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Association between Nondisjunction and Maternal Age in Meiosis-II Human Oocytes

Theresa Dailey,[1] Brian Dale,[2] Jacques Cohen,[1,3] and Santiago Munné[1,3]

[1]The New York Hospital—Cornell University Medical Center, New York; [2]Stazione Zoologica Anton Dohrn, Naples; and [3]The Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center, Livingston, NJ

Summary

The relationship between advanced maternal age and increased risk of trisomic offspring is well known clinically but not clearly understood at the level of the oocyte. A total of 383 oocytes that failed fertilization from 107 patients undergoing in vitro fertilization were analyzed by FISH using X-, 18-, and 13/21-chromosome probes simultaneously. The corresponding polar bodies were also analyzed in 188 of these oocytes. The chromosomes in the oocyte and first polar body complement each other and provide an internal control to differentiate between aneuploidy and technical errors. Two mechanisms of nondisjunction were determined. First, nondisjunction of bivalent chromosomes resulting in two univalents going to the same pole and, second, nondisjunction by premature chromatid separation (predivision) of univalent chromosomes producing either a balanced (2 + 2) or unbalanced (3 + 1) distribution of chromatids into the first polar body and M-II oocytes. Balanced predivision of chromatids, previously proposed as a major mechanism of aneuploidy, was found to increase significantly with time in culture ($P < .005$), which suggests that this phenomenon should be interpreted carefully. Unbalanced predivision and classical nondisjunction were unaffected by oocyte aging. In comparing oocytes from women <35 years of age with oocytes from women ≥40 years of age, a significant increase ($P < .001$) in nondisjunction of full dyads was found in the oocytes with analyzable polar bodies and no FISH errors. Premature predivision of chromatids was also found to cause nondisjunction, but it did not increase with maternal age.

Introduction

Genetic analysis of abortuses and live-born offspring has shown that older women are at a higher risk of delivering trisomic fetuses. These risks have been estimated to increase from 1.9% of clinically recognized pregnancies in women 25–29 years of age to 19.1% in women >39 years of age (Hassold and Chiu 1985). This difference is even more profound at the 8-cell stage. An increase of X, Y, 18, 13, and 21 aneuploidies from 4% in women 25–34 years of age to 37% in women ≥40 years of age has been found by using FISH (Munné et al. 1995*a*). Most aneuploidies are considered to be the result of nondisjunction in maternal meiosis I (Hassold and Chiu 1985; Warburton et al. 1986; Hassold et al. 1987; May et al. 1990; Antonarokis et al. 1991; Nothen et al. 1993). The accepted theory is that oocytes of older women are more sensitive to nondisjunction during the first meiotic division. However, a relationship between maternal age and aneuploidy in mature oocytes has not been demonstrated. Oocytes have been examined by karyotyping, but only two reports have demonstrated a slight correlation between aneuploidy and maternal age (Plachot et al. 1988; Macas et al. 1990), while most others failed to show significant differences (see review by Delhanty and Penketh 1990; De Sutter et al. 1991; Pellestor 1991; Selva et al. 1991; Tarin et al. 1991; Edirisinghe et al. 1992; Almeida and Bolton 1993; Angell et al. 1993, 1994; Kamiguchi et al. 1993). This lack of significant difference may be largely due to problems inherent to unrepresentative patient populations and karyotyping.

In addition to the question of the relationship between aneuploidy and maternal age, there is a debate over the mechanism causing aneuploidy in the human oocyte. Two mechanisms have been described that explain the occurrence of aneuploidy: (1) the classical mechanism of nondisjunction of bivalent chromosomes and (2) predivision of sister chromatids at meiosis I (Angell 1991) (fig. 1). Studies using karyotyping have found that nondisjunction of bivalent chromosomes is the most common mechanism of aneuploidy, while Angell and coworkers (Angell 1991; Angell et al. 1993, 1994) have suggested that aneuploidy is solely caused by predivision of chromatids.

