discussion

Interpretation of Results

Although far from being an optimal source, reject oocytes from IVF patients are currently the only source of oocytes available for study on a population basis. Rigorous selection criteria were used to remove those

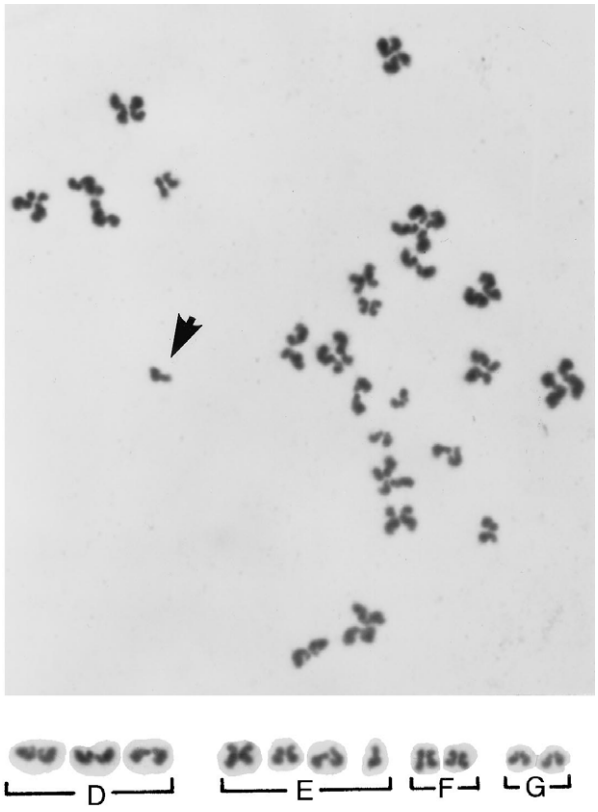

Figure 2 23+½ MII oocyte (patient 3120), designated as MII,23,X,+cht(17–18). Methyl-green/DAPI showed that the extra chromatid was not of chromosome 16 origin.

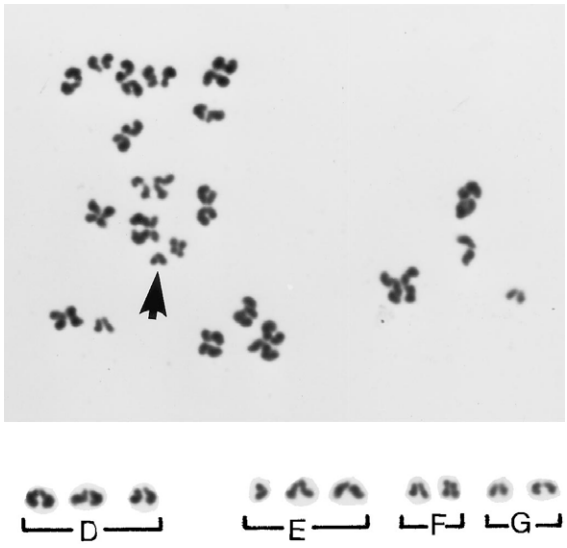

Figure 3 22+½ MII oocyte (patient 4808), designated as MII,22,X,−16,+16cht. Chromatid 16 is identified by methyl-green/DAPI staining.

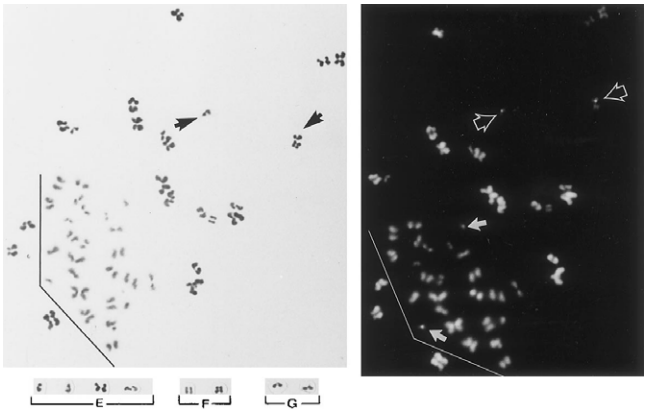

Figure 4 22+½+½ MII oocyte (patient 4483), designated as MII,22,X,−16,+16cht+16cht. Geimsa staining on the left shows morphology of chromosome 16 chromatids in MII (patient 4483). Methyl-green/DAPI staining on the right is shown by arrowheads pointing to heteromorphic brightly staining centromeric regions of chromosome 16 chromatids in MII (*black arrowheads with white outline*) and first polar body (*white arrowheads*). Heteromorphic differences in the centromeric regions of both chromatids are suggested.

