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MODERN TRENDS

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Effects of male age on the frequencies of germinal and heritable chromosomal abnormalities in humans and rodents

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Objective: To review evidence regarding the effects of male age on germinal and heritable chromosomal abnormalities using available human and rodent studies and to evaluate possible underlying mechanisms.

Design: Review of English language-published research using MEDLINE database, excluding case reports and anecdotal data.

Result(s): There was little evidence from offspring or germ cell studies for a generalized male age effect on autosomal aneuploidy, except in rodents. Sex chromosomal nondisjunction increased with age in both human and rodent male germ cells. Both human and rodent data showed age-related increases in the number of sperm with chromosomal breaks and fragments and suggest that postmeiotic cells are particularly vulnerable to the effects of aging. Translocation frequencies increased with age in murine spermatocytes, at rates comparable to mouse and human somatic cells. Age-related mechanisms of induction may include accumulation of environmental damage, reduced efficiency of DNA repair, increased genomic instability, genetic factors, hormonal influences, suppressed apoptosis, or decreased effectiveness of antioxidants and micronutrients.

Conclusion(s): The weight of evidence suggests that the increasing trend toward fathering at older ages may have significant effects on the viability and genetic health of human pregnancies and offspring, primarily as a result of structural chromosomal aberrations in sperm. (Fertil Steril® 2004;81:925–43. ©2004 by American Society for Reproductive Medicine.)

Key Words: Paternal age, human, rodent, chromosomal abnormalities, structural aberrations, aneuploidy, sperm FISH, review

The modern trend toward fathering children at older ages has raised public health concerns about the possible effects of paternal age on the viability and genetic health of human pregnancies and offspring. Increased life expectancies and availability of assisted reproductive technologies are increasing the opportunity for men to father children at older ages (1). Since 1980, there has been an almost 25% increase in the number of men aged 35 to 54 years fathering children, according to the U.S. National Center for Health Statistics (2, 3). In contrast to female fertility, which begins to decline by a woman's early 30s due to the precipitous loss of functional oocytes, spermatogenesis continues well into male senescence, and men of advanced age typically retain their fecundity (4).

The incidence of abnormal reproductive outcomes is known to be higher among older fathers, although there are methodological difficulties in separating maternal and paternal effects within a study as couples' ages and lifestyles are strongly correlated. Abnormal outcomes associated with advanced paternal age include pregnancy loss (5, 6), developmental and morphological birth defects (7–9), neurological disorders (10, 11), various syndromes (12, 13), and diseases of complex etiology such as childhood cancer (14). These abnormalities are likely due to an increase in chromosomal or gene mutations with increasing paternal age (12), yet specific etiologies remain unknown.

Concern that chromosomal damage increases with age in the male germ line derives, in part, from studies of somatic cells showing significant age-related increases in the frequency of various abnormal cytogenetic end points, including increases in translocations (15, 16), acentric fragments (16, 17), telomere shortening and loss (18–20), aneuploidy (21),

chromosomal loss (22, 23), and micronuclei (24). Although genetic damage in somatic cells can negatively impact the immediate health of an individual, leading to diseases such as cancer, genetic damage in reproductive cells has potentially lasting consequences on the viability and health of the immediate offspring, as well as the fitness of future generations.

The purpose of this review is to examine and evaluate the evidence regarding the effects of paternal age on germinal and heritable chromosomal abnormalities from human and rodent studies. First, evidence for age effects on numerical chromosomal abnormalities is evaluated with regard to chromosome-specific variation, age effects on meiosis I (MI) vs. meiosis II (MII) nondisjunction, and putative confounders of the male age effects on aneuploidy. Second, evidence for age effects on structural chromosomal aberrations is evaluated with regard to the paternal age response for stable vs. unstable aberrations and somatic vs. germinal effects. Third, we discuss possible mechanisms underlying the observed paternal age effects on chromosomal abnormalities.

MATERIALS AND METHODS

We included all research on humans and rodents published in English through June 30, 2002, by searching MEDLINE electronic database sources. We excluded case reports, case series, and anecdotal data. All categories of structural and numerical chromosomal abnormalities in both male germ cells and offspring were evaluated.

