ANDROLOGY

The Effects of Age and Abnormal Sperm Count on the Nondisjunction of Spermatozoa

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Purpose: *The effect of paternal age on the nondisjunction of sex chromosomes is controversial. Also, the prevalence of chromosomal anomalies in infertile patients is controversial, it has been reported that the sex chromosomal aneuploidy rate following treatment with intracytoplasmic sperm injection (ICSI) is higher than in naturally conceived pregnancies. We investigated the influence of paternal age and oligozoospermia on the nondisjunction of spermatozoa.*
Methods: *We determined the rate of aneuploidy for gonosomes and autosomes, using two-color fluorescence in situ hybridization (FISH) of the X and Y chromosomes and chromosomes 12 and 18 in 10 donors under 25 years of age who had a normal sperm count ($\geq 20 \times 10^6$/ml), 10 donors over the age of 39 years with idiopathic infertility and normozoospermia ($\geq 20 \times 10^6$/ml), and 5 oligozoospermic donors ($< 20 \times 10^6$/ml).*
Results: *There was no obvious relationship between increasing age and autosomal disomy (disomy 12 and disomy 18). Neither autosomal disomy nor diploidy was increased in any group. The frequency of X-, Y-, XX-, and YY-bearing sperm did not differ significantly among groups, but the frequency of XY-bearing sperm was significantly higher in the older infertile group than in the control donors.*
Conclusions: *The incidence of nondisjunction of paternal sex chromosome in meiosis I was higher in older men with idiopathic infertility. The present results suggest that the risk of producing XXY fetuses is higher among men >39 years of age with idiopathic infertility.*

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INTRODUCTION

Chromosomal aneuploidy is a common cause of spontaneous abortion and intrauterine fetal death. Because aneuploidy most often arises in germ cells, the prevalence of nondisjunction has been estimated by screening for aneuploidy in human germ cells. Human sperm chromosoma aberration has been investigated using the zona-free hamster egg penetration test (1), which can detect structural chromosomal aneuploidies. Fluorescence in situ hybridization (FISH) has recently been used to detect chromosomal aneuploidies in human spermatozoa. FISH allows cytogenetic analysis of a larger number of spermatozoa in a shorter period of time and does not require sperm-fertilizing ability. Furthermore, the use of multicolor FISH makes it possible to distinguish between meiosis I and meiosis II nondisjunction in sex chromosomes. Autosomal disomy can arise from nondisjunction in both meiosis I and meiosis II. In sex chromosomes, nondisjunction during meiosis I results in an XY disomy, whereas nondisjunction during meiosis II results in an XX disomy or a YY disomy.

Intracytoplasmic sperm injection (ICSI) has recently been used to treat male infertility. Because this method avoids natural sperm selection, it is important to investigate the aneuploidy sperm rate of ICSI-treated patients. Constitutional chromosomal abnormalities have been found in 4.6–10.6% of oligozoospermic patients (2–4). Cytogenetic analysis has shown that the incidence of sex chromosome aberrations is signifi-

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cantly increased in fetuses conceived by ICSI (5,6). These observations suggest that oligozoospermic patients should be counseled about the risk of transmitting de novo chromosomal aberrations and fertility problems to their offspring. To our knowledge, four previous studies have examined the incidence of disomic spermatozoa in infertile men. Although Guttenbach *et al.* (7) and Miharu *et al.* (8) reported that the incidences of disomy and diploidy were no higher in infertile patients than in healthy males, Moosani *et al.* (9) found a significant increase in the frequencies of disomy on chromosome 1 and XY disomy in chromosomaly normal infertile men. Bernardini *et al.* reported that the frequency of disomic sperm chromosomes 1, 17, and sex chromosomes was significantly higher in patients with severe oligoasthenoteratozoospermia than in fertile donors and patients with idiopathic infertility (10).

