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Increased rate of nondisjunction in sex cells derived from low-quality semen

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Abstract The relationship between chromosomal nondisjunction and semen quality was studied in two groups of males who differ highly in their semen quality: 12 individuals with low-quality semen caused by varicocele, and 8 subjects with high-quality semen, selected from sperm donors for in vitro fertilization. Chromosomal nondisjunction was inferred from the rate of disomy found in mature sperm cells. To determine the rate of disomy, we applied fluorescence in situ hybridization using satellite-specific probes for chromosomes 1, 15, 18, X and Y. In sperm cells of males with low-quality semen, the mean rate of disomy for each of the autosomes and of hetero-disomy for the sex chromosomes (XY) was significantly higher than that observed in the high-quality semen samples: more than 15-fold higher for chromosomes 1 and 15, and 7-fold higher for chromosomes 18 and XY. Yet, the homo-disomy rate for each of the sex chromosomes (XX and YY) was almost the same in both types of semen. The large discrepancy between the low- and high-quality semen in the rate of sex chromosome hetero-disomy versus the similar rate of homo-disomy strongly suggests that the abnormal chromosomal segregation in meiocytes of males with low-quality semen resulted from chromosomal nondisjunction at the first meiotic division. The results indicate that men showing poor semen quality are at an increased risk for meiotic nondisjunction, similar to women at the end of their reproductive years.

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Introduction

Trisomy is the most common aneuploidy in man, found in about 5% of all clinically recognized pregnancies (Hassold and Jacobs 1984; Hassold et al. 1993; Abruzzo and Hassold 1995). Trisomy usually results from the fusion of an unbalanced gamete carrying an extra chromosome (a disomic gamete) with a balanced one (a monosomic gamete). Different trisomic genotypes vary widely in their implantation capability, *in utero* survival, and ability to cross the pregnancy-birth barrier (Kline and Stein 1985; Jacobs 1990, 1992).

A few trisomic genotypes, particularly those involving the sex chromosomes as well as several specific autosomes (chromosomes 13, 18, and 21), often survive to term and are revealed in newborns. These cases are accompanied by mental disorders and inborn defects. Other types of trisomy are lost because of prenatal selection: some are lost spontaneously in the course of pregnancy, others cause very early developmental failure and are aborted before the pregnancy is clinically recognized (Jacobs 1990; Jacobs and Hassold 1995). Thus, trisomy in man is a common cause of inborn defects, a high rate of abortion, and reproductive failure.

Most human chromosomes have been implicated in the trisomic state, either in the embryo or in the newborn. The frequency of trisomy varies widely from one chromosome to another, for example, trisomy for chromosome 16 has been found in more than 7% of all spontaneous abortions (Jacobs 1990; Jacobs and Hassold 1995), whereas for chromosome 1 it was found only in one eight-cell embryo following in vitro fertilization (Watt et al. 1987). Most probably, there is an earlier and a stronger selection against trisomic zygotes that display severe phenotypic defects.

In most cases of trisomy detected in aborted material and in newborns, the additional chromosome was traced back to maternal meiotic nondisjunction (ND) and only rarely to paternal meiotic ND (Hassold and Jacobs 1984; Pellestor 1991; Hassold et al. 1993; Abruzzo and Hassold 1995; Jacobs and Hassold 1995; Eichenlaub-Ritter 1996). Accordingly, one may assume that the risk for ND in sper-

matogenesis is rather small. Alternatively, it might be argued that the apparent rarity of ND during spermatogenesis reflects a stronger and earlier selection against paternally derived trisomic zygotes with severe phenotypic consequences (Hall 1990; Fisher et al. 1995). This is corroborated by the finding that in some trisomies involving sex chromosomes (47,XYY and 47,XXY), where the phenotypic effect is relatively small, there is a high frequency of paternally originated extra chromosomes (MacDonald et al. 1994). In addition, there is a high frequency of individuals exhibiting Turner's syndrome (45,X0), the only monosomy in man that crosses the pregnancy-birth barrier, where the state of monosomy results from a paternal ND event – in most cases, a prezygotic one (Hassold et al. 1992; Lorda-Sanchez et al. 1992). These findings support the view that ND in the course of spermatogenesis is not an uncommon phenomenon.