RESULTS

Human Offspring Studies of Paternal Age Effects on Aneuploidy

Aneuploidy is the most common heritable chromosomal abnormality in our species with approximately 0.3% of newborns bearing extra or missing chromosomes (25). The estimated frequency of aneuploidy at conception is ~100 times higher than at birth, given that the primary reproductive health consequence of aneuploidy is pregnancy loss, which occurs in windows of development that depend on the specific chromosome involved. All chromosomal aneuploidies have been represented among spontaneous abortuses, with 45,X and trisomies 16, 21, and 22 comprising nearly 60% of the total (25). Some fraction of sex chromosomal aneuploidies and a few autosomal trisomies (e.g., trisomy 13, 18, and 21) survive to birth, with newborns exhibiting developmental and morphological defects characteristic of the trisomy involved.

Cytogenetic data from human oocytes, fertilized eggs, preimplantation embryos, and sperm indicate that most constitutional aneuploidy arises *de novo* in the parental germ cells from errors during meiosis. Meiosis is a highly conserved pathway among eukaryotic organisms, yet our species suffers an exceptionally high burden of aneuploidy. Despite the high frequency and clinical importance of human aneuploidy, the underlying molecular mechanisms of induction and the contributing risk factors remain uncertain.

Maternal age is a strong risk factor for trisomic pregnancies, and there is considerable variation in the age response among chromosomes, ranging from small age effects (e.g., on the large chromosomes of groups A and B) to linear (e.g., trisomy 16) and exponential increases (e.g., trisomy 21) (25–27). These findings suggest that the mechanisms involved in chromosomal nondisjunction are not homogeneous across chromosomes (28), at least for women.

Effects of Paternal Age on Autosomal Trisomy

For men, progress has been slow in characterizing the age response for individual trisomies due to the relatively low number of paternally derived trisomy cases. Trisomy 21 accounts for more than 95% of Down syndrome cases (29), and affects about 1 in 700 pregnancies. Paternally derived trisomy 21 accounts for 5%–10% of Down syndrome cases (30). Evidence for a paternal age effect on trisomy 21 in offspring remains inconclusive (Table 1). Several groups have reported positive associations with increased paternal age (38–44). For example, McIntosh et al. (38) observed about a twofold higher risk for trisomy 21 among fathers more than 50 years of age compared to fathers 25–29 years, after adjusting for maternal age. Most studies, however, have not found a paternal age effect for cases of trisomy 21 (46, 47, 51, 53, 55, 57). It has been hypothesized that a paternal age effect for trisomy 21 may be confined to cases of paternal MI error (58, 59), yet studies that classified cases according to the meiotic stage of origin did not find a paternal age effect (29, 52).

As shown in Table 1, the weight of evidence also indicates that there is no clear association between paternal age and the incidence of several other autosomal aneuploidies commonly found in human fetuses and newborns (e.g., trisomy 13, 15, 16, and 18). For trisomy 18 (Edwards syndrome), which affects about 1 in 8,000 live births, Robinson et al. (54) reported higher paternal ages in six cases of trisomy 18 vs. a control group. Hatch et al. (37) also observed a trend toward greater numbers of trisomy 18 with increasing paternal age, but the effect did not reach statistical significance and again the numbers of affected offspring were small ($n = 7$). A larger study of 118 trisomy 18 live births found no paternal age effect (56).

Effects of Paternal Age on Sex Chromosomal Aneuploidies in Human Offspring

Compared to the autosomes, sex chromosomal aneuploidies (e.g., 47,XXY, 47,XYY, 47,XXX, 45,X) have a much more substantial paternal contribution (25). Sex chromosomal aneuploidies are the most common chromosomal abnormality among human live births, the combined affect being about 1 in 500 live births. It has been estimated that

TABLE 1

Epidemiological studies of the association between paternal age and aneuploid human offspring.