The issue of whether older men are at an increased risk of producing aneuploid offspring remains unresolved (11, 12). Studies of 21 trisomy have suggested that maternal age is related to the etiology of nondisjunction (13). The incidence of trisomic pregnancies is 2–4% among women aged 20 to 25 years compared with approximately 33% among women aged 43 years or older (14). The origin of the supernumerary chromosome of the trisomy (21 trisomy, 16 trisomy, 13 trisomy, XXX, and XXY) has been investigated in DNA marker studies (15–17). The results of studies examining the effect of paternal and maternal age are conflicting. MacDonald *et al.* (18) reported that the incidence of 47,XXY increased significantly with increases in maternal age but was not associated with the mean paternal age. In contrast, Lorda-Sanchez *et al.* (19) reported that the incidence of paternally derived 47,XXY increased significantly with increases in paternal age. Paternally derived trisomy is relatively rare. Therefore disomic human sperm is a better source for investigating the effect of paternal age on the etiology of nondisjunction (11). Although the majority of maternally derived trisomies are believed to be related to maternal meiosis I errors, the majority of paternally derived trisomies appear to result from paternal meiosis II errors. FISH analysis of sperm nuclei in the gonosomal chromosomes could provide information regarding the meiotic stage of sex chromosomal nondisjunction during spermatogenesis.

In the present study, we used FISH to elucidate the effect of paternal age and the sperm count on the occurrence of chromosomal nondisjunction and to determine which meiotic stage of sex chromosomal nondisjunction is related to paternal age.

MATERIALS AND METHODS

Semen Sample

Semen samples were obtained from 25 male donors: 10 healthy donors under the age of 25 years, 10 patients over the age of 39 years with idiopathic infertility, and 5 donors with oligozoospermia ($<20 \times 10^6$/ml). All subjects gave consent for the use of semen specimens to be used for research purposes. Idiopathic infertility was diagnosed when a clinical pregnancy was not achieved after at least 2 years of sexual activity with a partner whose fertility workup was normal. Sperm shape abnormalities, sperm motility (>50%), and sperm concentrations ($\geq 20 \times 10^6$/ml) (20) were within the normal ranges in both healthy donors and patients with idiopathic infertility. Abnormalities in sperm shape were within the normal range in the oligozoospermic patients in this study, but the sperm concentration was abnormal (5×10^6 to 20×10^6/ml).

Preparation of Spermatozoa

Semen samples were washed three times in a phosphate-buffered saline (PBS) solution and twice with ethanol:glacial acetic acid (3:1). The cell pellet was diluted again with ethanol:glacial acetic acid (3:1) and air-dried on slides.

Decondensation of the sperm is essential for in situ hybridization. The DNA of human spermatozoa is tightly compacted in the nucleus with protamines, arginine-rich nuclear proteins which are specific to spermatozoa. Proper decondensation of sperm chromatin is necessary for FISH. Decondensation can be performed with a number of agents, including dithiothreitol (DTT), Triton X-100, lithium diidosalicylate, and trypsin (21–26). In the present study, decondensation was performed with 25 m*M* DTT (Sigma, St Louis, MO). Slides were incubated for 5 to 60 min in a freshly made solution of 25 m*M* DTT. We did not use trypsin to preserve spermatozoa morphology.

Preparation of Mitotic Chromosome Spreads

Mitotic chromosomes from normal male and female peripheral blood lymphocytes were used as controls to examine the accuracy of the FISH procedure for each DNA probe.

DNA Probes

DNA probes were purchased from Oncor, Inc. (Gaithersburg, MD). Probes were labeled with either

biotin or digoxigenin. Y chromosome-specific α-satellite DNA (DYZ3, digoxigenin labeled) and X chromosome-specific α-satellite DNA probes (DXZ1, biotin labeled) were used for gonosomes. Chromosome-specific α-satellite DNA probes (D12Z3, biotin labeled, and D18Z1, digoxigenin labeled) were used for autosomes.

Fluorescence In Situ Hybridization

The FISH procedure was slightly modified from the original detection protocol of chromosome in situ hybridization system recommended by Oncor Inc. Slides were denatured in 70% formamide/2× standard saline citrate (SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate) for 2 min at 70°C and immediately dehydrated in cold (4°C) 70 and 100% ethanol solutions. The DNA probe (1 ml) (DYZ3 and DXZ1 or D12Z3 and D18Z1) was combined with 20 μl of Hybrisol VII (Oncor). The probe solution was denatured at 70°C for 5 min and immediately chilled in an ice bath. The probe solution (20 μl) was then placed on slides and covered with a coverslip. The preparations were incubated in a humidified chamber for 12 to 18 hr at 37°C. The slides were washed at 43°C in 65% formamide/2× SSC for 15 min, in 2× SSC for 8 min, and in 4× SSC for 5 min.