In addition one should consider that an aneuploid secondary spermatocyte, having to undergo complex maturation processes before differentiating into a functional gamete, might have a lower viability than an aneuploid oocyte. Indeed, in man, there is a strong body of evidence indicating that failure in meiotic pairing in spermatogenesis is accompanied by the production of defective, less viable gametes (Hendry et al. 1975; Chandley et al. 1976; Egozcue et al. 1983; Rosenmann et al. 1985; Luciani et al. 1987). Thus, strong selection against trisomic zygotes carrying an extra paternal chromosome coupled with low viability of defective male meiocytes apparently masks the ND errors occurring in male meiosis.

Assuming that in men, as in women (Hassold and Chiu 1985; Warburton et al. 1986; Hassold et al. 1987; May et al. 1990; Antonarakis et al. 1991; Nothen et al. 1993; Eichenlaub-Ritter 1994), it is the first meiotic division that is more susceptible to ND, then most ND errors occurring in males would result in the production of four aneuploid meiocytes (a pair of disomics and a pair of nullisomics). Taking into account that the aneuploid constitution of a spermatocyte interferes with the gamete's maturation, and/or leads to a reduction in sperm viability, then men at high risk for meiotic ND are expected to show not only increased frequency of aneuploidy in their sperm cells (accompanied by reproductive failure), but also a reduction in semen quality. Semen quality in man is a widely variable trait, expressing total semen volume, spermatozoa concentration, motility, vitality and morphology (Jequier and Crich 1986; Carlsen et al. 1992; World Health Organization 1992; Olsen et al. 1995; Sherins 1995).

In the present study we tested the hypothesis that semen quality is related to meiotic ND, i.e., males with low-quality semen comprise a high-risk group for ND, whereas males with high-quality semen comprise a low-risk group. To test our assumption we checked the frequency of disomy for five chromosomes (1, 15, 18, X and Y) in samples of sperm cells from high- and low-quality semen. A total of 20 healthy individuals who belong to two well-defined groups in terms of semen quality participated in the study. Based on the scatter curve describing the variable trait of semen quality in man, members of the first group scored at the upper level of the curve (high-quality-semen) whereas those of the second group scored at the lower level of that curve (low-quality semen). The first group comprised 8 individuals selected from donors who contributed sperm for clinical purposes and their sperm profile was optimal; the second group comprised 12 individuals with reduced sperm quality because of varicocele, a major cause of male infertility, which does not affect any other phenotypic trait (Haans et al. 1991; Marmar et al. 1992; Gorelick and Goldstein 1993). The present study indicates that men having poor-quality semen are at high risk of meiotic ND originating from the first meiotic division.

Materials and methods

Semen samples: collection and classification

Ejaculates were obtained from 20 males (25–40 years old) who differed in their semen quality: 8 individuals (samples 1–8) were selected from sperm donors with high-quality semen, while the other 12 individuals (samples 11–18 and 31–34) were selected from a group of varicocele patients with poor-quality semen (Table 1). Each individual was subjected to a routine chromosomal analysis to confirm a normal (46,XY) male karyotype, using phytohemagglutinin-stimulated lymphocytes.

Slide preparation

Following ejaculation, samples were kept at room temperature for about 30 min to allow liquefaction and then washed twice with RPMI 1640 (Biological Industries, Beit Haemek, Israel). Following two more washes, each with a fresh 3:1 methanol:acetic acid solution for 8 min, the cell suspensions were dropped onto precleaned glass slides, air-dried, and stored at –20° C until use.

Pretreatment of sperm nuclei (decondensation)

Slides were incubated in a small volume (100 µl) of freshly diluted 10 mM dithiothreitol (Sigma) in 0.05 M TRIS buffer at –4° C for 25–50 min. Following incubation, the slides were washed twice

Table 1 Characteristics of high- and low-quality semen samples based on the indicated parameters

Parameters	High-quality semen (samples 1–8)	Low-quality semen (samples 11–18 and 31–34)
Volume (ml)	> 2.0	≤ 2.0
Concentration (spermatozoa/ml)	$> 60 \times 10^6$	$\leq 30 \times 10^6$
Total count (per ejaculate)	$> 120 \times 10^6$	$\leq 60 \times 10^6$
Vitality (%)	≥ 70	≤ 50
Motility (% with forward progression within 60 min)	≥ 50	≤ 30
Morphology (% with normal forms)	> 50	< 30

with 2 × saline sodium citrate (SSC; 0.15 M NaCl, 0.015 M sodium citrate) for 10 min, passed through a cold ethanol series (70%, 80%, 100%), air-dried, and observed under phase contrast microscopy.