Type of aneuploidy	No. of cases	Type of cases	Location	Birth years	Paternal age effect[a]	Reference
47,XXY	20	Live births, prenatal	Switzerland	1989	↑ $P<.05$	(31)
	66	Live births	UK/USA	Not given	NS	(32)
	64	Live births, prenatal	UK/USA	Not given	NS	(33)
	18	Live births, prenatal	UK/USA	Not given	NS	(34)
	290	Live births	UK	Not given	NS	(35)
45,X	18	Live births	California	1987–1990	NS	(36)
Trisomy 22	37	Spontaneous abortions	New York	1974–1986	↓ $P=.01$	(37)
Trisomy 21	969	Live births	British Columbia	1952–1973	↑ OR = 2 for ≥50 y	(38)
	60	Prenatal diagnoses	Germany	Not given	↑ $P<.001$ for ≥41 y	(39)
	1,279	Live births	Japan	1952–1968	↑ $P<.01$ for ≥55 y	(40)
	218	Live births	Copenhagen, Denmark	1960–1971	↑ $P<.01$ for ≥55 y	(41)
	551	Live births	British Columbia	1964–1976	↑ $P<.05$	(42)
	Not available[b]	Not available[b]	France	Not available[b]	↑ $P<.05$	(43)[c]
	693	Live births	Norway	1967–1978	↑ $P<.05$ for ≥50 y	(44)
	1,244	Live births	Ohio	1970–1980	↓ $P<.002$	(45)
	42	Spontaneous abortions	New York	1974–1986	↑ NS[d]	(37)
	394	Prenatal diagnoses	New York	1977–1984	NS	(46)
	318	Live births	Lima, Peru	1970–1989	NS	(47)
	4,000	Live births	29 states in US	1961–1966	NS	(48)
	226	Live births	Atlanta	1968–1976	NS	(49)
	1,858	Live births	US, all states	1974	NS	(49)
	Not given	Live births	New York	1977–1984	NS	(50)
	38	Live births	NA[e]	Not given	NS	(51)
	492	Live births	British Columbia	1952–1963	NS	(42)
	56[f]	Live births	NA[e]	1970–1982	NS	(52)
	6,384	Live births, stillbirths	Atlanta	1968–1980	NS	(9)
	853	Live births	New York	1963–1974	NS	(53)
	9	Live births	Switzerland	1989	NS	(54)
	611	Live births	France	1969–1980	NS	(55)
	67[f]	Not available[b]	Not available[b]	Not available[b]	NS	(29)
Trisomy 20	12	Spontaneous abortions	New York	1974–1986	↓ NS[d]	(37)
Trisomy 19	0	Spontaneous abortions	New York	1974–1986	No cases	(37)
Trisomy 18	6	Live births	Switzerland	1989	↑ $P<0.05$	(54)
	7	Spontaneous abortions	New York	1974–1986	NS[d]	(37)
	118	Live births	Kuwait	1980–1997	NS	(56)
Trisomy 17	5	Spontaneous abortions	New York	1974–1986	Too few to analyze	(37)
Trisomy 16	142	Spontaneous abortions	New York	1974–1986	NS	(37)
Trisomy 15	37	Spontaneous abortions	New York	1974–1986	NS	(37)
UPD 15[g]	7	Live births	Switzerland & UK	1983	↑ $P<.0005$	(54)
Trisomy 14	23	Spontaneous abortions	New York	1974–1986	NS	(37)
Trisomy 13	39	Spontaneous abortions	New York	1974–1986	↓ NS[d]	(37)
Trisomy 10–12[h]	12	Spontaneous abortions	New York	1974–1986	NS	(37)

~55% of the sex chromosomal aneuploidies at birth are paternal in origin, but the fractions differ: ~80% for 45,X (Turner syndrome); ~6% for 47,XXX (Triple X syndrome); 100% for 47,XYY (Hyper Y syndrome); ~50% for 47,XXY (Klinefelter syndrome) (60). Klinefelter syndrome affects about 1 in 500 male births (61) and is the most common cause of hypogonadism and infertility in men. Turner syndrome affects about 1 in 5,000 live female births, because it is highly lethal by 28 weeks gestation (62). Those that survive to birth have relatively minor complications, perhaps due to undetected mosaicism, including decreased birth weight and neck webbing, although many are phenotypically normal. The 47,XXX females and 47,XYY males each occur with a frequency of about 1 per 1,000 live births and are not usually associated with clinically recognizable phenotypes or infertility (62).