One of the aims of this study was to elucidate the maternal age effect on the rates of aneuploidy resulting from nondisjunction during meiosis I by studying meiosis-II oocytes and their corresponding polar bodies. In

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Address for correspondence and reprints: Dr. Santiago Munné, Saint Barnabas Medical Center, 101 Old Short Hills Road, Suite 501, West Orange, NJ 07052. E-mail: munne@usa.pipeline.com

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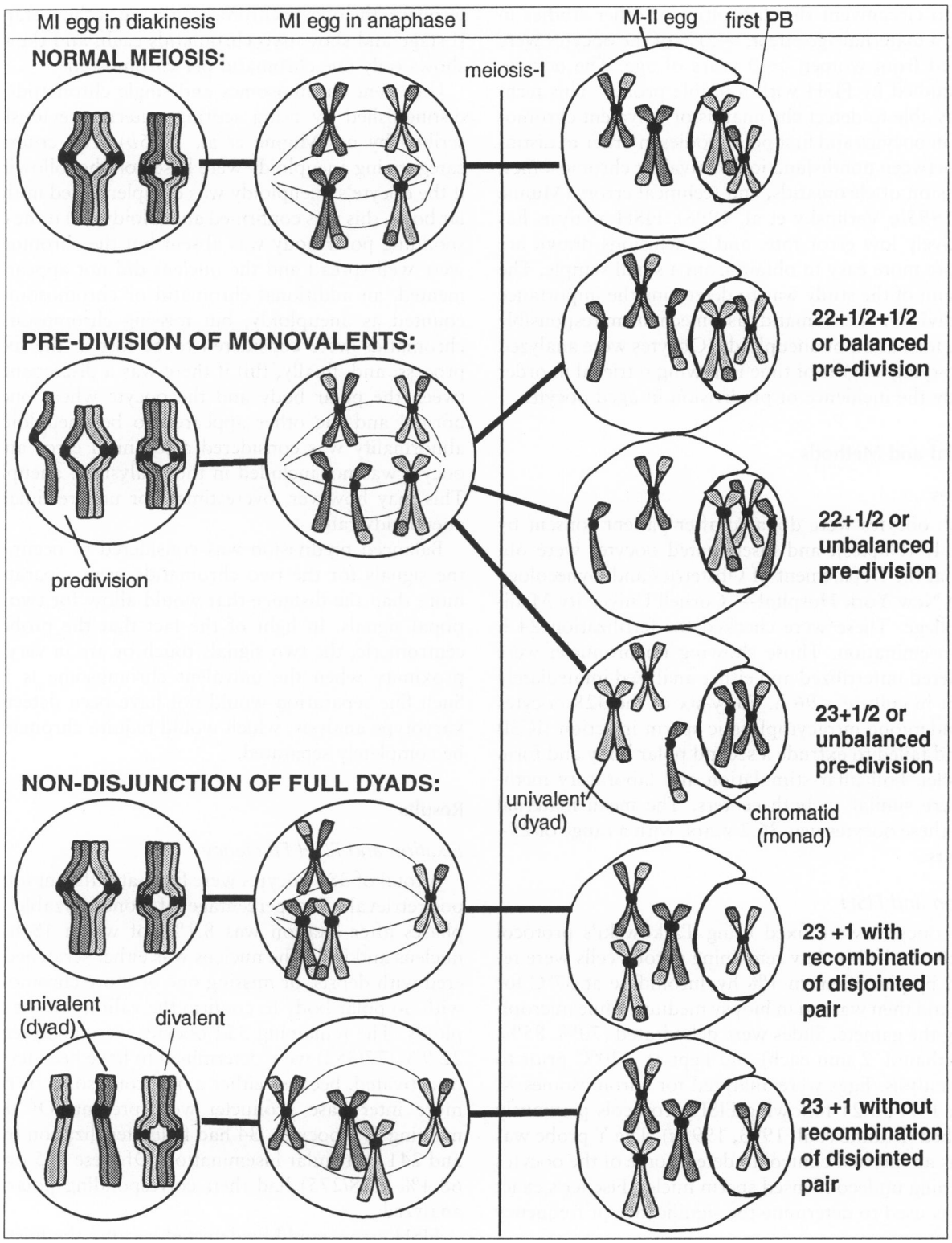