Biotin-labeled probes were detected with rhodamine–avidin (Biomeda, Foster City, CA; diluted 1:100 in 4× SSC and 1% BSA), and digoxigenin-labeled probes were detected with anti-digoxigenin-fluorescein (Boehringer Mannheim, GmbH; diluted 1:200 in 4× SSC and 1% bovine serum albumin). After being incubated for 45 min at 37°C with rhodamine–avidin and anti-digoxigenin-fluorescein, slides were washed with 4× SSC, 4× SSC/0.1% Tween 20, 4× SSC, and 2× SSC. Sperm nuclei were counterstained with 20 μl of DAPI (4′, 6′-diamidino-2-phenylindole; 1 μg/ml; Sigma, St. Louis, MO, USA).

Counting and Statistical Analysis

We examined randomly selected portions of the slide. Sperm nuclei were considered to be disomic for a specific chromosome if hybridization yielded two compact distinct signals of equal size separated by a distance of at least one diameter of the signal size within that cell (24). Sperm nuclei with indistinct margins or with diffuse signals were excluded from scoring. FISH preparations were analyzed under a fluorescent microscope (NIKON FXA) equipped with either an FITC/rhodamine/DAPI triple-band pass filter set, FITC single-band pass filter set, or rhodamine single-band pass filter set, each of which was selected for a different objective. Two-color FISH for gonosomes does not distinguish sex chromosome disomy from diploidy. Therefore, the sex chromosome disomy rate was adjusted based on an estimated meiotic error ratio of 1:3, which reflects the ratio at which diploidy occurs during meiosis I and meiosis II (8,27). The chi-square test was used to examine the ratio of X-to Y-bearing sperm. The distribution of signals scored for X, Y, 12, and 18 was assessed using Fisher's exact probability test. The Mann–Whitney *U* test was used to compare the disomy rates of donors under 25 years of age, donors over 39 years of age, and oligozoospermic patients.

RESULTS

Human lymphocyte mitotic chromosomes exhibited a signal in the centromeric region of both chromosome 12 and chromosome 18 in male and female preparations probed with biotin-labeled D12Z3 and digoxigenin-labeled D18Z1. When mitotic chromosome preparations were probed with digoxigenin-labeled DYZ3 and biotin-labeled DXZ1, signals were located in the centromere of the single X and single Y chromosome in male preparations. In female preparations, the signals were located in the centromeres of both X chromosomes.

We scored approximately 102,371 sperm nuclei in each semen sample. More than 2000 sperm nuclei in each DNA probe set were counted. X-bearing sperm accounted for 44.64 to 54.55% of the total sperm, and Y-bearing sperm accounted for 45.19 to 55.04% of the total sperm (Table I). The ratio of X- to Y-bearing sperm was 1:1 except for donors Y-5, Y-8, Y-10, A-3, A-5, and A-7 ($P < 0.05$). However, the mean ratio of X- to Y-bearing sperm was 1:1. The adjusted frequency of sex chromosomal disomy ranged from 0.04 to 0.17% for the XX disomy, from 0.00 to 0.19% for the YY disomy, and from 0.02 to 0.35% for the XY disomy (Table II). Nullisomic sperm for sex chromosomes ranged from 0.00 to 0.20%. In the absence of an internal control DNA probe (for autosomes), the excess number of nullisomic sperm was scored because it was not possible to differentiate between nullisomy and nonhybridization without an internal control. Since an internal probe was not used to detect gonosomal disomy, the nullisomic sperm rate for sex chromosomes was assumed to be higher than the exact rate (25).