As shown in Table 1, paternally derived cases of 47,XXY were found to be associated with advanced paternal age in one study (31), but this has not been confirmed by larger studies (32–35). Although there is an association between 47,XXX and advanced maternal age (62), to our knowledge no studies have investigated the effects of age on paternally derived cases of 47,XXX or 47,XYY. Moreover, no paternal

TABLE 1 Continued.

Type of aneuploidy	No. of cases	Type of cases	Location	Birth years	Paternal age effect[a]	Reference
Trisomy 9	15	Spontaneous abortions	New York	1974–1986	↑ NS[d]	(37)
Trisomy 8	20	Spontaneous abortions	New York	1974–1986	NS	(37)
Trisomy 7	17	Spontaneous abortions	New York	1974–1986	↑ NS[d]	(37)
Trisomy 3–6	24	Spontaneous abortions	New York	1974–1986	NS	(37)
Trisomy 2	24	Spontaneous abortions	New York	1974–1986	NS	(37)
Trisomy 1	0	Spontaneous abortions	New York	1974–1986	No cases	(37)

[a] Up or down arrow indicates positive or negative correlation with increasing paternal age, respectively. NS = not significant at $P \leq 0.05$.
[b] Data not available because paper not in English or journal article not currently accessible. Results are from the paper's abstract.
[c] Cases from couples undergoing artificial insemination or IVF-D.
[d] Trend not significant at $P \leq 0.05$.
[e] Not applicable because cases derived from multiple population sources.
[f] Juberg et al. analyzed 36 MI and 20 MII cases separately and combined; Savage et al. 22 MI and 27 MII cases.
[g] Uniparental disomy (UPD) involving chromosome 15.
[h] No cases of trisomy 11 were found out of 491 autosomal trisomies evaluated.

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age effect was observed in a study of paternally derived cases of 45,X (36), but the sample size was small (n = 18).

Young Paternal Age as a Risk Factor for Aneuploid Offspring

An intriguing finding among some epidemiological studies is the increased incidence of certain birth defects and trisomies such as Down syndrome among teenage parents (Table 1). McIntosh et al. (38) observed a striking pattern of elevated risk for various birth defects in the offspring of fathers <20 years of age compared to fathers aged 25–29 years, after controlling for maternal age. This effect was particularly strong for cases of Down syndrome, oral clefts, neural tube defects, and hypospadias.

Analysis of 969 cases of Down syndrome showed that the risk was fourfold higher among teenage fathers compared to fathers 25–29 years (odds ratio [OR] = 3.8; 95% confidence interval [CI] = 1.8–8.1), whereas the oldest fathers (≥50 years) had twice the risk as the fathers 25–29 years (OR = 2.0; 95% CI = 1.0–3.9). Roecker and Huether (45) also reported an increased risk for Down syndrome among fathers aged 15–19 years. Hatch et al. (37) reported higher incidences of trisomy 13, 20, and 22 among spontaneous abortuses fathered by very young men; however, only the trend for trisomy 22 was statistically significant.

Limitations of Human Offspring Studies

Determining the effects of paternal age on aneuploidy from epidemiological studies of human offspring can be limiting for several reasons: [1] there are few affected offspring for each syndrome available for study, [2] there is potential bias because of indeterminate loss of chromosomally abnormal embryos in utero, and [3] there are established difficulties separating maternal and paternal effects (e.g., couples' ages are strongly correlated, as are lifestyles). Direct studies of male gametes have circumvented many of these problems.

Human Sperm Studies of Age Effects on Aneuploidy

Human–Sperm/Hamster–Egg Cytogenetic Method

The earliest information on the chromosomal content of human sperm derives from the human–sperm/hamster–egg cytogenetic method (hamster–egg method) (63). Capacitated human sperm are fused with hamster oocytes whose zona pellucidae have been enzymatically removed, and the sperm chromosomes are examined at essentially the first metaphase using conventional banding methods. Using the hamster–egg method, 2%–3% of sperm from normal men were found to be aneuploid (64). Rates of sperm aneuploidy varied widely among healthy men but two large hamster–egg studies did not find an effect of donor age (65, 66), yet few donors more than age 40 years were evaluated. Martin and Rademaker (64), however, detected a significant negative correlation with increasing donor age, which was a pattern previously noted in some offspring studies. In contrast, Sartorelli et al. (67) evaluated seven donors aged 59–74 years and observed significantly higher frequencies of hyperploid sperm compared to five donors 23–39 years, and the effect was not restricted to any particular chromosomal group.