Probes

For mono-color detection we used five biotinylated probes (D1Z5, D15Z1, D18Z1, DXZ1 and DYZ3), each specific for a given chromosome (chromosomes 1, 15, 18, X, and Y, respectively). For two-color detection we used a biotinylated DXZ1 probe and a digoxigenin-labeled DYZ3 probe. For three-color detection, a mixture of biotinylated and digoxigenin-labeled D18Z1 probe, a biotinylated DXZ1 probe and a digoxigenin-labeled DYZ3 probe were used. The labeled probes were all obtained from Oncor.

In situ hybridization

We followed the fluorescence in situ hybridization (FISH) protocol as previously described (Bar-Am et al. 1992) with slight modifications. Briefly, slides were first denatured in 70% formamide, 2 × SSC at 73° C for 2 min. Prior to hybridization, the labeled probes in the hybridization mixture were heated to 75° C, and put on ice. The hybridization mixture (10 µl) was applied to each slide under a coverslip and hybridization proceeded overnight at 37° C. After incubation, the slides were washed at 42° C in 70% formamide, 2 × SSC for 20 min, then in 2 × SSC for 10 min, and finally in 4 × SSC, 0.1% Tween-20 for 5 min.

Detection

Mono-color detection of samples 1–5 and samples 11–15 was performed using FITC (fluorescein isothiocyanate) conjugated with avidin DCS (Vector Laboratories) in 4 × SSC, 1% BSA. Signals were enhanced once with 5 µg/ml biotinylated goat anti-avidin (Vector Laboratories) in the same dilution buffer, followed by another layer of FITC-avidin. Each incubation was followed by three washes of 3 min each in 4 × SSC, 0.1% Tween-20. Slides were counterstained with propidium iodide and mounted with anti-fade medium (1 mg of *p*-phenylenediamine in 1 ml glycerin buffer pH = 8). For signal detection and evaluation, we used an Olympus BH2 fluorescence microscope with appropriate filter combination for fluorescein and propidium iodide (450–490 nm).

Two-color detection of samples 6–8 and 16–18 was obtained after three successive incubation steps, using in each one the following mixture of reagents diluted in 4 × SSC, 1% BSA: (a) Texas red-avidin (Vector Laboratories) (5 µg/ml) and mouse anti-digoxigenin (Boehringer) (10 µg/ml); (b) biotinylated anti-avidin (5 µg/ml) and sheep FITC-anti-mouse (Sigma) (10 µg/ml); and (c) Texas red-avidin (5 µg/ml) and FITC-anti-sheep (Vector Laboratories) (10 µg/ml). Each incubation was carried out at 37° C for 30 min. Following each step, the slides were washed in three changes of 4 × SSC, 0.1% Tween-20 for 2 min each. After washing, slides were mounted in an anti-fade solution containing 1 µg/ml (DAPI). For simultaneous visualization of FITC (green), Texas red (red), and DAPI (blue), an Olympus BH2 fluorescence microscope fitted with a triple band pass filter (Chromatechnology) was used.

Three-color detection of samples 16–18 and 31–34 was obtained after three successive incubation steps. In these was used the following mixture of reagents (all obtained from Vector Laboratories) diluted in 4 × SSC, 1% BSA: (a) FITC conjugated with avidin and anti-digoxigenin rhodamine (5 µg/ml); (b) biotinylated goat anti-avidin (5 µg/ml); and (c) the same as in (a). Each incubation was carried out at 37° C for 30 min. After each step, the slides were washed in three changes of 4 × SSC, 0.1% Tween-20 for 2 min. Slide mounting and signal visualization were as described previously for the two-color detection. The three-color detection was performed to allow estimation of the level of diploid cells from the frequency of double-disomy cells.

Results

High-quality semen – low rate of disomy

Sex cells (spermatozoa) from high-quality semen (samples 1–8) exhibited very low rates of disomy, with mean ± SE values (%) of 0.08 ± 0.02 for chromosome 1, 0.09 ± 0.02 for chromosome 15, 0.12 ± 0.02 for chromosome 18, and 0.20 ± 0.05 for the sex chromosomes (XY+XX+YY) (Fig. 1 a–d, Table 2). These values are at the very bottom edge of the variation range found in other male populations previously investigated (Tables 2, 3). We found no significant differences between the various homologous pairs studied nor between the different individuals examined (Fig. 1 a–d).

The low levels of disomy in each of the high-quality semen samples cannot be attributed to low hybridization efficiency, since the hybridization efficiency [100 – (% nullisomy – % disomy)] in these samples was high: a mean of 98.6 ± 0.5, with specific efficiency values of 98.8 for chromosomes 1, 97.7 for chromosomes 15, 98.1 for chromosomes 18, and 99.9 for chromosomes XY.