Table I. Frequency of X, Y, XX, YY, and XY Sperm

Donor	Sperm cells (%)					
	X	Y	XX	YY	XY	Null[a]
<25 years old						
Y-1	47.54	52.13	0.09	0.09	0.14	0.00
Y-2	49.27	50.10	0.04	0.10	0.10	0.20
Y-3	51.91	48.07	0.09	0.05	0.09	0.09
Y-4	50.59	49.05	0.12	0.04	0.10	0.10
Y-5	54.22	45.19	0.15	0.15	0.16	0.08
Y-6	53.27	46.40	0.09	0.09	0.09	0.05
Y-7	48.32	50.92	0.19	0.09	0.19	0.09
Y-8	53.50	46.24	0.13	0.04	0.04	0.04
Y-9	51.70	47.93	0.05	0.01	0.09	0.09
Y-10	54.36	45.23	0.14	0.05	0.10	0.14
>39 years old						
A-1	52.23	47.06	0.09	0.23	0.37	0.00
A-2	51.41	48.01	0.10	0.14	0.29	0.05
A-3	44.64	55.04	0.08	0.04	1.16	0.04
A-4	47.74	52.81	0.15	0.09	0.16	0.05
A-5	54.55	45.10	0.10	0.05	0.15	0.05
A-6	52.06	47.38	0.09	0.19	0.24	0.05
A-7	54.16	45.40	0.13	0.09	0.18	0.04
A-8	52.68	46.76	0.09	0.14	0.24	0.09
A-9	47.62	52.10	0.05	0.09	0.09	0.05
A-10	47.40	52.13	0.19	0.10	0.14	0.04
Oligozoospermia patients						
O-1			0.10	0.03	0.06	
O-2			0.12	0.12	0.20	
O-3			0.33	0.22	0.33	
O-4			0.15	0.25	0.37	
O-5			0.03	0.03	0.06	

[a] Indicates sperm lacking a sex chromosome. Null sperm include monosomic sperm for sex chromosomes and hybridization failure.

The detected disomy rate ranged from 0.04 to 0.16% for chromosome 18 and from 0.04 to 0.21% for chromosome 12 (Table III); the diploid sperm rate ranged from 0.04 to 0.10%. No significant difference was found among donors in the distribution of disomy detected on chromosomes 12 and 18 and diploid sperm. The mean frequency of disomy for chromosome 12, chromosome 18, and the sex chromosomes is shown with the reported frequencies in Table IV. The most frequent disomy in the present study was the meiotic I sex chromosome disomy (XY disomy). The frequency of XY disomy was significantly higher than the frequencies of chromosome 12 disomy, chromosome 18 disomy, XX disomy, and YY disomy ($P < 0.05$). The overall frequency of sex chromosome disomies (XX disomy + YY disomy + XY disomy) was significantly higher than the frequency observed for disomy 12 and disomy 18 ($P < 0.01$).

Age had no effect on the frequencies of XX and YY disomies (Table II). The frequency of the XY disomy was higher in the older group than in the younger donors. The mean frequency of XY sperm in donors older than 39 years of age was significantly higher in donors younger than 25 years of age (Fig. 1).

DISCUSSION

Multicolor FISH is widely regarded as the gold standard for detection of disomic sperm (28) and has been used to determine the frequency of disomic sperm associated with chromosomes 12, 18, X, and Y (8, 11, 24, 25, 29–32) (summarized in Table IV). The reported and examined rate of disomy ranges from 0.02 to 0.35% and differs according to the chromosome and among donors. Human cytogenetic data also suggest that nondisjunction does not affect all chromosomes equally and that the chromosome-specific nondisjunction rate is related to sex (33). Several studies have shown that XY disomy is significantly more frequent than XX, YY, or autosomal disomy (11,30,32). The XY disomy was also significantly more frequent than autosomal disomy (chromosome 12 and chromosome 18), XX disomy, and YY disomy in the present study.

Table II. Adjusted Frequency of XX, YY, and XY Sperm in 20 Donors[a]

Donor	Sperm cells (%)		
	XX	YY	XY
<25 years old			
Y-1	0.05	0.05	0.12
Y-2	0.03	0.09	0.09
Y-3	0.08	0.04	0.08
Y-4	0.11	0.03	0.09
Y-5	0.14	0.14	0.15
Y-6	0.07	0.07	0.08
Y-7	0.18	0.08	0.18
Y-8	0.10	0.00	0.02
Y-9	0.04	0.00	0.08
Y-10	0.13	0.04	0.09
>39 years old			
A-1	0.05	0.19	0.35
A-2	0.08	0.12	0.28
A-3	0.06	0.02	0.15
A-4	0.14	0.07	0.15
A-5	0.09	0.04	0.20
A-6	0.07	0.17	0.23
A-7	0.11	0.07	0.17
A-8	0.08	0.13	0.23
A-9	0.04	0.08	0.08
A-10	0.17	0.08	0.13
Mann-Whitney *U* test, *P* value[b]	0.91	0.91	0.01

[a] The frequencies of disomy for sex chromosomes are adjusted by the diploid frequency.
[b] Mann–Whitney *U* test for difference between young donors (<25 years old) and older donors (>39 years old).