Sperm Fluorescence In Situ Hybridization for Aneuploidy

In the early 90s, fluorescence in situ hybridization (FISH) technology was adapted for the detection of sperm aneuploidy (68). This technique hybridizes DNA probes labeled with a fluorescent dye to complementary target sequences at specific chromosomal sites within decondensed sperm nuclei. The effectiveness of sperm FISH has improved with the

TABLE 2

Sperm FISH studies of paternal age effects on the frequency of disomic sperm involving the autosomes.

Chromosome involved	Total men studied	Age range of donors (y)	Total sperm in study	Sperm per donor	*P* value[a]	Reference
Chr. 1	10	21–52	225,846	10,000	↑ .01	(69)
	18	23–58	180,000	10,000	NS[b]	(70)
	24	20–49	240,000	10,000	NS	(71)
	7	22–42	11,089	1,500	NS	(72)
	3	>80	6,940	1,500	NS	(73)
Chr. 6	18	24–74	194,024	10,000	NS	(74)
Chr. 7	24	20–49	240,000	10,000	NS	(71)
Chr. 8	14	22–59	205,218	10,000	NS	(75)
Chr. 12	25	<25, >39	50,000	2,000	NS	(76)
	10	21–52	115,000	10,000	NS	(69)
Chr. 13	18	23–58	180,000	10,000	NS	(70)
	10	22–37	200,497	10,000	NS	(77)
Chr. 14	11	<30, >60	110,000	10,000	NS	(78)
Chr. 17	3	>80	6,940	1,500	NS	(73)
Chr. 18	45	19–35	450,000	10,000	↓ .009	(79)
	25	<25, >39	50,000	2,000	NS	(76)
	24	18–60	390,096	12,000	NS	(80)
	3	>80	5,646	1,500	NS	(73)
Chr. 21	11	<30, >60	110,000	10,000	↑ .001[c]	(78)
	38	24–57	398,681	10,000	NS	(81)
	18	23–58	180,000	10,000	NS	(70)
	18	24–74	194,024	10,000	NS	(74)
	10	22–37	200,497	10,000	NS	(77)

[a] Up or down arrow indicates positive or negative correlation with increasing age, respectively. NS = not significant at $P \leq .05$.

[b] Using two FISH probes simultaneously on chromosome 1.

[c] Significant for two of the three donors over 60 years.

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increased availability of chromosome-specific DNA probes and with increased emphasis on the importance of scoring criteria (60). From a technological standpoint, multicolor FISH had major advantages over the hamster–egg method: [1] FISH assays can use frozen archived samples whereas the hamster technique requires fresh samples; [2] FISH assays require less time and labor to analyze more sperm (e.g., ~50 times more cells can be scored by one technician for FISH vs. the hamster technique); and thus, [3] FISH assays cost less than the hamster–egg method.

As shown in Table 2, sperm FISH data showed little evidence for a paternal age effect on sperm autosomal aneuploidies across chromosome groups. Most of the studies involved chromosomes 1, 18, and 21. Martin et al. (69) reported an age-related increase in disomy 1 in sperm for men aged 21–52 years, but this finding was not confirmed in other studies (70–73). The study by Martin et al. (68) used a probe for the large (~15 Mb) heterochromatic block of classic satellite DNA at 1q12, which was found to be prone to breakage using a FISH assay that distinguished breaks from numerical abnormalities in human sperm (82). Thus, the age-related increase in "disomy 1" observed by Martin et al. may be the result of breaks rather than extra copies of chromosome 1 in sperm. In fact, our laboratory recently demonstrated a significant age-dependent increase in the frequency of breaks within the 1q12 region of sperm (unpublished data).