In these high-quality semen samples the mean frequency of sex chromosome hetero-disomy (XY; 0.10 ± 0.03%) was similar to the sum of both types of homo-disomy (XX+YY; 0.12 ± 0.03%) (Fig. 1 e–g ; Table 3). Considering that ND of sex chromosomes at the first meiotic division results in hetero-disomy whereas in the second one it results in homo-disomy, these findings clearly indicate that in meiocytes of high-quality semen, the rates of ND in the first and second meiotic divisions are similar.

Cells of high-quality semen exhibited a somewhat higher tendency for ND between sister chromatids of the Y chromosome than between those of the X chromosome. This tendency is evident from the higher frequency of YY cells as compared with XX cells (Fig. 1 e, f; Table 3). However, the two sex chromosomes differed from each other in this respect at a low level of significance (χ^2 = 4.27; $P < 0.05$).

Low-quality semen – high rates of disomy

In contrast to sex cells from high-quality semen (samples 1–8), those of low-quality semen (samples 11–18) revealed high frequencies of disomy for all chromosomes studied (Fig. 1 a–d). The mean ± SE frequency values (%) of disomy were 1.35 ± 0.11 for chromosome 1, 1.67 ± 0.14 for chromosome 15, 0.85 ± 0.12 for chromosome 18, and 0.90 ± 0.14 for the sex chromosomes (Table 2).

The differences in disomy rates between semen samples of low and high quality were highly significant (Fig. 1 a–d). Compared with cells of high-quality semen, the mean disomy frequencies in cells of low-quality semen were more than 15 times higher for chromosomes 1 and 15, 7 times higher for chromosome 18, and 4.5 times higher for the sex chromosomes (Table 2). The disomy rates in spermatozoa derived from low-quality semen were also much higher than those described elsewhere for sperm cells de-