Table III. Aneuploidy Frequency for Chromosomes 12 and 18[a]

Donor	Disomic and monosomic sperm for chromosome 12 and 18(%)				
	(12,18,18)	(12,12,18)	(12,—)	(—,18)	(12,12,18,18)
Y-1	0.10	0.05	0.15	0.05	0.10
Y-2	0.09	0.09	0.05	0.00	0.09
Y-3	0.08	0.04	0.00	0.00	0.04
Y-4	0.12	0.05	0.00	0.10	0.05
Y-5	0.04	0.10	0.04	0.04	0.04
Y-6	0.10	0.10	0.00	0.00	0.05
Y-7	0.04	0.17	0.00	0.08	0.04
Y-8	0.12	0.04	0.04	0.04	0.10
Y-9	0.04	0.12	0.04	0.04	0.04
Y-10	0.10	0.10	0.00	0.04	0.04
A-1	0.05	0.10	0.05	0.10	0.10
A-2	0.05	0.05	0.05	0.00	0.05
A-3	0.10	0.10	0.05	0.10	0.05
A-4	0.04	0.09	0.04	0.00	0.04
A-5	0.12	0.10	0.05	0.05	0.05
A-6	0.16	0.21	0.05	0.00	0.05
A-7	0.13	0.09	0.00	0.09	0.05
A-8	0.04	0.12	0.00	0.04	0.04
A-9	0.08	0.04	0.04	0.04	0.04
A-10	0.12	0.05	0.12	0.05	0.05

[a] Results represent the percentage of observed sperm for each subject.

Meiosis I errors are predominant in maternally derived nondisjunction, whereas meiosis II errors are predominant in paternally derived nondisjunction. In addition, a reduced recombination rate is reportedly associated with both maternally and paternally derived meiosis I errors (19,34). A reduced recombination rate and a reduced chromosomal map have been detected in maternally derived nondisjoined chromosomes 21 and X (meiosis I error). Hassold *et al.* demonstrated an absence of recombination in the pseudoautosomal region of X and Y chromosomes in most cases of paternally derived 47,XXY (35). Reduced recombination is common in cases of both paternal and maternal meiosis I nondisjunction (36). This reduction tends to be observed in acrocentric chromosomes or chromosomes that have a relatively short portion to pair (X–Y pairing). Thus, pairing failure seems to influence the etiology of nondisjunction.

Analysis of DNA polymorphism has provided information about the meiotic stage of nondisjunction in autosomal chromosomes (15). For male sex chromosomes, meiosis I nondisjunction is easily distinguished from meiosis II nondisjunction because XX disomy and YY disomy are equivalent to meiosis II nondisjunction and XY disomy is equivalent to meiosis I nondisjunction. The present data suggest that meiosis I errors are more common than the meiosis II errors in male gonosomal disomy. Studies investigating the DNA polymorphic markers for XXY syndrome and XXX syndrome (19,37–39) have shown that the maternal XX disomy rate is the same as the paternal XY disomy rate and that the maternal XX disomy rate is significantly higher than the paternal XX disomy rate. If we assume that the genomic imprinting effect for the X chromosome does not alter the viability of paternally derived XXX embryos (40), the prevalences of XXX and XXY embryos suggest that male XY nondisjunction predominates. The incidence of YY-bearing sperm in the present study (0.09%) was similar to the frequency of 47,XYY trisomy in clinically recognized pregnancies (0.05%) (41). The frequency of XY-bearing sperm (0.17%) was three times greater than the incidence of 47,XXY trisomy (0.05%). The difference in the frequencies of YY- and XY-bearing sperm may explain the difference in the incidences of 47,XYY (0.07%) and 47,XXY (0.14%) fetuses, as determined from analysis of amniocentesis results (42). In the present study, the null sperm category included monosomic sperm for sex chromosomes and hybridization failures. Thus the rate of monosomic sperm for sex chromosomes would be lower than the null sperm frequency (0.00 to 0.20%). Determining the precise