Figure 1 Mechanisms of nondisjunction. Normal meiotic division and three mechanisms of nondisjunction are shown. The normal M-I oocyte and first polar body, each with two chromatids, are shown in the top row. Balanced predivision of chromatids is shown in the second row. Unbalanced predivision is shown in the third and fourth rows. All three types of predivision can produce normal or abnormal oocytes after meiosis II. Nondisjunction of full bivalents may involve crossing-over or be arecombinational, but in both cases, the result is of disomic or nullisomic eggs.

order to circumvent the difficulties of other studies in finding a maternal age effect, >20% of the oocytes were obtained from women ≥40 years of age. The oocytes were studied by FISH with multiple probes. This technique is able to detect chromatids of univalent chromosomes in oocytes and first polar bodies in order to distinguish between nondisjunction of bivalent chromosomes, predivision of chromatids, and technical errors (Munné et al. 1995*b*; Verlinsky et al. 1996). FISH analysis has a relatively low error rate, and conclusions drawn are therefore more easy to obtain from a small sample. The other aim of the study was to determine the importance of predivision of chromatids as a mechanism responsible for the formation of aneuploidy. Oocytes were analyzed after varying lengths of time following retrieval in order to study the incidence of predivision in aged oocytes.

Material and Methods

Oocytes

Fresh oocytes were donated after patient consent by IVF Italy (Naples), and inseminated oocytes were obtained at the Department of Obstetrics and Gynecology of The New York Hospital— Cornell University Medical College. These were checked for fertilization 24 h after insemination. Those showing no pronuclei were considered unfertilized and either analyzed immediately or kept in culture ≤96 h. Forty-six of the 328 oocytes had undergone intracytoplasmic sperm injection (ICSI) and had failed to extrude a second polar body and form pronuclei. Follicular stimulation and laboratory methods were similar in both centers. The mean maternal age of these oocytes was 36.2 years, with a range of 25–45 years.

Fixation and FISH

The oocytes were fixed using Tarkowski's protocol (Tarkowski 1966). Any remaining corona cells were removed by placement in 1% hyaluronidase at 37°C for 5 min and then washed in biopsy medium while micropipetting the gamete. Slides were dehydrated (70%, 85%, 95% ethanol, 2 min each) and kept at −20°C prior to their analysis. Eggs were analyzed for chromosomes X, Y, 18, 13, and 21 following FISH protocols previously described (Munné et al. 1993, 1995*a*). The Y probe was used as an internal control to detect some of the oocytes containing undecondensed sperm nuclei. Fischer's exact test was used to determine the significance of frequency distributions.

Scoring Criteria

Because of the differences in the chromatin among polar bodies, oocytes, and sperm, the three nuclei can be distinguished. The polar body chromosomes appear as a compacted metaphase, degenerated, or in interphase; the oocyte chromosomes are at the metaphase-II stage and show two chromatids each; and the sperm shows only one chromatid per chromosome.

Univalent chromosomes and single chromatids were distinguished by using scoring criteria previously described by us (Munné et al. 1995*b*). The criteria for categorizing aneuploidy were based on the following: (i) if the oocyte's aneuploidy was complemented in the polar body, this was confirmed aneuploidy; (ii) if the corresponding polar body was absent but the chromosomes were well spread and the nucleus did not appear fragmented, an additional chromatid or chromosome was counted as aneuploidy, but missing chromosomes or chromatids were considered to be lost in the fixation process; and, finally, (iii) if there was a discrepancy between the polar body and the oocyte where one was normal and the other appeared to be aneuploid, the abnormality was considered a technical error and the oocyte was not included in the analysis of aneuploidy. This may however, overestimate or underestimate the aneuploidy rate.

Balanced predivision was considered to occur when the signals for the two chromatids were separated by more than the distance that would allow for two additional signals. In light of the fact that the probes are centromeric, the two signals touch or are in very close proximity when the univalent chromosome is intact. Such fine separation would not have been detected by karyotype analysis, which would require chromatids to be completely separated.

Results

Fixation and FISH Efficiency

A total of 383 oocytes were fixed at different intervals postretrieval. The percentage of nonanalyzable metaphases after fixation was 8.1%, of which 18 had no nucleus and in 13 the nucleus was either scratched, covered with debris, or missing one or more chromosomes with no polar body to confirm the validity of the hypoploidy. The remaining 352 oocytes were analyzed, and 22.9% (77/352) were determined to have been fertilized or activated, because either a Y chromosome or two or more interphase pronuclei were present. Of the remaining 275 oocytes, 34 had failed fertilization by ICSI and 241 by regular insemination. Of these 275 oocytes, 68.4% (188/275) had their corresponding polar body analyzed.