A study by Robbins et al. (79) found a significant decrease in disomy 18 sperm with increasing donor age. No donor age effect was detected in nearly all studies of disomy 21 "Down" sperm (70, 74, 77, 81), except for a relatively small study by Rousseaux et al. (78), who found higher frequencies of sperm disomy 21 for two men more than 60 years of age compared to men less than 30 years. Age studies of autosomes 6, 7, 8, 12, 13, 14, 17, and 18 have yielded no evidence for an age-related increase in sperm disomy frequencies (Table 2).

There is some evidence for an age-related increase in sex chromosomal aneuploidies in sperm (Table 3). Nine of 11 sperm FISH studies found an age effect on sex chromosomal aneuploidies (69, 73–76, 79–81, 83). The two studies that did not detect an age effect either used a single chromosome method that could not distinguish disomic from diploid sperm (84) or evaluated a narrow age range for men less than 40 years (77).

TABLE 3

Sperm FISH studies of paternal age effects on the frequency of aneuploid sperm involving the sex chromosomes.[a]

Sperm disomy	Total men studied	Age range of donors (y)	Total sperm in study	Sperm per donor	Fold increase (age groups)	*P* value[b]	Reference
Meiosis I error							
XY	38	24–57	398,681	10,000	2.3 (20–29 vs. 50–59)	↑ .006	(81)
	24	18–60	390,096	12,000	2.6 (18–29 vs. 50–60)	↑ .007	(80)
	25	<25, >39	50,000	2,000	1.8 (<25 vs. >39)	↑ .01	(76)
	3	>80	5,646	1,500	2.1 (<30 vs. >80)	↑ Not given[c]	(73)
	18	24–74	194,024	10,000	—	↑ NS[d]	(74)
	45	19–35	450,000	10,000	—	NS	(79)
	18	23–58	181,556	10,000	—	NS	(83)
	14	22–59	205,218	10,000	—	NS	(75)
	10	21–52	115,000	10,000	—	NS	(69)
	10	22–37	200,497	10,000	—	NS	(77)
Meiosis II errors							
disomy X	14	22–59	205,218	10,000	2.3 (22–36 vs. 43–59)	↑ .005	(75)
	24	18–60	390,096	12,000	2.2 (18–29 vs. 50–60)	↑ .02	(80)
	45	19–35	450,000	10,000	ND[e]	↑ .002	(79)
	18	24–74	194,024	10,000	—	↑ NS[d]	(74)
	38	24–57	398,681	10,000	—	NS	(81)
	25	<25, >39	50,000	2,000	—	NS	(76)
	18	23–58	181,556	10,000	—	NS	(83)
	10	21–52	115,000	10,000	—	NS	(69)
	10	22–37	200,497	10,000	—	NS	(77)
	3	>80	5,646	1,500	—	NS	(73)
disomy Y	14	22–59	205,218	10,000	3.0 (22–36 vs. 43–59)	↑ .0001	(75)
	18	23–58	181,556	10,000	1.6 (20–29 vs. ≥45)	↑ <.02	(83)
	10	21–52	115,000	10,000	1.7 (21–37 vs. 39–52)	↑ .04	(69)
	24	18–60	390,096	12,000	2.2 (18–29 vs. 50–60)	↑ .06	(80)
	18	24–74	194,024	10,000	—	↑ NS[d]	(74)
	45	19–35	450,000	10,000	—	NS	(79)
	38	24–57	398,681	10,000	—	NS	(81)
	25	<25, >39	50,000	2,000	—	NS	(76)
	10	22–37	200,497	10,000	—	NS	(77)
	8	18–40	8,061	1,000	—	NS[f]	(84)
	3	>80	5,646	1,500	—	NS	(73)

[a] All studies except one reported an effect on the sex chromosomes (XX, YY, XY or sum of XX+YY+XY).
[b] NS = not significant; all *P* values represent positive correlations with increasing age (indicated by up arrows).
[c] *P* value not given. Reported slight increase in XY sperm with age compared to published data for men <30 years.
[d] Sum of disomy XX, YY and XY was significant at *P*=.05, mainly due to disomies XX and XY.
[e] Data not presented in paper.
[f] Single chromosome FISH assay. YY sperm could not be distinguished from diploid sperm.