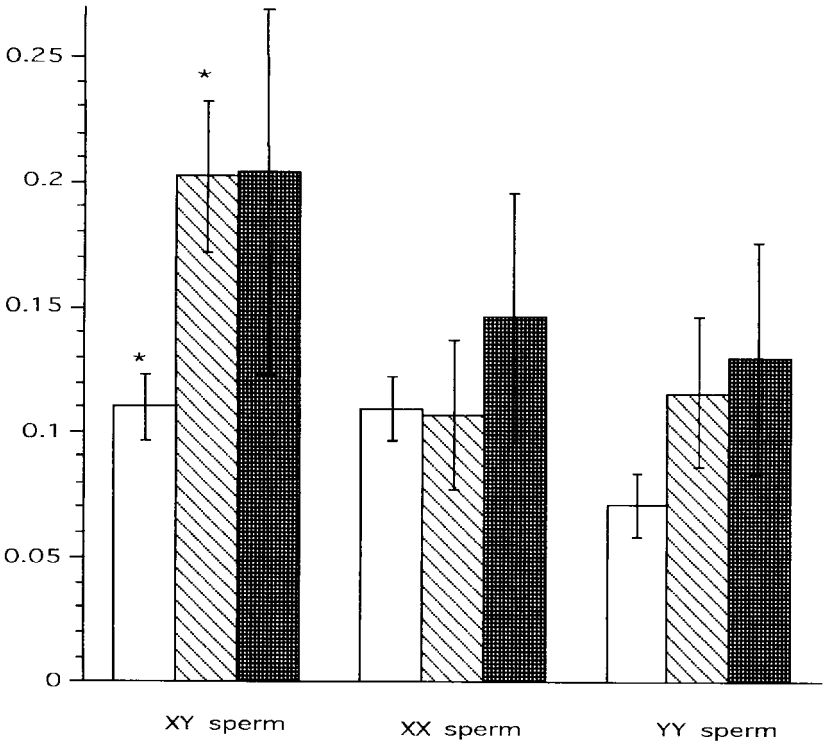

Fig. 1. Frequency of XY, XX, and YY sperm from donors <25 years old, donors >39 years old, and oligozoospermic patients. The frequencies shown are not adjusted for diploidy. Results represent the mean ± SE. Frequency of XY sperm differs significantly between young donors and aged donors: □, <25 years old; ▨, >39 years old; ▩, oligozospermic patients. *$P < 0.05$.

monosomic frequency in oocytes and the fetus and the origin of monosomy is difficult because most monosomic pregnancies tend to be aborted before implantation. In the XO syndrome, which is the only monosomic condition that can be easily analyzed, 80% of the lost X chromosomes are of paternal origin (39). Although the reason for this finding has not yet been clarified, three possibilities have been proposed. A genomic imprinting effect is observed in early stages of normal embryonal development and this effect on the X chromosome may alter the viability of XO fetuses (40,43). In contrast to the maternally derived X chromosome, the paternally derived X chromosome may be inactive during meiotic division. These findings suggest that genomic imprinting influences the early stages of fetal development. Another possibility is that the number of monosomic oocytes is reduced during meiotic division of oocytes. Finally, the paternal X chromosome may be more easily removed than the maternal X chromosome during postzygotic division, which appears to be the most probable explanation because the paternal X chromosome is at risk of being lost between fertilization and the first cleavage (44). Further investigations of oogenesis and fetal development are needed to evaluate these possible explanations.

Table IV. Reported Disomy Rates for Chromosomes 12, 18, X, and Y Using Multicolor FISH

Study	12	18	XX	YY	XY
Guttenbach *et al.* (29)			0.04	0.06	0.09
Williams *et al.* (24)		0.08	0.04	0.06	0.09
Miharu *et al.* (8)			0.07	0.04	
Bischoff *et al.* (25)	0.30	0.25	0.38	0.08	0.13
Springs *et al.* (30)	0.16	0.11	0.07	0.21	0.15
Chevret *et al.* (32)			0.04	0.01	0.34
Griffin *et al.* (11)			0.02	0.03	0.10
Springs *et al.* (31)	0.16	0.11	0.07	0.21	0.15
Present study	0.07	0.08	0.08	0.11	0.17

