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Variation in the frequency and type of sperm chromosomal abnormalities among normal men

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Summary. The chromosomal constitution of 1582 human sperm from 30 normal men of proven fertility was investigated after sperm penetration of hamster eggs. A minimum of 30 sperm chromosome complements were analysed per donor so that the distribution and variation in the frequency and type of sperm chromosomal abnormalities could be assessed. The mean frequency of sperm chromosomal abnormalities in individual men was 10.4% (± 6.0%) with a range of 0–24.7%. For numerical abnormalities the mean was 4.7% (± 2.9%) with a range of 0–10% and for structural abnormalities the mean was 6.2% (± 6.0%) with a range of 0–23.1%. The 95% confidence intervals for the mean of an individual male were 0–10.5% for numerical abnormalities, 0–18.2% for structural abnormalities, and 0–22.4% for total abnormalities. There was a significant excess of hypohaploid complements compared with hyperhaploid complements. Since hypohaploid complements could be caused by technical artefact, a conservative estimate of aneuploidy was obtained by doubling the frequency of hyperhaploid sperm, yielding an estimate of 2.4% aneuploidy. The proportion of X-bearing (53%) and Y-bearing (47%) sperm did not differ significantly. These results were compared to the other two large studies of sperm chromosome complements from normal men.

Introduction

The frequency of chromosomal abnormalities in human gametes has been a topic of interest and speculation for many years. Investigators have extrapolated from information on liveborn offspring and spontaneous abortions to estimate the frequency of chromosomal anomalies in human gametes and at conception (Jacobs 1971; Boue et al. 1975; Roberts and Lowe 1975; Hook 1980).

In the last few years direct information on the chromosomal constitution of human sperm, oocytes, and early embryos has become available because of access to unfertilized and fertilized human oocytes from in vitro fertilization clinics and because of the introduction of a new technique that allows analysis of human sperm chromosomes after penetration of hamster eggs (Rudak et al. 1978). These new developments have provided an opportunity to determine the actual frequency of chromosomal abnormalities in human gametes and data to study the mechanisms underlying the production of chromosomal anomalies and the factors that influence their frequency.

To date the data from in vitro fertilization clinics has been based on very small numbers because of the scarcity of the material and because of the ethical dilemmas involved in studying human embryos (Angell et al. 1983; Michelmann and Mettler 1985; Rudak et al. 1985; Zenzes et al. 1985; Martin et al. 1986). More information has been generated from the study of human sperm complements using the hamster egg system, but this research is still in its infancy since only three laboratories have reported on large numbers of human sperm complements (Martin et al. 1983; Brandriff et al. 1985; Kamiguchi and Mikamo 1986). Moreover, these studies have been performed on small numbers of men. We studied 33 normal men (Martin et al. 1983); Brandriff et al. (1985) studied 11 normal men; and Kamiguchi and Mikamo (1986), only 4 men. Thus it has not yet been possible to determine adequately the variation in the frequency of sperm chromosomal abnormalities among normal men.

The major problem with our study of 1000 human sperm complements from 33 normal men (Martin et al. 1983) was the large variation in the number of sperm complements analysed per male. Since the range in the number of sperm complements analysed per donor was 4–134, we could not assess the variation in the frequency of chromosomal abnormalities among males adequately. To overcome this problem, we have studied 30 donors with a minimum sample size of 30 sperm karyotypes per donor. This report presents our results on the variation in the frequency and type of sperm chromosomal abnormalities in these 30 normal men.

Materials and methods

Thirty healthy men of proven fertility participated in the study. The men had no history of radiotherapy, chemotherapy, or exposure to a recognized mutagen or clastogen. Seventeen of the men were new donors and 820 sperm chromosome spreads were analysed from these new donors. The other 13 donors were previously studied and reported (Martin et al. 1983).

The techniques for human sperm and hamster egg preparation, culture of fertilized eggs, and chromosome preparation have been described previously (Martin 1983). The chromosomes were Q-banded with quinacrine dihydrochloride.