which were inevitably degenerate or otherwise aberrant, and, since the final criterion for inclusion depended on the presence of a clearly analyzable metaphase plate with "healthy" chromosomes, there is no reason why this should introduce any bias in the final selection of the 200 metaphases. Time in culture was not considered to have any impact on the MII-chromosome results. For clinical reasons, the majority of oocytes were fixed at ~70 h after aspiration. Nevertheless, the single-chromatid abnormalities were found in a similar frequency in those few oocytes fixed at 30 h and 48 h and even in one oocyte from a separate survey (Angell 1995) fixed at 0 h. Clearly, a "control" population of oocytes is not feasible.

The results confirm previously published observations (Angell 1991; Angell et al. 1994) that none of the MII-metaphase oocytes have the 24 *whole* chromosomes that would have been expected if the classic mechanism of nondisjunction operated at MI division in human oocytes. The only chromosome abnormalities compatible with MI-division errors were single chromatids that must have arisen through an atypical but frequently observed form of chromosome misdivision that arises when univalent chromosomes or unstable bivalents divide precociously at MI division instead of at MII division. Detailed studies of many different species throughout the plant and animal kingdom—particularly of hybrids (Wilson 1925; Darlington 1937; White 1954)—and, recently, elegant studies of the XO mouse (Hunt et al. 1995) show that, at MI anaphase, if univalents are lying far away from the equator, they will be included with the bivalents and pass to one or other of the two poles

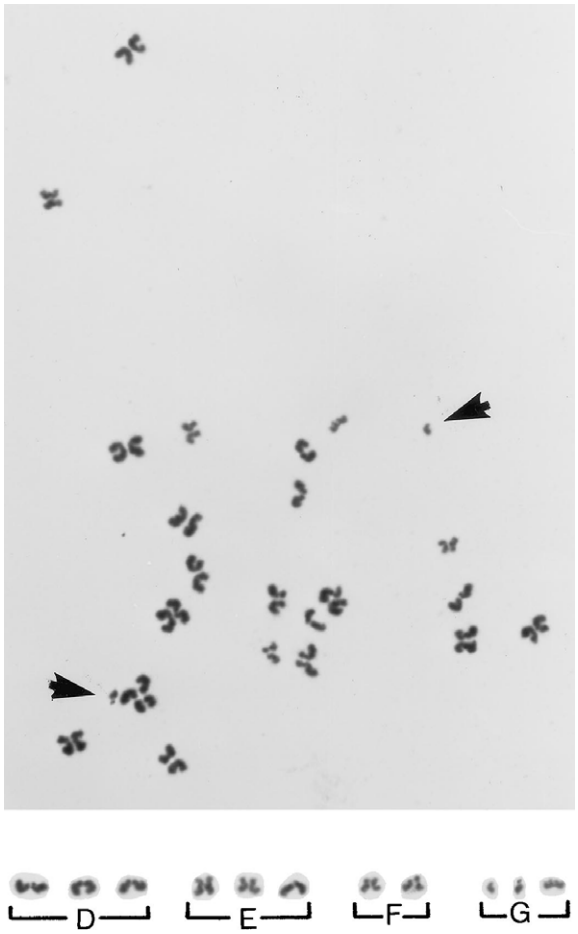

Figure 5 22+½+½MII oocyte (patient 6431), designated as MII,22,X,−G,+Gcht,+Gcht. Heteromorphic differences in satellited regions of the two chromatids may be present.

(fig. 7*a* and *b*). If lying near the equator, they will move onto the plate, orientate themselves axially, and divide, at MI instead of at MII, into their two chromatids, which then pass to opposite poles. At MII division, the only

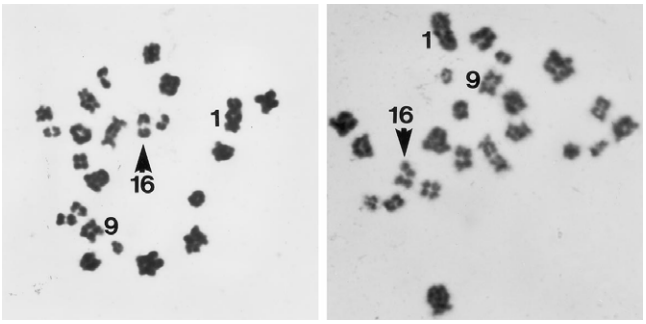

Figure 6 MI oocytes from a 28.2-year-old (*left*) and a 31.4-year-old (*right*). Chromosomes 1, 9, and 16 are identified by methyl-green/DAPI staining.