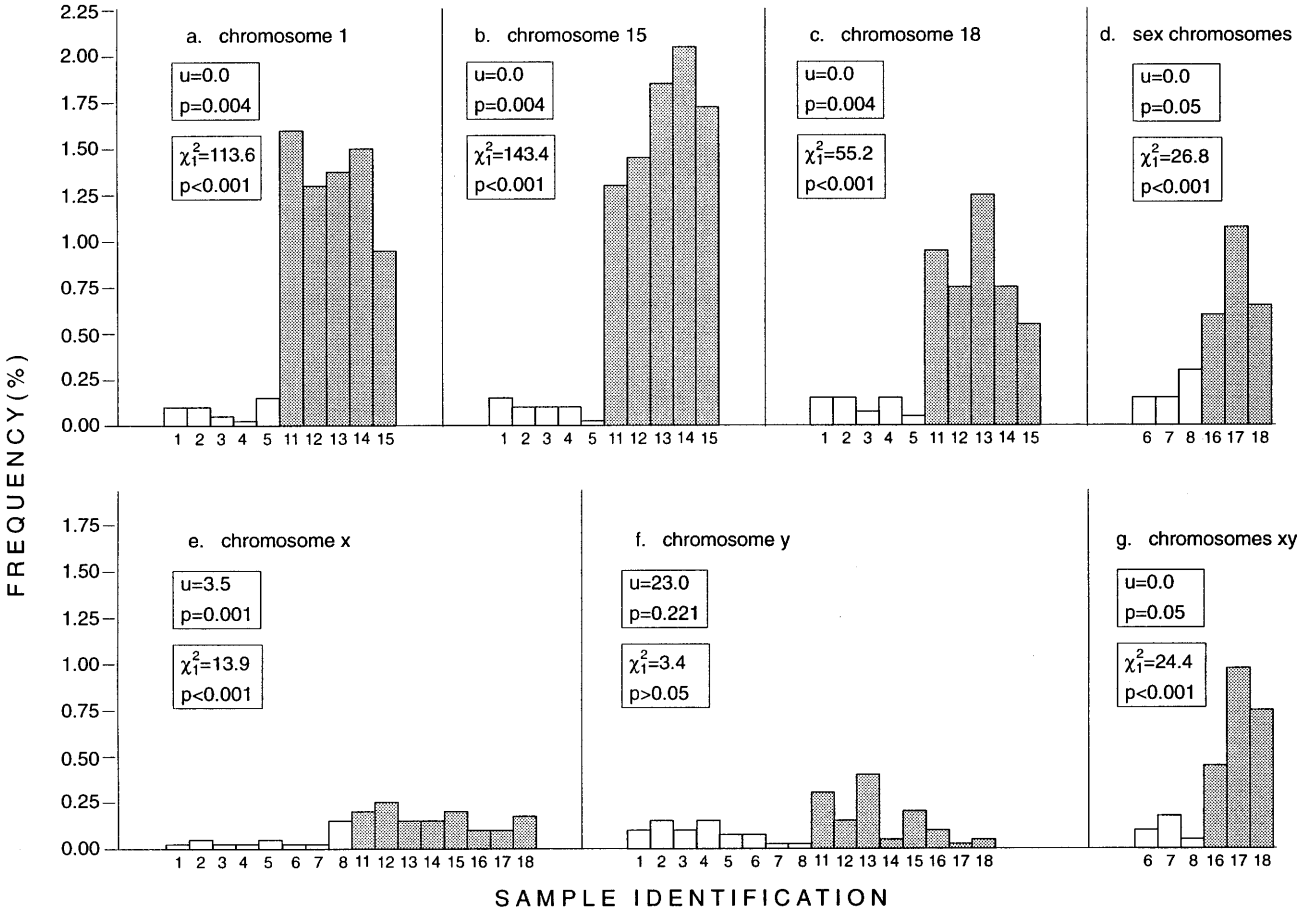

Fig. 1 a–g Disomy frequency (%) for chromosomes 1, 15, 18 and the sex chromosomes in sperm cells of individuals exhibiting high-quality (samples 1–8; *empty bars*) and low-quality (samples 11–18; *solid bars*) semen. Each estimate was based on at least 2000 cells. Both the Mann-Whitney *U*-test and the χ^2 test were used to compare the two groups of samples. Note the large difference between the two groups in **a**–**d** [reflecting nondisjunction (ND) errors in both meiotic divisions] and in **g** (reflecting ND errors in the first meiotic division) versus their similarity in **e** and **f** (reflecting ND errors in the second meiotic division)

Table 2 Mean frequencies (%) of disomy for autosomes 1, 15, 18, and for the sex chromosomes in sperm samples derived from various groups of individuals

Male population	Study	Chromosome 1	Chromosome 15	Chromosome 18	XY+XX+YY
Males with high-quality semen[a]	Present study	0.08 (± 0.02)	0.09 (± 0.02)	0.12 (± 0.02)	0.20 (± 0.05)
Fertile males[b]	Martin et al. (1993)	0.06	0.14	–	–
	Goldman et al. (1993)	–	–	–	0.41
	Han et al. (1993)	–	–	–	0.70[e]
	Robbins et al. (1993)	0.14	–	–	–
	Schattman et al. (1993)	–	–	–	0.30[e]
	Williams et al. (1993)	–	–	0.08	0.28[e]
	Bischoff et al. (1994)	–	0.20	0.19	0.57[e]
	Miharu et al. (1994)	0.14	–	–	–
	Wyrobek et al. (1994)	–	–	–	0.14[e]
	Chevret et al. (1995)	–	–	–	0.52[e]
	Martin et al. (1996)	0.11	–	–	0.32
Males with low-quality semen[c]	Present study	1.35 (± 0.11)	1.67 (± 0.14)	0.85 (± 0.12)	0.90 (± 0.14)
Infertile males[d]	Miharu et al. (1994)	0.13	–	–	–
	Moosani et al. (1995)	0.18	–	–	0.50[e]

[a] Individuals exhibiting high-quality semen (samples 1–8)
[b] Individuals with proven fertility (based on other studies using FISH)
[c] Individuals exhibiting low-quality semen (samples 11–18)
[d] Individuals showing reproductive failure (based on other studies using FISH)
[e] Calculated from data given by the author

Table 3 Mean frequencies (%) of sex chromosome hetero-disomy (XY) and of both types of homo-disomy (XX and YY) in sperm samples derived from various groups of individuals

Male population	Study	XY	XX	YY
Males with high-quality semen[a]	Present study	0.10 (± 0.30)	0.03 (± 0.02)	0.09 (± 0.02)
Fertile males[b]	Goldman et al. (1993)	0.23	0.08	0.10
	Han et al. (1993)	0.21	0.28	0.21
	Schattmen et al. (1993)	0.17	0.04	0.09
	Williams et al. (1993)	0.09	0.08	0.11
	Bischoff et al. (1994)	0.12	0.37	0.08
	Miharu et al. (1994)	–	0.13	0.08
	Wyrobek et al. (1994)	0.06	0.04	0.04
	Chevret eta l. (1995)	0.42	0.05	0.05
	Martin et al. (1996)	0.16	0.07	0.18
	Spriggs et al. (1996)	0.16	0.03	0.13
Males with low-quality semen[c]	Present study	0.74 (± 0.16)	0.16 (± 0.02)	0.16 (± 0.05)
Infertile males[d]	Miharu et al. (1994)	–	0.16	0.11
	Moosani et al. (1995)	0.33	0.08	0.09