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Table 1. Individual results of sperm chromosomal analysis

Donor	Age	No. of sperm analysed	Total no. X	Total no. Y	No. (%) abnormal	Abnormal complements
1	22	55	36	18	3 (5.5%)	24,X,+G 22,−C or −Y 23,X,cbs(X)(q)
2	24	73	37	36	3 (4.1%)	24,Y,+C 24,Y,+16,cte:(B,F):dic,qr,ctg(2)(q) 22,Y,−G
3	22	41	24	17	4 (9.6%)	24,X,+C 24,Y,+2B,−D 24,Y,+16 21,X,−3,−7,−13,dic(3;7),+2ace
4	24	68	34	34	9 (13.2%)	24,X,+21 22,Y,−5 22,Y,−15 22,Y,−16 22,Y,−20 23,X,+ace 23,X,+ace 23,X,Cp^- 23,Y,−20,+mar
5	24	38	21	17	3 (7.9%)	24,X,+21 24,Y,+C 23,Y,csb(F)
6	26	71	33	38	4 (5.6%)	24,XX 22,X,−E 23,Y,csb(7)(q) 23,Y,csb(14(q2)
7	29	31	15	16	1 (3.2%)	22,Y,−15
8	29	72	33	39	7 (9.7%)	24,Y,+22 22,X,−7,+ace 22,Y,−12 22,X,−17 23,X,cte(1;14):tr,cte(3;5):tr 23,Y,+ace 23,X,−2,−3,+mar,+ring,+3ace
9	28	35	20	15	3 (8.6%)	22,X,−D 22,Y,−18 22,Y,−20
10	25	73	34	39	5 (6.8%)	22,Y,−12 22,Y,−12 22,Y,−21 22,Y,del(15)(q21→qter) 23,X,csb(22)(q13)
11	31	37	25	12	0 (0%)	
12	32	137	68	68	16 (11.7%)	24,X,+B 24,Y,+9 25,Y,+3,+15 24,X,+21 24,Y,−14 22,X,−F 22,Y,−C 21,−C or −Y,−G,ctg(3) 23,X,csb(9)(q1) 23,X,ctg(C)(q) 23,Y,cte(B;D):dic,qr 23,X,9q−,+2ace 23,Y,csb(5)(q3) 23,X,t(2;13)(q33;q33) 23,Y,csb(3) 23,X,csb(13)(q22)
13	30	51	29	22	3 (5.9%)	22,X,−22 23,Y,csb(10)(p13) 23,X,t(9;14)(p22;q22)
14	30	30	14	16	1 (3.3%)	23,X,cbs(C)(q)

Table 1 (continued)

Donor	Age	No. of sperm analysed	Total no. X	Total no. Y	No. (%) abnormal	Abnormal complements
15	32	121	67	54	9 (7.4%)	24,X,+E,del(3)(p2→pter) 24,Y,+1 24,Y,+C (2 cells) 22,X,−13 21,X,−1,−13 21,X,−18,−22 23,X,csg(3)(p21) 23,Y,csb(7)(q11)
16	35	44	24	19	4 (9.1%)	23,−X or −Y,+2 22,Y,−16 21,X,−11,−17 23,Y,+ace
17	35	50	25	25	3 (6.0%)	22,Y,−13 22,Y,−22 21,Y,−3,−7,csb(1)(q21)
18	38	81	39	41	20 (24.7%)	24,Y,+9 22,Y,−6 22,Y,−16 22,Y,−20 21,−C or −Y,−D,−G,1p,+mar,+9ace 22,X,−14 23,Y,cte(3;11):qr,dic 23,X,4q+ 23,X,ctg(7)(q) 23,Y,cte(4;12):qr,10q− 23,Y,csb(11)(q13),ctg(16)(q11) 23,X,ctg(12)(q13) 23,X,csb(20) 23,X,+ace 23,X,cte(4;12);dic,tr+ace 23,X,+ace 23,Y,+ace (3 cells) 23,Y,csb(19)
19	35	34	14	20	6 (17.6%)	24,X,+G 22,X,−22 23,Y,dic(3)(8p),+ace(8q),+ace 23,Y,−12,9q−,+mar,+3ace 23,Y,csb(4)(cen) 22,Y,−20,Dq−
20	37	41	18	22	4 (9.8%)	22,Y,−20 22,−X or −Y 23,Y,+ace 23,Y,csb(4)(q21)
21	41	72	39	32	5 (6.9)	22,−X or −Y,del(9)(p22→pter) 23,X,csg(1)(q22) 23,X,cte(2;16):qr 23,Y,dic(4;12)(p16;q14),+ace(12q14→qter) 23,X,csb(8)(q22)
22	40	30	20	10	3 (10.0%)	23,X,+ace 23,Y,csb(1)(q12) 23,Y,csb(8)(q)
23	44	39	27	11	6 (15.4%)	22,−X or −Y 22,Y,−D 23,X,csb(19) 23,X,+ace (3 cells)
24	40	42	23	19	4 (9.5%)	22,Y,−18 21,X,−7,−8 23,X,cbs(2)cen,del(4)(cen→pter) 23,Y,cbs(6)(q1)
25	42	30	20	10	3 (10.0%)	22,X,−19 23,Y,csb(1)(p31) 23,Y,+ace