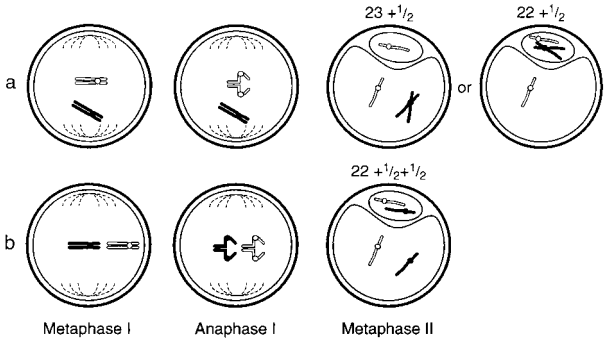

Figure 7 *a*, Origin of 23+½ and 22+½ MII oocytes, through precocious division of one univalent at the MI division. *b*, Origin of 22+½+½ MII oocytes, through precocious division of both univalents.

option is for each chromatid to move, at random, to either pole. Consequently, in human oocytes, one-half of the 23+½ oocytes and one-quarter of the 22+½+½ oocytes will be hyperhaploid gametes and will become trisomic after fertilization. If this mechanism is to explain the common human trisomies of MI-division origin, then, first, oocyte and trisomy data must concord, and, second, there must be evidence in favor of univalents at MI metaphase.

Correlation between Trisomy and Oocyte Data

a. Distribution and frequency.—Chromatid abnormalities in the oocytes showed a distribution pattern and frequency that were remarkably similar to those found in trisomies, chromosome 16 being by far the most frequent, followed by chromosomes 21 and 22 and chromosomes 13-15. The overall frequency of potential single trisomies after fertilization (calculated on the basis of 5 22+½ oocytes, 30 22+½+½ oocytes, and 6 oocytes with multiples) was ~7%, higher than the 4%, at conception, estimated from the spontaneous-abortion data. The potential frequency of trisomy 16 after fertilization was 2.125%, also slightly higher than the estimated trisomy 16 frequency of 1.33% at conception, which also was based on spontaneous-abortion data (Hassold and Jacobs 1984). However, presumed loss of trisomy conceptions at subsequent stages of development could certainly account for the difference between the two sets of data.

Data from spontaneous abortions show that doubly trisomic individuals are more frequently trisomic for chromosome 21 than for chromosome 16 (Warburton et al. 1980), a fact attributed to different survival capabilities of chromosomally abnormal conceptions. The oocyte data present a similar situation, in which oocytes with multiple chromatid abnormalities have chromosomes 21 and 22 represented more frequently than those in the chromosome 16–18 group. In this situation, the change in pattern may reflect a basic difference in sequence of occurrence of misdivision events, those which involve chromosome 16 arising well in advance of those involving the smaller chromosomes, followed in turn by any others in the complement.

b. Maternal age.—Despite the advanced age of the patients receiving infertility treatment, there was a significant increase in the ages of those bearing oocytes with chromatid abnormalities, compared with those with chromosomally normal oocytes. Of greatest interest was the fact that the mean age of the patients with chromatid abnormalities for chromosome 16 was significantly younger than that for those bearing abnormalities for other chromosomes. This parallels the data from spontaneous abortions in which the maternal age of trisomy 16 individuals is significantly less than that for individuals with trisomies of the other small chromosomes (Hassold et al. 1984; Risch et al. 1986). A further close parallel with the spontaneous-abortion data was the highly significant increase in both the age of patients bearing an oocyte with more than one chromosome with a chromatid error and the age of women bearing doubly trisomic conceptions (Hassold and Jacobs 1984; Risch et al. 1986).