[a] Individuals exhibiting high-quality semen (samples 1–8)
[b] Individuals with proven fertility (based on other studies using FISH)
[c] Individuals exhibiting low-quality semen (samples 11–18)
[d] Individuals showing reproductive failure (based on other studies using FISH)

rived either from fertile males (Table 2) or even from males who had experienced reproductive failure (Table 2).

Low-quality semen – high rates of ND at the first meiotic division

In spermatozoa from low-quality semen samples the rate of sex chromosome hetero-disomy (XY) was similar ($P > 0.50$) to the total rate of sex chromosome disomy (XY+XX+YY), and significantly higher ($P < 0.001$) than the rate of homo-disomy (XX+YY) (Fig. 1 d–g, Table 4). Thus, based on the behavior of the sex chromosomes, we assume that the high rate of disomy in spermatozoa of the low-quality semen reflected ND errors at the first meiotic division rather than at the second one.

Moreover, as judged by the frequencies of XX and YY cells, the rate of ND errors at the second meiotic division was only slightly higher in sex cells of low-quality semen than in those of high-quality semen (Fig. 1 e, f; Table 3).

Table 4 Comparisons (χ^2 test) between levels of disomy for various sex chromosome combinations in a population of spermatozoa derived from low-quality semen (total of samples 11–18); *n* number of cells scored, *m* mean frequency (%)

	XY+XX+YY $n = 6{,}297$ $m = 0.90$	XX $n = 16{,}297$ $m = 0.16$	YY $n = 16{,}297$ $m = 0.16$	XX+YY $n = 16{,}297$ $m = 0.32$
XY $n = 6{,}297$ $m = 0.74$	$\chi^2 = 0.98$ $P > 0.50$	$\chi^2 = 46.4$ $P < 0.001$	$\chi^2 = 46.4$ $P < 0.001$	$\chi^2 = 27.7$ $P < 0.001$
YY $n = 16{,}297$ $m = 0.16$	$\chi^2 = 66.9$ $P < 0.001$	$\chi^2 = 0.0$ $P > 0.95$	–	–
XX $n = 16{,}297$ $m = 0.16$	$\chi^2 = 66.9$ $P < 0.001$	–	–	–

The frequencies of XX and YY in sperm cells derived from males with low-quality semen were similar in range to the values reported in other male populations (Table 3).

Low-quality semen – chromosome-specific rates of ND

Considering the total population of spermatozoa derived from the low-quality semen, two levels of disomy rates were observed: a high one (above 1%), revealed by homologous pairs 1 and 15, and a somewhat lower one (below 1%), shown by pairs XY and 18 (Fig. 1 a–d; Table 5). While homologous pairs at the same level did not deviate from one another, a significant difference ($P < 0.001$) was observed between pairs from the two different levels (Table 5). Thus, it appears that the rate of disomy in spermatozoa from the low-quality semen is chromosome specific.

Table 5 Comparisons (χ^2 test) between levels of disomy for chromosomes 1, 15, 18, and for both sex chromosomes in a population of spermatozoa derived from low-quality semen (total of samples 11–18); *n* the number of cells scored, *m* mean frequency (%)

	XY+XX+YY $n = 6{,}297$ $m = 0.90$	XY $n = 6.297$ $m = 0.74$	Chromosome 18 $n = 10{,}010$ $m = 0.85$	Chromosome 15 $n = 10{,}047$ $m = 1.67$
Chromosome 1 $n = 10{,}006$ $m = 1.37$	$\chi^2 = 7.1$ $P < 0.01$	$\chi^2 = 13.5$ $P < 0.001$	$\chi^2 = 11.5$ $P < 0.001$	$\chi^2 = 3.4$ $P > 0.05$
Chromosome 15 $n = 10{,}047$ $m = 1.67$	$\chi^2 = 17.5$ $P < 0.01$	$\chi^2 = 26.5$ $P < 0.001$	$\chi^2 = 27.3$ $P < 0.001$	–
Chromosome 18 $n = 10{,}010$ $m = 0.85$	$\chi^2 = 0.08$ $P > 0.70$	$\chi^2 = 0.68$ $P > 0.30$	–	–