Table 1 (continued)

Donor	Age	No. of sperm analysed	Total no. X	Total no. Y	No. (%) abnormal	Abnormal complements
26	55	30	13	17	5 (16.7%)	22,X,−7 22,X,−22 21,Y,−B,−22 23,X,ctb(4;5):qr,+ace 23,Y,csg(3)(p2),csg(16)(q22),csg(19)(q13), csg(22)(q11),cte(4;11):dic,qr
27	45	30	15	15	3 (10.0%)	22,Y,−19 21,X,−B,−13 23,Y,+ace
28	51	30	15	15	6 (20.0%)	22,X,−19 23,Y,ctg(12)(q15) 23,Y,csb(16)(cen) 23,X,csb(1)(q21) 23,Y,csb(14)(q23) 23,Y,+2ace
29	50	44	20	24	8 (18.2%)	23,X,csb(4)(q23) 23,Y,ctg(16)(q) 23,X,csb(1)(p31) 23,X,csg(6)(q15) 23,X,csg(1)(q21),ctg(4)(q),ctg(5)(q),6q−,+−20,+marF 23,Y,csg(2)(q31) 23,Y,csb(2)(q31) 23,Y,csg(11)(p14),csb(14)(cen)
30	45	52	24	28	12 (23.1%)	21,Y,−12,−D,csg(17)(q21) 23,X,csb(2)(p23) 23,Y,csb(2)(q12) 23,X,csg(3)(p21) 23,Y,csg(3)(q21) 23,Y,csb(5)(q14) 23,Y,csb(5)(p12) 23,Y,csb(9)(p11) 23,Y,csb(12)(q23) 23,Y,2csb(Y)(cen+q11) 23,X,multiple breaks and rearrangements 23,Y,multiple breaks and rearrangements

Abnormal chromosome spreads were verified by a microscope analysis of the region surrounding the egg to eliminate artefacts from broken cells and by karyotyping the hamster chromosome complement to ensure normality. Structural aberrations were classified according to the international system (ISCN 1978). If a sperm complement had both numerical and structural abnormalities, the abnormalities were counted in each of these two categories. If a sperm contained more than one numerical abnormality, it was counted only once as an aneuploid sperm. Similarly, if a sperm contained more than one structural abnormality, it was also counted only once. A chromosome gap (csg) was distinguished from a chromosome break (csb) by the width of the staining discontinuity, using the rule that breaks were separated by five or more chromatid widths and by lateral displacement. Spreads were said to have a deletion (del) when a given chromosome had a terminal deletion and the missing chromosomal piece could not be identified in the complement. Acentric fragments (ace) were acentric pieces of chromosomes that could not be identified.

Results

This study is based on 1582 sperm chromosome complements from 30 normal men. The age of the men varied from 22 to 55 years with a mean of 34.7 years. The ability of sperm from individual donors to penetrate hamster eggs varied from 10% to 100% (proportion of hamster eggs penetrated), with a mean of 48%. The mean number of sperm complements analysed per donor was 53, with a range of 30–137.