FISH errors could be detected because the missing or extra chromosomes were only located in the oocyte or polar body. Of the 188 oocytes with polar body analyzed, 20 (10.6%) were considered FISH errors. Ten of them were oocytes classified as normal but with their corresponding polar bodies being abnormal, eight oocytes were abnormal with normal polar bodies, and in

Table 1

Abnormal X, 18, 13/21 Chromosomal Events in Relation to Time in Culture

Time in Culture (h)	No.	Maternal Age (years)	Extra or Missing Univalents (%)	Predivision of Chromatids (%)	
				2 + 2	1 + 3
0–6	38	38.0	7.8	5.2	2.6[a]
24–48	139	36.2	6.5	23.0*	2.9
≥72	87	35.4	6.9	33.3**	4.6[a]

[a] Not significant.
* $P < .05$.
** $P < .005$.

two cases both polar body and oocyte were missing one of the 13/21 pairs. The overall efficiency of this method, estimated by subtracting the fixation errors (8.1%) and FISH errors (10.6%), was therefore 81.3%.

Aged Oocytes and Balanced Predivision of Chromatids

Oocytes were analyzed in groups according to postretrieval time in culture: fresh or <6 h ($n = 38$), 24–48 h ($n = 139$), and ≥72 h ($n = 87$) (table 1). All 275 oocytes were included in this analysis to ensure a large sample size, since the polar body was not paramount for determining balanced predivision. The hypothesis that the rate of premature balanced predivision of chromatids would increase as oocytes aged in culture was confirmed. Balanced predivision occurred in 5.2% of fresh oocytes and increased to 33.3% in oocytes aged ≥72 h ($P < .005$). All analyzed chromosomes were affected, and there was no statistically significant difference in the rate of separation by chromosome. Nondisjunction of bivalent chromosomes and unbalanced predivision of chromatids, on the other hand, did not increase significantly with time in culture (table 1).

On the basis of these data, we conclude that most balanced predivision is an artifact and not an abnormality that occurs frequently in fresh oocytes. Therefore, to evaluate the actual balanced predivision rate, one should only use the data obtained from fresh mature oocytes.

Maternal Age and Oocyte Aneuploidy

Aneuploidy evaluation was performed only when oocytes and polar bodies could be analyzed simultaneously and when no FISH errors were detected ($n = 168$). The oocytes were classified into three groups according to maternal age: 26–34 years ($n = 67$); 35–39 years ($n = 68$); and ≥40 years ($n = 33$). The age cutoffs were arbitrarily chosen and were similar to those used by Munné et al. (1995*a*). A similar proportion of fresh and aged eggs were used for each of these three groups. For instance, in the group of 26–34 year olds, 15% of the oocytes were fresh, 55% were 24–48 h old, and 30% were ≥72 h old. For the group of 35–39 year olds, these proportions were 18%, 47%, and 35%, respectively, and for the group of 40–45 year olds, these proportions were 21%, 64%, and 15%, respectively. There were no significant differences between these distributions.

Oocytes, aneuploid for chromosomes X, 18, and/or 13/21, are shown in table 2 according to their maternal age. The data show that nondisjunction of bivalent chromosomes increased from 1.5% of the analyzed chromosomes from women 25–34 years of age to 24.2% of the analyzed chromosomes from women ≥40 years of age ($P < .001$). A marked difference was noted when these results were compared to the phenomenon of unbalanced predivision, since this was not affected by maternal age. Figure 2 shows an oocyte with a normal X, 18, 13, 21 chromsome complement, while figure 3 shows an oocyte with simultaneous nondisjunction of bivalent chromosomes, balanced and unbalanced predivision of chromatids.

Polyploidy

Twenty-four oocytes were polyploid. Five of those, three triploid and two tetraploid oocytes, also showed a diploid polar body. The other 19 did not have a polar body, and 4 were triploid, 14 tetraploid, and 1 hexaploid. There was no increase in polyploidy with either increased maternal age (11% in the youngest and 12% in the oldest group) or length of time in culture (11% in fresh oocytes and 7% after ≥72 h).