Table 6 Frequencies (%) and numbers (in *parentheses*) of cells showing mono-disomy for chromosomes 18, XY, X and Y, and double disomy for chromosomes 18 and XY in cell samples from low-quality semen (samples 16–18 and 31–34), following three-color FISH with specific probes for chromosomes 18, X and Y

Sample no.	No. of cells screened	Disomy 18	Disomy XY	Disomy X	Disomy Y	Double disomy 18+XY
16	2031	0.64 (13)	0.44 (9)	0.10 (2)	0.10 (2)	0.15 (3)
17	1997	0.90 (18)	0.90 (18)	0.10 (2)	0.10 (2)	0.00 (0)
18	2001	0.75 (15)	0.70 (14)	0.15 (3)	0.15 (3)	0.10 (2)
31	2004	0.80 (16)	0.75 (15)	0.10 (2)	0.25 (5)	0.20 (4)
32	2011	1.24 (25)	0.79 (16)	0.05 (1)	0.15 (3)	0.20 (4)
33	2010	0.89 (18)	0.84 (17)	0.10 (2)	0.10 (2)	0.15 (3)
34	2008	1.00 (20)	0.75 (15)	0.10 (2)	0.05 (1)	0.05 (1)
Mean ± SE	14062	0.89 ± 0.07	0.74 ± 0.05	0.10 ± 0.01	0.13 ± 0.02	0.12 ± 0.03

In addition, in cells from low-quality semen, the hybridization efficiencies for chromosomes 1 and 15 (means of 99.4 and 99.9, respectively) were similar to those obtained for chromosomes XY and 18 (means of 99.9 and 99.2, respectively).

The significant differences between various chromosomal pairs in the rate of ND exclude the possibility that the disomic cells found in the low-quality semen samples are in fact diploid cells. Indeed, a three-color FISH assay, allowing the simultaneous identification of chromosomes X, Y and 18, confirmed the high rates of disomy 18 and XY in cells from poor-quality semen, and showed clearly that each occurred in a separate cell (Table 6).

Discussion

Sex cells of males with high-quality semen showed low levels of disomy for chromosomes 1, 15, 18, X and Y. These levels appear especially low when compared with those found in sex cells of males with proven fertility although not classified according to their semen quality (for references see Tables 2, 3). In contrast, the levels of disomy obtained in sex cells derived from low-quality semen (varicocele patients) were very high: The rates of ND in all four chromosomal pairs studied (pairs 1, 15, 18 and XY) were much higher than those previously reported for sex cells of either fertile or infertile males (for references see Tables 1, 2). Compared with high-quality semen the mean disomy rate per chromosome in sex cells of low-quality semen was tenfold higher (0.12% vs. 1.19%). Assuming that the four chromosomal pairs tested are representative of other pairs, then the risk for ND among varicocele patients is indeed very high.

The increased rate of ND in varicocele patients is not associated specifically with the varicocele phenotype, but rather with low-quality semen in general (Finkelstein et al. in preparation). In this respect, it is interesting to refer to males suffering from Hodgkin's disease. These patients, even if they have fathered children, usually show reduced semen quality at the time of diagnosis, prior to any physical or chemical treatment (reviewed in Botchan et al. 1997). It has recently become evident that Hodgkin's patients also show elevated rates of ND in their sex cells at diagnosis prior to any clinical treatment (Robbins et al. 1997; Finkelstein et al. in preparation).

The relationship between semen quality and ND is further demonstrated in two recent studies dealing with the risk of sex chromosome ND in spermatogenesis of 47,XYY males. These studies, both relying on FISH assays, arrived at different findings: one, using sperm of an individual with poor semen quality, showed that the trisomy status in the germ cells leads to elevated rates of XY disomy in sex cells (Blanco et al. 1997); the second, using sperm samples derived from two subjects having normal semen parameters, showed no increase in XY disomy in their sex cells (Chevret et al. 1997). Thus, the close relationship between the rate of ND and semen quality, is evident even with the background of a trisomic chromosomal complement.

Semen quality in humans shows tremendous interindividual variability as well as intraindividual changes (Carlsen et al. 1992; Auger et al. 1995; Olsen et al.1995; Sherins 1995). Furthermore, infertile males, similar to males with proven fertility, show a large inter- and intraindividual variation in this trait (Meschede et al. 1995).