The individual results of sperm chromosomal analysis are shown in Table 1. Examples of normal and abnormal sperm complements are shown in Figs. 1 and 2, respectively, The results have been analysed in two different ways. First the types of abnormalities in the entire sample were studied, as a basis for comparison with our previous study (Martin et al. 1983). Then the data from the individual males were analysed to describe this population of 30 normal men and compare our results with those of Brandriff et al. (1985) and Kamiguchi and Mikamo (1986).

A summary of the types of numerical and structural abnormalities observed in the total sample is presented in Table 2. The mean frequency of sperm chromosomal abnormalities was 10.3% with 4.7% being numerical abnormalities and 6.2% being structural abnormalities. Ten sperm complements contained both numerical and structural abnormalities, and these were counted in each category. There was a significant excess of hypohaploid complements compared with hyperhaploid complements $\chi^2 = 14.9$, $P < 0.001$). Since hypohaploid complements could be caused by technical artefacts, a con-

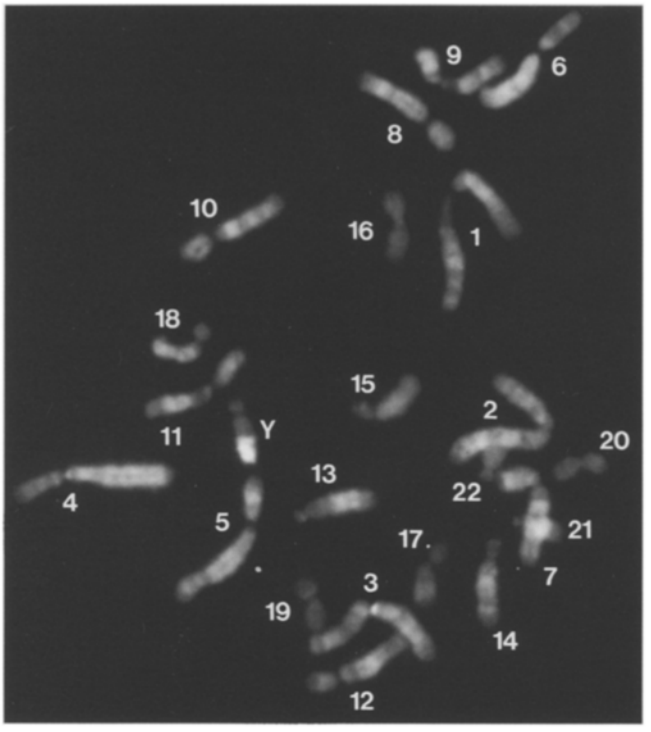

Fig. 1. Chromosome spread of a normal human spermatozoon with a 23,Y complement (×3000)