Discussion

Maternal Age Effect

Nondisjunction increases with maternal age in cleaved embryos (Munné et al. 1995*a*) and clinically recognized pregnancies (Hassold and Chiu 1985; Warburton et al. 1986). Most of these aneuploidies originate at maternal

Table 2

X, 18, and 13/21 Aneuploidy Events in Human Oocytes, when Polar Body and Oocyte Were Simultaneously Analyzed

	Maternal Age (years)		
Oocytes Analyzed	25–34	35–39	40–45
X balanced predivision	1	1	0
18 balanced predivision	0	2	2
13/21 balanced predivision	6	7	0
Multiple balanced predivisions	3	3	2
Extra chromatid 18 and 13/21 balanced predivision	0	0	2
Extra 18 and missing X chromatids and 13/21 balanced predivision	0	1	0
Extra chromatid 18	0	1	0
Missing X univalent	0	0	1
Extra chromatid 13/21	1	0	0
Extra 13/21 univalent	0	1	0
Missing 13/21 univalent	0	2	5
Extra 13/21 and extra 18 univalents	1	0	0
Extra X, extra 18, and missing 13/21 univalents	0	0	1
Missing 18 and extra 13/21 univalents	0	0	1
Extra X univalent and missing 13/21 chromatid	0	1	0
Extra 13/21 chromatid, missing X, extra 13/21, and extra 18 univalents	0	1	0
Subtotal:			
Balanced predivision (A)	10 (14.9)[a]	14 (20.6)[a]	6 (18.1)[a]
Unbalanced predivision (B)	1 (1.5)[a]	4 (5.9)[a]	2 (6.0)[a]
Nondisjunction of univalents (C)	1 (1.5)*	5 (7.4)**	8 (24.2)
Oocytes with B and C aneuploidy (D)	0 (0)	2 (2.9)	0 (0)
Total	67	68	33

Note.—Numbers in parentheses are percentages.
[a] Not significant.
* $P < .001$ (difference between first column and third).
** $P < .05$ (difference between second column and third).

meiosis I (Hassold et al. 1987, 1995; May et al. 1990; Antonorakis et al. 1991, 1993; Zaragoza et al. 1994). Exceptions are trisomy 18, which occurs mostly at maternal meiosis II (Fisher et al. 1995), and trisomies 47XXY and 47XYY, which are 50% and 100% paternally derived, respectively (Hassold et al. 1988; McDonald et al. 1994). Previous determinations of karyotypes in M-II oocytes failed to confirm a unequivocal relationship between maternal age and aneuploidy. These findings were probably affected by three problems. First, the population of women studied was relatively young and relatively homogeneous. The present study used a population of oocytes with a sufficient maternal age range to capture both younger and older patients. A second issue concerned the inherent disadvantages of karyotyping and chromosome banding of oocytes. For instance, only about half of the karyotyped oocytes analyzed prior to 1991 (Pellestor 1991) yielded results, and, in those, very few chromosome-specific aneuploidies were identified. In contrast, the present study was able to analyze specific aneuploidies in >80% of unfertilized oocytes. Third, karyotyping cannot use the first polar body as an internal control, because its nuclear integrity is time dependent, and its chromosomes are usually in interphase. Polar body ploidy can therefore be analyzed reliably only by FISH (Munné et al. 1995*b*). The present results show for the first time a significant relationship between maternal age and nondisjunction of bivalents during meiosis I.