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Sperm FISH data on teenage men is very limited because many believe that one should not encourage sperm studies of peripubertal men. The youngest age for which sperm aneuploidy frequencies have been determined have generally been for men in their 20s. In a study of the effects of smoking, Rubes et al. (85) evaluated sperm from a large group of 18-year-old men using FISH for chromosomes 8, X, and Y. The frequencies of sperm aneuploidy among these teenage men did not appear higher than would be expected compared to published data for men in their 20s. Animal models such as the mouse (86) may be useful for studies of sperm aneuploidy in peripubertal males.

Effects of Age on Meiosis I vs. Meiosis II Sperm Aneuploidy

The meiotic origin of chromosomal nondisjunction appears to vary considerably between men and women and among chromosomes (25, 26, 87). Results from more than 1,000 human offspring showed that almost all maternally derived cases of trisomy 15, 16, and 22 originated in meiosis I (MI), yet most cases of trisomy 7 and 18 resulted in meiosis II (MII) (25). For paternal cases, 100% of XXY, trisomy 2, and trisomy 22 originated in MI, whereas 100% of XXX, XYY, and trisomy 15 originated in MII. Paternal trisomy 21 cases appear to be more evenly distributed between MI and MII (25).

TABLE 4

Sperm FISH studies of paternal age effects on the frequency of diploid sperm.

Total men studied	Age range of donors (y)	Total sperm in study	Sperm per donor	*P* value[a]	Approximate fold increase	Reference
11	<30, >60	110,000	10,000	↑ <.001[b]	2.0[c]	(78)
18	24–74	194,024	10,000	↑ .002[d]	1.5[e]	(74)
45	19–35	450,000	10,000	↓ .001[f]	ND[g]	(79)
14	22–59	205,218	10,000	↓ .006[h]	1.8[i]	(75)
38	24–57	398,681	10,000	NS	—	(81)
25	<25, >39	50,000	2,000	NS	—	(76)
24	18–60	390,096	12,000	NS	—	(80)
24	20–49	240,000	10,000	NS	—	(71)
18	23–58	181,556	10,000	NS	—	(83)
10	21–52	225,846	10,000	NS	—	(69)
10	22–37	200,497	10,000	NS	—	(77)
3	>80	12,586	1,500	NS	—	(73)

[a] Up or down arrow indicates positive or negative correlation with increasing age, respectively. NS = not significant at $P \leq .05$.
[b] Positive correlation for one of the three donors over 60 years using autosomal probes.
[c] Men age >60 years vs. <30 years.
[d] Positive linear correlation for total sperm diploidy (sum of MI and MII).
[e] Men age 70–79 years vs. 20–29 years.
[f] XY diploid sperm.
[g] Data not presented in paper.
[h] Effect due to one young outlier, which if excluded, then $P = .13$.
[i] Men age 28.9 ± 5.0 years vs. 46.8 ± 3.1 years.

Sloter. Effects of male age on chromosomal abnormalities. Fertil Steril 2004.

Sperm FISH studies that use probes for the X and Y chromosomes were able to determine the meiotic origin of the nondisjunction event. The XY "Klinefelter" sperm originate in MI. Of 10 sperm FISH studies that investigated the effects of age on XY sperm, five found a positive paternal age effect (Table 3), with about a two- to threefold higher frequency of XY sperm by age 50 years (73, 74, 76, 80, 81). Three of these studies reached statistical significance (76, 80, 81). Griffin et al. (80) and Bosch et al. (74) also detected effects on MII.

Sperm disomies X and Y originate in MII. Six of 11 FISH studies detected age effects on MII (Table 3). In two studies the effect was confined to disomy Y (69, 83), and in another the effect was confined to disomy X (79). Two other studies by Griffin et al. (80) and Robbins et al. (75) detected positive effects on both disomy X and disomy Y. The effects on MII ranged from about a two- to threefold increase by age 50 years, the same as the effect on MI.

Age Effects on Sperm Diploidy

Triploidy accounts for 1%–3% of all recognized pregnancies, and 15%–20% of all chromosomally abnormal miscarriages, making triploidy one of the most frequent chromosomal abnormalities in human conceptions (88). In general, paternally derived triploid abortuses are more common among younger couples, whereas maternal triploids are more frequent among older couples (88).