These extremely close correlations between trisomy and oocyte data, with respect to chromosome distribution, frequency, and maternal/patient age, argue strongly that the chromatid errors found in MII-metaphase oocytes are the nondisjunction products that lead to trisomies after fertilization. However, the data are at variance with other cytogenetic studies of oocytes, in the literature reviewed by Jacobs (1992), in which hyperhaploidy involving an extra whole chromosome has been claimed, although it has not been substantiated by convincing photographic evidence. Although Kamiguchi et al. (1993) reported chromatid abnormalities identical to the 23+½ and 22+½ abnormalities described above, they also found that 4.3% of the oocytes were hyperhaploid, with an extra whole chromosome, which they illustrated in four clear karyotypes. On theoretical grounds, oocytes with an extra whole chromosome could be expected in some MII-metaphase oocytes, but the facts that there were none in our population of 200 oocytes and that other unequivocal evidence for their presence in the literature is so small suggest that they must form a very small proportion of MI-division nondisjunction abnormalities.

Kamiguchi et al. (1993) also described metaphases equivalent to our 22+½+½ abnormalities, which they believed were in vitro–degenerative effects. Munné et al. (1995) and Dailey et al. (1996) also suggested that our 22+½+½ abnormalities must be artifacts of in vitro culture and specifically related to time in culture. How-

ever, there are several clear differences between the "balanced predivision" 22+½+½ abnormalities described by Dailey et al. and the 22+½+½ abnormalities in the MII metaphases that I have described. First, the balanced predivision abnormalities described by Dailey et al. are in vitro effects found predominantly in the first polar body, whereas the 22+½+½ abnormalities in the present study are found in both the MII metaphase and the first polar body, at the same time (see fig. 4). Second, at the genetic level the two situations are quite different: if MII metaphases were to have single chromatids arising through in vitro culture, they would be genetically identical, whereas those that have arisen as a consequence of MI misdivision will show heteromorphic differences, where present. Examples of heteromorphic differences are suggested in the satellited regions of the chromosomes 21 and 22 chromatids shown in figure 5 and also in the size differences of the heteromorphic regions of the chromatids for chromosome 16, as revealed by methyl-green/DAPI staining, both in the MII metaphase and polar-body chromosomes, shown in figure 4. Third, 71% of the chromosome preparations by Dailey et al. were analyzed, in contrast to the present study, in which only 30% could be unequivocally analyzed. Therefore, the discrepancies between the two sets of data may lie in the fact that many more oocytes were excluded (as summarized in Material and Methods) in the present study. In particular, oocytes were excluded if they either showed excessive separation of the chromatids at the centromere or had chromosome or chromatid breaks at the centromere, either of which could give anomalous FISH results unless parallel studies to determine the exact morphology of the chromosomes were performed.

The Case for Univalents at MI Metaphase

The data presented above argue that, as a woman ages, the vulnerable chromosomes must be processed, at MI division, as univalents and not as bivalents. However, recombination studies show that only a small proportion of MI-division trisomies are nonrecombinants, whereas the vast majority have at least one crossover event. The key feature, then, of the recombination data, in relation to aging, must lie in the different pattern of recombination in the affected chromosomes in trisomies in which, in the affected chromosomes in trisomy 16 (Hassold et al. 1995), recombination is normal at the distal chromosome end but is absent at the proximal end. This absence of an anchoring recombinant in the pericentromeric region of the chromosome is what creates meiotic configurations that emerge from the prolonged dictyate stage prior to ovulation, to function as univalents instead of bivalents. The question, then, must be, Is there visible evidence of functional univalents at MI metaphase?

Direct observations of meiotic configurations and chiasma in MI-metaphase chromosomes, in relation to age, in the mouse (Henderson and Edwards 1968; Luthardt et al.1973; Polani and Jagiello 1976) and Chinese hamster (Sugawara and Mikamo 1983) show a decrease in chiasma frequency and an increase in univalent frequency with increasing maternal age. However, Speed (1977) drew attention to the more contracted state of bivalents in older mice and suggested that both the apparent increase in the numbers of univalents seen at MI metaphase and the decrease in chiasma counts were attributable to this morphological change of the bivalents with age.