Variation in semen quality among males with proven fertility can account for the wide interindividual variation in disomy rate found in various studies using either the FISH assay (Robbins et al. 1993; Bischoff et al. 1994; Miharu et al. 1994; Moosani et al. 1995) or the sperm karyotyping complementation technique (Templado et al. 1996). Similarly, the wide interstudy variation in the rate of ND in males classified according to their reproductive status can be explained on the basis of variation in semen quality between sperm donors (for review of FISH studies see Tables 2, 3, and Blanco et al. 1996; for review of sperm karyotyping studies see Templado et al. 1996). Indeed, our data show that men with a given semen quality – high or low – show a narrow range of interindividual variation in the rate of disomy.

In cells of poor-quality semen the high rates of sex chromosome disomy resulted from ND of chromosomes X and Y rather than from ND of their sister chromatids. If the behavior of the sex chromosomes is representative of all other chromosomal pairs, then the segregation errors in spermatogenesis of males with poor semen quality are generated at the first meiotic division, similar to most age-dependent errors in oogenesis (Abruzzo and Hassold 1995; Jacobs and Hassold 1995; Eichenlaub-Ritter 1996).

Molecular studies using polymorphic DNA markers showed that the age-dependent segregation errors at the

first meiotic division in female meiosis are associated with reduced recombination in the nondisjoint chromosomes. Thus far, reduced recombination has been documented in age-dependent maternally derived first meiosis trisomies of sex chromosomes (MacDonald et al. 1994), chromosomes 21 (Sherman et al. 1994), 16 (Hassold et al. 1995), 15 (inferred from uniparental disomy; Mascari et al. 1993; Robinson et al. 1993), and 18 (Fisher et al. 1995).

Assuming that the first meiosis errors in spermatogenesis are generated by a mechanism similar to the one underlying the first meiosis errors in oogenesis, then men with poor semen quality are expected to undergo reduced recombination, compared with males with high-quality semen. In this case, men with low-quality semen, when having the possibility of fathering children, pass to their offspring fewer recombinant chromosomes than men with high-quality semen, and thus may shorten the male genetic linkage map. Indeed, in paternally derived cases of 47,XXY, the two paternal sex chromosomes show reduced recombination in the pseudoautosomal region (Hassold et al. 1991).

Another theory suggests that the age-dependent segregation errors at the first meiotic division in females arise from incorrect release of distal chiasmata (Hawley et al. 1994; Lamb et al. 1996). Obviously, a distal chiasma, in contrast to a proximal or an interstitial one, leads to reduced recombination. Accordingly, the mechanism underlying age-dependent first meiosis ND errors in females is not based on alteration in the pattern of recombination. Rather it is based on reduced capability of the aged meoicyte to cope successfully with the more complicated configurations of distal chiasmata, which under optimal cellular conditions, are released adequately. Hence reduced recombination characterizes only the nondisjoint chromosomes and not the whole genome. If this theory is applied to male meiosis, meiocytes associated with the production of poor semen are expected to be less effective in ensuring first meiosis postrecombination events to proceed normally.

Alteration in recombination resulting from changes in number and localization of chiasmata, similar to nonscheduled and/or incorrect release of chiasmata, are most probably chromosome dependent and may vary from chromosome to chromosome. Thus both the aforementioned mechanisms can generate a chromosome-specific disposition for ND, found in our study in cells derived from semen of poor quality. The increased disposition for ND of chromosomes 1 and 15 found in cells of poor quality semen can be attributed to the presence of the large heterochromatic block on the former and the nucleolar organizer on the latter – two factors that were already suspected to interfere with prerecombination alignment and/or with postrecombination separation of homologs (reviewed in Spriggs et al. 1996). However, if meiocytes of males producing high-quality semen, in contrast to those associated with poor-quality semen, can cope successfully with pre- and/or postrecombination difficulties (discussed previously), then they are expected to escape the hampering effects of these two factors on proper chromosomal segregation. Indeed in cells of high-quality semen, chromosomes 1 and 15 showed low rates of ND, similar to those of chromosomes 18 and XY.

Be the mechanism as it may, it appears that men showing poor semen quality comprise a high-risk group for meiotic ND. Hence, semen quality, being a highly variable trait, should be considered in studies aiming to estimate the rates of ND in various groups of males. In addition, the association between low-quality semen and the increased risk for ND should be considered in clinical applications aiming to assist couples when the male shows poor semen quality. This is especially relevant in the clinical use of the intracytoplasmic sperm injection technique (Palermo et al. 1996).

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