In the present study the frequency of sex chromosomal nondisjunction (meiotic I nondisjunction) was increased in older normozoospermic patients with idiopathic infertility. XX and YY disomies were not age dependent, but the rate of XY disomy was significantly

related to age ($P < 0.05$), suggesting that paternal gonosomal meiotic I nondisjunction is age dependent. Although some studies have demonstrated a significant increase in the frequency of XY disomy in infertile men with abnormal semen analysis (oligozoospermia, teratozoospermia, or asthenozoospermia) and older men (9,11), the largest molecular study on the parental origin of sex chromosomal trisomy found no evidence of a direct relationship between nondisjunction and paternal age (18). The failure of previous molecular studies to demonstrate a paternal age effect on nondisjunction of sex chromosomes may be due to the limitations of statistical analysis of the incidence of paternally derived trisomy (11, 12). The age-dependent increase in the frequency of XY disomy (sex chromosomal meiosis I) also suggests an age dependent mechanism for nondisjunction. Both maternal and paternal sex chromosomes are susceptible to meiosis I nondisjunction and this effect is age dependent, which suggests that a common mechanism exists for meiosis I in spermatogenesis and oogenesis. Fitzgerald *et al.* (45) reported that the incidence of sex chromosomal aneuploidy was positively related to age in cultured lymphocytes from both sexes. They reported that premature division of the X-chromosome centromere was strongly associated with X-chromosome aneuploidy. This phenomenon has been explained by age-related dysfunction in the X-chromosome centromere, suggesting an age-related mechanism for chromosome separation, especially in the sex chromosome. The present study failed to identify a significant age-related increase in disomy 12 and disomy 18. Further study of autosomes is necessary to determine whether paternal age affects the disomic frequency.

The present study showed no increase in the frequency of sex chromosomal disomy in oligozoospermic patients. Although some previous studies found no difference in the rates of disomy and diploidy between infertile patients and healthy males (7, 8), others observed an increased risk of interstitial deletions on the long arm of the Y chromosome (46) and an increased frequency of disomic sex chromosomes and chromosome 1 in spermatozoa (9). Furthermore In't Veld *et al.* (47) reported that the frequency of aneuploid sperm was markedly increased in one oligoasthenoteratozoospermic patient. FISH analysis of this patient showed the virtual absence of normal haploid sperm ($<2\%$) and the presence of diploid (40%) and triploid sperm (24%). In another study, an increased frequency of mitosis with separated centromeres was found in four members of a subfertile family in Spain (48). The frequency of aneuploid sperm is assumed to differ among patients. Thus, some patients may be particularly susceptible to meiotic and mitotic nondisjunction. Because there were only 5 oligozoospermic patients in the present study and there was considerable variability, further studies with more patients are needed to determine which oligoospermic patient risks producing aneuploid sperm.

Although Martin *et al.* (49) reported that analysis of human sperm karyotype indicated no significant relationship between the frequency of chromosomally and morphologically abnormal spermatozoa, sperm karyotyping depends on the fertilizing ability of sperm. FISH analysis is now the best method to detect the aneuploid frequency of human sperm that demonstrate no fertilizing ability. Although intracytoplasmic sperm injection (ICSI) has been used for oligozoospermic and teratozoospermic patients, this therapeutic approach avoids natural sperm selection. Therefore, ICSI-treated patients risk producing chromosomally abnormal offspring (4,5,47). The present data suggest that patients with idiopathic infertility do not have a higher risk of producing autosomal chromosomally abnormal offspring than fertile males. However, older infertile normozoospermic patients had an increased risk of producing XXY fetuses in the present study. Some authors have suggested that the frequency of disomic spermatozoa is higher in oligozoospermic and teratozoospermic patients (10, 47). These observations suggest that ICSI-treated patients should be informed of the risk of chromosomal aberration prior to undergoing treatment.

CONCLUSIONS

The present results suggest that sex chromosomal nondisjunction occurs more frequently than autosomal nondisjunction in spermatogenesis and that the rate of sex chromosomal and autosomal nondisjunction does not appear to be increased in oligozoospermic patients in this study. The frequency of male-origin nondisjunction of sex chromosomes during meiosis I showed an age-dependent increase.

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