Mechanisms of Aneuploidy

The second aim of this research was to provide insight into the mechanisms of nondisjunction in human oocytes. Our approach indicates that nondisjunction of bivalent chromosomes and predivision of chromatids can be distinguished by FISH. The results show that both mechanisms contribute to aneuploidy, but only nondisjunction of bivalent chromosomes increased with maternal age. Predivision of chromatids was not affected by maternal age but by time in culture. It has been reported that balanced

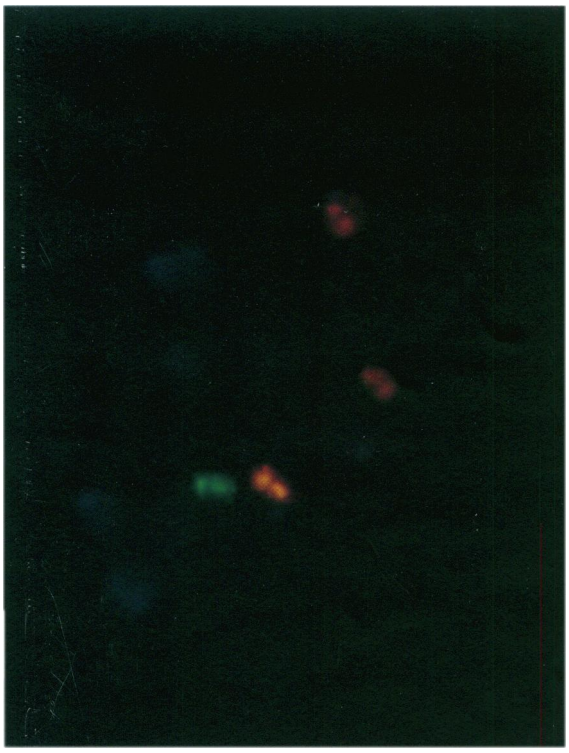

Figure 2 FISH with fluorochrome-labeled X (yellow), 18 (green), and 13/21 (red) chromosome–specific DNA probes. An oocyte with one X-, one 18-, and two 13- or 21-univalent chromosomes is shown. Each univalent shows a double-dotted hybridization signal corresponding to two chromatids (normal oocyte). The corresponding polar body is not visible in this picture.

predivision increases when the oocyte ages (Rodman 1971; Eichenlaub-Ritter et al. 1988; Munné et al. 1995*b*), a phenomenon that was confirmed in the current study. Predivision may be the result of spindle instability during oocyte aging (Szöllösi 1983). The frequency of balanced predivision should therefore be considered only in fresh oocytes. Because of the scarcity of this material, we could not accurately establish its true incidence. The suggestion that nondisjunction of bivalents is a significant mechanism of aneuploidy coincides with findings in most studies involving karyotyping (Plachot et al. 1988; Delhanty and Penketh 1990; Macas et al. 1990; DeSutter et al. 1991; Pellestor 1991; Selva et al. 1991; Tarin et al. 1991; Edirisinghe et al. 1992; Almeida and Bolton 1993; Kamiguchi et al. 1993).

The present results do not confirm conclusions drawn by Angell and coworkers, who found that predivision, balanced and unbalanced, was the only source of nondisjunction in human oocytes (Angell 1991; Angell et al. 1993, 1994). The discrepancies between the present and Angell's results may be explained in three ways. First, the present data on oocytes aged in culture demonstrate that there is a significant increase in balanced predivision with increasing time in culture. In light of the fact that the oocytes analyzed by Angell and coworkers were kept in culture for 3 d before analysis, it is possible that the high rates of balanced predivision reported were the result of nuclear degeneration. Second, our rate of balanced predivision per chromosome at 72 h of oocyte aging is higher than those reported by Angell et al. (1993, 1994) for the same aging time. This is probably due to the use of different scoring criteria for balanced predivision between laboratories. Third, most of the predivision in their work involved chromosome 16, which was not analyzed here. Trisomy 16 is unique in that it is the only trisomy that increases linearly with maternal age (Risch et al. 1986; Morton et al. 1988). Recent recombination studies of trisomy 16 by Hassold et al. (1995) support predivision of chromatids as a possible mechanism of trisomy 16 formation. We are currently investigating aneuploidy 16 by using FISH analysis on fresh oocytes to minimize the drawbacks mentioned above.