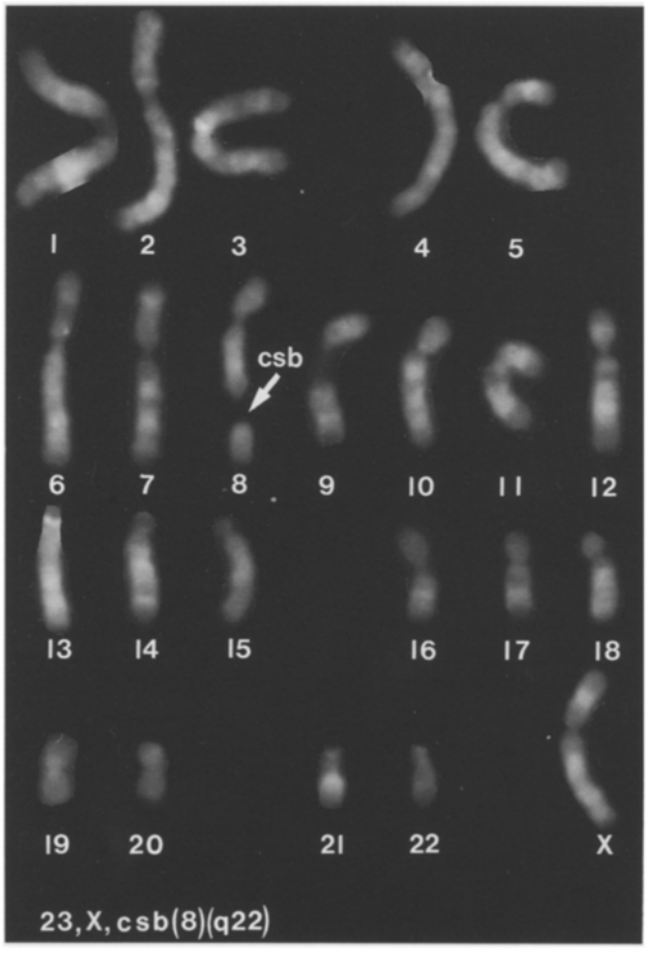

Fig. 2. Karyotype of an abnormal human spermatozoon with a 23,X,csb8(q22) complement (×3000)

Table 2. Summary of types of abnormal complements in the total sample of 1582 human sperm

Total abnormal	163	(10.3%)	
Numercial abnormalities[a]	75	(4.7%)	
Hyperhaploid	20	(1.3%)	19 ($N+1$) 1 ($N+2$)
Hypohaploid	53	(3.4%)	43 ($N-1$) 9 ($N-2$) 1 ($N-3$)
Multiple aneuploidy	2	(0.1%)	
Structural abnormalities[a]	98	(6.2%)	
Chromosome break	35	(2.2%)	
Chromosome gap	11	(0.7%)	
Fragment	19	(1.2%)	
Deletion	7	(0.4%)	
Duplication	1	(0.06%)	
Translocation	2	(0.1%)	
Dicentric	3	(0.2%)	
Complex exchanges (including chromosome and chromatid)	14	(0.9%)	
Chromatid gaps	3	(0.2%)	

[a] 10 sperm complements contained both numerical and structural abnormalities and these were counted in each of the categories

Table 3. Descriptive statistics of sperm chromosomal abnormalities in the individual men

	Mean (± SD)	95% confidence interval for mean individual male
Total abnormalities	10.4% (± 6.0%)	0%–22.4%
Numerical abnormalities[a]	4.7% (± 2.9%)	0%–10.5%
Hyperhaploid sperm	1.2% (± 1.6%)	0%– 4.4%
Hypohaploid sperm	3.6% (± 2.7%)	0%– 9.0%
Structural abnormalities[a]	6.2% (± 6.0%)	0%–18.2%

[a] Sperm complements that contained both numerical and structural abnormalities were counted in each of these categories

servative estimate of aneuploidy can be obtained by doubling the frequency of hyperhaploid sperm. There were 20 hyperhaploid complements and two complements that had both extra and missing chromosomes. Doubling the frequency of these two categories yielded an estimate of aneuploidy of 2.8%. The majority of structural abnormalities were chromosome breaks (2.2%), although chromosome gaps, fragments, deletions, duplications, translocations, dicentrics, complex exchanges, and chromatid gaps were also present.

A summary of the descriptive statistics for the individual men is presented in Table 3. The range in the total frequency of sperm chromosomal abnormalities for the individual men was 0–24.7%, with a mean of 10.4% and a standard deviation of 6.0%. The 95% confidence interval for the mean of an individual man was 0–22.4%. For numerical abnormalities the mean was 4.7% with a standard deviation of 2.9%, a range of 0–10% and a 95% confidence interval for the mean of an individual male of 0–10.5%. The mean frequency of hyperhaploid sperm in the individual donors was 1.2% with a standard deviation of 1.6%, a range of 0–5.3%, and a 95% confidence interval of 0–4.4%. These rates were doubled to provide an estimate of the mean frequency of aneuploidy in the individual men, with a mean of 2.4% (± 3.2), a range of 0–10.6%, and a 95% confidence interval of 0–8.8%. For structural abnormalities the mean was 6.2% with a standard deviation of

6.0%, range of 0–23.1% and a 95% confidence interval of 0–18.2%. These descriptive statistics are summarized in Table 3. Based on Z tests for skewness and kurtosis, the data were normally distributed in this population for the total frequency of sperm chromosomal abnormalities, for complements with numerical abnormalities, and hypohaploid complements. For structural abnormalities and hyperhaploid complements, the skewness was significantly different from 0, with the majority of donors having a low frequency of structural abnormalities and more than half of the donors having no hyperhaploid complements.