Equivalent material in humans can only be obtained at the time of gynecological intervention, and, consequently, the small amount of data available is all from women in the latter half of their reproductive life, and data from young women are extremely scarce and limited to one case (Polani et al. 1982). Estimates of chiasma frequency have varied from 32 to 55 (average 43.7) (Jagiello et al. 1976) and are lower than the mean of 50.6 in the male (Hulten 1974). The accuracy of chiamsa counts in MI-metaphase oocytes in older women has been queried (Angell 1995) in view of the contracted, fuzzy appearance of the bivalents even in material retrieved under optimum conditions. However, univalents can be observed with greater accuracy, and their frequency has been shown to correlate with increasing age. Homologous univalents lying close together in secondary association are regularly seen in human MI-metaphase material, but there are insufficient data to show any correlation with increasing maternal age (Angell 1995), although such an association has been established in the mouse (Henderson and Edwards 1968). The two MI metaphases in figure 6 show several of the smaller chromosomes—chromosome 16 in particular—as secondarily associated univalents.

The available data on MI-metaphase chromosomes from human oocytes from older patients contrasts dramatically with the one available pieces of data on an MI metaphase from a young woman. This remarkable oocyte from a 14-year-old Down syndrome girl (Polani et al. 1982) provided a preparation that had uncontracted and clear bivalents without true or secondarily associated univalents, and it was possible to count 71 or 72 chiasma. Since young girls rarely require relevant gynecological operations, no further data are available from this age group. However, this anecdotal case, together with the data from older women, suggests that, as in the mouse (Speed 1977), MI-metaphase chromosomes in oocytes from young women are long and extended but, with increasing age, become contracted, have less clarity, and are often seen as secondarily associated univalents. Since chromosome 16 is recognized as

a model for the study of trisomy formation in relation to maternal age (Hassold et al. 1995), its appearance as secondarily associated univalents, as seen in figure 6, would suggest that this kind of configuration, when combined with a vulnerable recombinant pattern, must create the critical factors that lead to univalent behavior and subsequent precocious division of the components of the bivalents at MI division. It is possible that age-related changes to the structural proteins of the chromosomes—particularly those proteins required for chromosome condensation—reduce the capacity of chromosomes to condense effectively into stable bivalent structures prior to MI division. Although the "production line" hypothesis (Henderson and Edwards 1968) assumes that the apparent reduction in chiasma that is seen with age in the mouse correlates directly with reduced recombination, the model outlined above assumes that the physical deterioration in the protein structure of the chromosomes causes the apparent reduction in chiasma frequency within the bivalents with age, as they prepare inadequately to enter MI division. In view of this similarity between mouse and human oocyte chromosomes, the mouse could prove a suitable model for examination, at the molecular level, of aging affects in meiotic chromosomes.

The mechanism of formation of MII trisomies is unclear, but the data presented above lend no support to Lamb et al.'s (1996) hypothesis that MII nondisjunction arises as a consequence of an error at MI. However, since the mean maternal age of the MII trisomies is even greater than that for MI trisomies, the physical deterioration of their chromosomes will be more extreme and more vulnerable to malfunction. Therefore, where there is excessive pericentric recombination, the forces needed to separate the more firmly anchored bivalents could lead to the centromeric structure itself being weakened to such an extent that it cannot function effectively at MII division. The foregoing observations on degenerating oocytes (Angell 1993, fig. 4) show a characteristic degeneration pattern, associated with increasing age, of chromosome and chromatid breaks specifically at the centromeric regions of the MII-metaphase chromosomes, sometimes resulting in the complete separation of this region. Recent oocyte-donation programs (Declining fertility: egg or uterus 1991; Navot 1991) have emphasized the enormous impact of maternal age on all aspects of oocyte quality, including those immediately responsible for chromosome abnormalities.

In conclusion, although the mechanism of formation of MII errors remains open to speculation, the situation with respect to MI-division nondisjunction is somewhat clearer. Several factors appear important in relation to the maternal age affect: First, vulnerable recombinants arise by chance distribution of recombination events at the pachytene stage of meiosis in fetal life. Second, the physical structure of all the chromosomes in the oocyte gradually deteriorates during the extended dictyate stage. If vulnerable recombinants are processed in oocytes at an age when the chromosomes are still young, then they are of no consequence, but, if they are ovulated in women of advanced maternal age, the vulnerable meiotic configurations that emerge after the extended dictyate stage will be unable to hold together effectively and will be able to function only as homolgous univalents and not as stable bivalents. Therefore, they will undergo precocious division at MI, and the resulting chromatids will have the potential to form unbalanced gametes at MII division and, hence, trisomies after fertilization.

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