The Rate of Aneuploidy in M-II Oocytes

Because of the increase in balanced predivision of chromatids with oocyte aging, we can only estimate the overall rate of aneuploidy. Since balanced predivision did not seem to increase with maternal age, a good estimate of it would be its frequency in fresh oocytes (5.2%). Another factor to consider is that predivided chromatids segregate at random producing unbalanced embryos in only a proportion of the predivision events. According to Angell et al. (1994), this proportion is one half. We have therefore estimated the oocyte aneuploidy rate for the chromosomes studied by using a formula that includes the two major pathways of nondisjunction: $1/2(\text{fresh } A) + \frac{1}{2}(B - D) + C$, where A is 5.2%, B is the rate of unbalanced predivision, C is the frequency of nondisjunction of bivalents, and D is the frequency of oocytes with both unbalanced predivision and nondisjunction of bivalents (table 2). This gives an estimate of 4.9%, 11.5%, and 29.8% aneuploid oocytes in the 25–34, 35–39, and 40–45 maternal age groups, respectively. This estimate compares favorably with FISH studies in human 8-cell embryos obtained from IVF (Munné et al. 1995*a*). For instance, for the 40–45 maternal age group, 37.2% of morphologically normal 8-cell embryos were aneuploid for the same group of chromosomes. Approximately 80% of the aneuploidies detected in those embryos could have originated from events at maternal meiosis I, while the rest occurred at maternal meiosis II or during spermatogenesis, events which are not identified by the present approach. This figure coincides with most parental and meiotic studies of aneuploidy (Hassold and Chiu 1985; Warburton et al. 1986; Hassold et al. 1987; May et al. 1990; Antonorakis et al. 1991; Nothen et al. 1993).

Although aneuploidy rates in oocytes and 8-cell em-

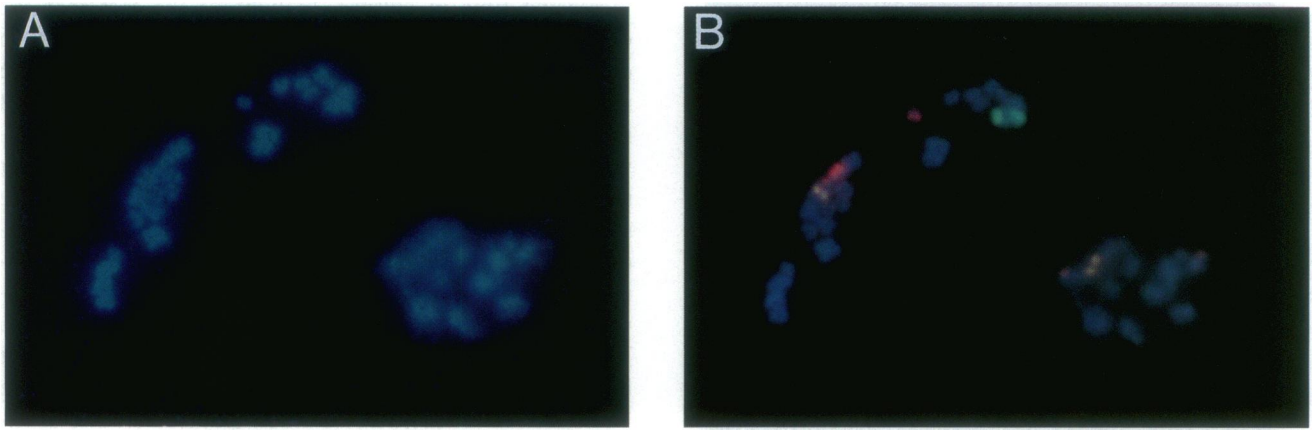

Figure 3 Observation of (*A*) DAPI staining and (*B*) DAPI plus hybridization signals of an oocyte metaphase (*right*) and its corresponding first polar body (*left*). The difference in nuclear condensation allows for the clear differentiation between the two sets of chromosomes. Panel *B* shows a clear example of nondisjunction of chromosomes 18 (green) and 13 or 21 (red); two chromosome-18 univalents (with two chromatids each) can be observed in the oocyte and none in the polar body, while three univalents of chromosomes 13 and 21 (one overlapping with the X univalent (yellow)) are found in the oocyte and only one univalent, split into two chromatids (balanced predivision), is found in the polar body.

bryos studied by FISH are quite similar, both are several times higher than the rate found in first-trimester pregnancies. For instance, fetuses of 9–14 wk in women 40–45 years of age have a frequency of 4.3% trisomy for chromosomes 13, 18, and 21 combined (Snijders et al. 1994). In comparison, we estimate that ~12% of the oocytes are trisomic for these chromosomes. Compared to embryo data, in which as many trisomies as monosomies were found, the trisomy rate was ~20% for these chromosomes (Munné et al. 1995*a*). There are a number of observations that could explain why the frequency of trisomies is three to five times higher than those encountered in fetuses from first-trimester pregnancies.