The mean proportion of X-bearing sperm was 53% with a standard deviation of 8.1%. Based on a Z test for skewness and kurtosis the proportion of X-bearing sperm in these 30 men was normally distributed. The proportion of X-bearing (53%) and Y-bearing (47%) sperm was not significantly different from a one-to-one ratio ($P = 0.09$, $Z-1.7$, one sample Z test with a continuity correction).

Discussion

Our total frequency of sperm chromosomal abnormalities in this study (10.3%) was similar to the frequency (8.5%) obtained in our last study of normal men (Martin et al. 1983). All three large studies of chromosomal abnormalities in human sperm from independent laboratories have reported similar overall frequencies of abnormalities: 9.4% in the study by Brandriff et al. from the USA (1985), 13.9% in the study by Kamiguchi and Mikamo from Japan (1986), and a mean of 10.4% for individual men in this study from Canada. Therefore an estimate of the mean frequency of chromosomal abnormalities in sperm might be approximately 10%.

The frequency of numerical abnormalities in this study (4.7%) was also similar to the 5.2% found by our previous study (Martin et al. 1983), but the ratio between hyperhaploid and hypohaploid complements differs. In our last study the frequency of hyperhaploid and hypohaploid complements was not significantly different; in this study there was a significant excess of hypohaploid complements. This excess was most likely caused by technical artefact. Different technicians were employed for the two studies, and it is possible that slight differences in the technique for chromosome fixation (for example, blowing on the slide) could increase the likelihood of chromosome loss. Thus, for those laboratories using Tarkowski's technique (1966) for chromosome fixation, it is best to estimate the frequency of aneuploidy by doubling the frequency of hyperhaploidy. In this study the frequency of hyperhaploid complements in the total sample was 1.3%, and a conservative estimate of aneuploidy is 2.8% (including sperm with multiple aneuploidy). A comparison of the mean frequency of hyperhaploid sperm complements in the individual men in the three large studies demonstrates considerable similarities. Brandriff et al. (1985) reported 0.7% hyperhaploid complements (range 0–2.2%), Kamiguchi and Mikamo (1986) reported 0.5% (range 0–1.1%) and we have a mean of 1.2% (range 0–5.3%) in the individual men in our study. This suggests that an estimate of the mean frequency of aneuploidy in human sperm is approximately 1%–3%. The range in the frequency of hyperhaploid sperm was quite small in all three studies. It is to be expected that the range in our study would be the highest, since we have studied a larger number of donors than Brandriff et al. (1985) or Kamiguchi and Mikamo (1986).