The first explanation may be that many trisomies are eliminated before the pregnancy is clinically recognized. There is evidence indicating that most of the embryo loss occurs early in the pregnancy. For instance, while 15% of clinically recognized pregnancies spontaneously abort, and only 3.2% do so after 8 wk of gestation, it has been reported that >20% of early pregnancies are lost after implantation and before the 3d wk of pregnancy (Simpson and Mills 1986; Simpson et al. 1987; Wincox et al. 1988). Therefore, a large proportion of spontaneous pregnancies are lost after implantation, and many more could be lost before implantation. The embryo loss is even higher in the infertile population undergoing IVF, where, even in the best IVF centers, no more than 30% of transferred embryos implant (Society for Assisted Reproductive Technology [SART] and American Society for Reproductive Medicine [ASRM] 1995). Our high rates of aneuploidy in oocytes and embryos fits with the interpretation that the earlier a pregnancy aborts the higher is the chance that the lost conceptus was chromosomally abnormal. This is consistent with chromosome studies of very early spontaneous abortions, which have used chorionic villus sampling techniques, since these have showed a rate of abnormality of ~70%, much higher than the 50% rate commonly cited for spontaneous abortions (Guerneri et al. 1987). Furthermore, about a third of the aneuploid oocytes found in the present study had two or more aneuploidy events, a combination that is generally lethal early in pregnancy (Guerneri et al. 1987).

A second explanation for the high rate of trisomic oocytes may be that our sample population is not necessarily representative of the general gamete population. One major difference is that the patients from whom the oocytes were obtained underwent hormonal stimulation, and a second is that our data are exclusively sampled from the infertile population. Gras et al. (1992) reported some preliminary evidence that more aneuploidy is found in the oocytes of stimulated than unstimulated cycles. In addition, germinal vesicle–stage human oocytes aspirated from unstimulated follicles are rarely aneuploid (1%–3%) after in vitro maturation to MII (Van Blerkom 1990), when compared to mature oocytes from stimulated follicles (22.9%) (Pellestor 1991). Other evidence comes from spontaneous abortions following IVF. Although in the best IVF programs the implantation rate per embryo is presumably similar or higher than spontaneous conception, 19% of clinically recognized IVF pregnancies spontaneously abort (SART and ASRM 1995) compared to only 12%–15% in the general population (Simpson and Mills 1986).

Finally, a third cause for the high rate of trisomy may be that the present results are the product of a technical

artifact. Although the sample of oocytes studied is still small, frequencies compare favorably with those from similar studies performed on spare embryos (Munné et al. 1995*a*). The present data also coincided with previous studies on oocytes in that an excess of aneuploidy was found for chromosomes of groups D and G when compared to the frequency expected for all chromosomes disjoined at the same rate (reviewed by Pellestor 1991; Plachot 1992). In addition, if only nondisjunction of univalents is taken into account, our rates of aneuploidy for these chromosomes compare favorably with oocyte data for the same group of chromosomes (reviewed by Pellestor 1991; Plachot 1992). Predivision, however, cannot be compared to oocyte data, because it was disregarded in most studies until Angell et al. brought it to the attention of the medical community (1991). When the present data on oocytes and previously reported results on embryos (Munné et al. 1995*a*) are considered together, it is possible to confirm a number of biological observations: these include the increase of nondisjunction with maternal age, an 80% maternal meiosis-I origin of aneuploidy, and an excess of aneuploidy for chromosomes 13 and 21. It is difficult to imagine a single technical artifact that could explain these established biological phenomena.

In conclusion, the present results support the existence of at least two mechanisms producing aneuploidy: nondisjunction of full monovalents and predivision of chromatids. The results also show a significant increase in nondisjunction of full monovalents with maternal age, while predivision was not affected. Instead, balanced predivision of chromatids increases with oocyte aging in culture. Because of this phenomenon, the contribution of predivision to the overall aneuploidy rate should only be estimated in fresh oocytes in future studies.

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