The frequency of structural abnormalities in this study (6.2%) was higher than in our previous study (3.3%, Martin et al. 1983). In all three large studies of human sperm chromosome complements there has been more variation in the frequency of structural abnormalities than numerical abnormalities among males. Brandriff et al. found a mean frequency of 7.7% structural abnormalities with a range of 1.9%–15.8% in 11 donors; Kamiguchi and Mikamo reported 13.0% with a range of 10.8%–17.4% in 4 donors; and we found a mean frequency of 6.2% with a range of 0%–23% in 30 donors. There is some concern that structural abnormalities might be caused by the culture system. This is particularly true of chromatid-type aberrations. However, chromatid-type aberrations comprise a minority of the structural aberrations in all three large studies: we determined that 10% of all structural abnormalities were of the chromatid type (chromatid gaps and chromatid exchanges). Brandriff et al. (1985) found 13% chromatid-type aberrations, and Kamiguchi and Mikamo found 20%. The most common type of structural aberration in all three studies has been chromosome breaks: 36% in this study, 55% in the study by Brandriff et al. (1985), and 45% in the study by Kamiguchi and Mikamo (1986). It is unlikely that the culture system caused the structural abnormalities in the human sperm chromosomes since in a comparison of 1000 chromosomal complements of hamster egg and human sperm pronuclei, we found only 0.5% structural abnormalities and no chromosome breaks in 1000 hamster egg spreads compared to 3.3% structural abnormalities (of which 67% were chromosome breaks) in human sperm complements within the same fertilized eggs (Martin 1984). Of course, it is possible that the structural abnormalities were produced in the human sperm and not in the hamster egg chromosomes because of the foreign environment in which the human sperm DNA had to decondense and replicate. To test this hypothesis, it would be necessary to analyse chromosomes of other species after penetration of hamster eggs. This research has been hampered by the lack of standard in vitro methods to capacitate sperm of other species. However, a number of publications have indicated success with in vitro capacitation and penetration of hamster eggs by bull sperm (Bousquet and Brackett 1982; Graham et al. 1986), guinea pig sperm (Yanagimachi 1972) and mouse and rat sperm (Hanada and Chang 1972). If these studies are extended to pronuclear chromosomal analysis of the sperm from these various species, we may be able to determine whether structural chromosomal abnormalities in sperm are induced by this culture system. Another line of evidence that suggests that the structural chromosomal abnormalities are inherent in the sperm and not induced by the hamster egg milieu is the fact that individual males have significantly different frequencies of structural abnormalities. This has been confirmed in all three large studies. In this study the range in the frequency of structural aberrations was 0–23% for individual males. If the hamster egg technique were inducing the abnormalities, a less variable frequency of abnormalities among men would be expected. Also Brandriff et al. (1985) have demonstrated that, for two donors, the frequency of chromosomal aberrations remained stable after an elapsed interval of over 1 year. Thus the frequencies of structural abnormalities appear to be donor-specific and may reflect genetic differences or varying exposures to clastogens.

The range in age in our 30 donors was 22–55 years. In another report (Martin and Rademaker 1987) we have

analysed the relationship between the frequency of sperm chromosomal abnormalities and the age of the donor. We found a significant negative correlation between age and the frequency of hyperhaploid complements and a highly significant positive correlation between structural chromosomal abnormalities and age. Thus, with increasing age there was a decreased risk of aneuploid sperm and an increased risk of sperm with structural chromosomal abnormalities. The differences in the frequencies of abnormal sperm reported in this study compared with our previous study (Martin et al. 1983) or the studies of Brandriff et al. (1985) and Kamiguchi and Mikamo (1986) may be explained in part by the different ages of the men studied. In our previous study (Martin et al. 1983) the mean frequency of hyperhaploidy was 2.4% and the mean frequency of structural chromosomal abnormalities was 3.3%. The mean age of this population was 29 years. In this study the mean age was 34.7 years and the mean frequency of hyperhaploidy (1.3%) was lower and the mean frequency of structural abnormalities (6.2%) was higher. This is consistent with our findings of a decreased frequency of hyperhaploidy and an increased frequency of structural chromosomal abnormalities with age. Brandriff et al. (1985) reported a similar mean age to our present study (32.9 years) and also a similar mean frequency of hyperhaploidy (0.7%) and structural chromosomal abnormalities (7.7%). Kamiguchi and Mikamo (1986) reported on only four men and did not include the mean age of these men.

The frequency of X-bearing sperm and Y-bearing sperm was 53% and 47%, respectively, in this study. Kamiguchi and Mikamo (1986) found the same ratio of X- and Y-bearing sperm. Brandriff et al. (1985) found almost exactly 50% of each type of sperm. In all three studies the ratio of X- and Y-bearing sperm did not differ significantly from the expected one-to-one ratio.

In summary, we have determined the distribution and variation in the frequency and type of chromosomal abnormalities in sperm from 30 normal men. Results from this study were compared to the results of two other large studies of human sperm chromosome complements. Hopefully studies from other laboratories will soon be reported to permit further comparisons of different populations of normal men.

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