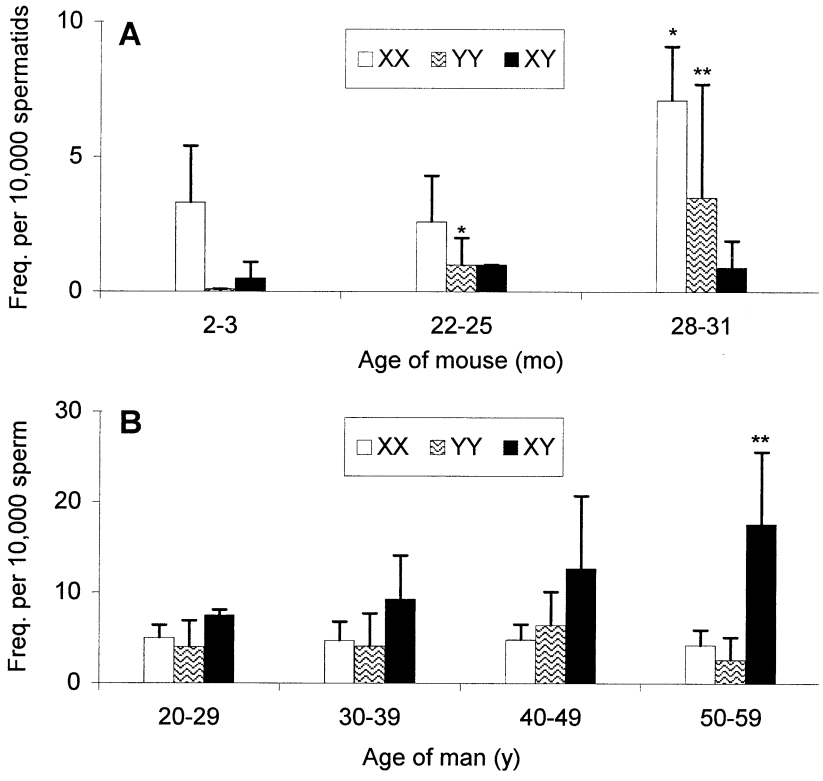
Paternally derived triploidy results from fertilization of a normal oocyte by two sperm (dispermy) or by a diploid sperm. The hamster–egg method is unable to distinguish diploid sperm from multiple fertilizations, but diploid sperm can be readily detected using multiprobe FISH assays. Table 4 presents the available human sperm FISH data for the effects of age on sperm diploidy frequencies. In a study of 18 men aged 24–74 years, Bosch et al. (74) observed a linear increase of 17% per 10-year interval of donor age in total sperm diploidy using a four-color FISH assay for chromosomes 6, 21, X, and Y. Using probes for chromosomes 14 and 21, Rouseaux et al. (78) detected about twofold higher frequencies of diploid sperm in a 64-year-old donor vs. a healthy control group less than 30 years of age. However, several other sperm studies, many with larger numbers of donors, did not find an age effect for sperm diploidy frequencies (69, 71, 73, 76, 77, 80, 81, 83). Two separate studies by Robbins et al. (75, 79) found a negative correlation with increasing paternal age, yet in one study the effect was primarily due to an outlier in the youngest age group.

Rodent Studies of Age Effects on Male Germ Cell Aneuploidy

Few studies to date have examined the effects of male age on chromosomal abnormalities in mouse sperm. Using an X-Y-8 sperm FISH assay on the spermatids of B6C3F1 mice, Lowe et al. (86) observed a 1.5 to 2-fold increase in disomy 8 frequencies in mice at more than 22 months of age

FIGURE 1

Effects of male age on the frequency of sex chromosomal aneuploidy in mouse and human sperm by meiotic stage of origin. XY sperm arise from MI disjunction errors, whereas disomy X and disomy Y originate in MII. (**A**) *Bars* (±SE) represent frequencies of sex chromosomal nondisjunction in B6C3F1 mouse spermatids of various ages using the multicolor X-Y-8 FISH assay (86). (**B**) *Bars* (±SE) represent frequencies of sex chromosomal nondisjunction in human sperm using a multicolor X-Y-21 FISH assay (81). *$P<.05$; **$P<.01$.



Sloter. Effects of male age on chromosomal abnormalities. Fertil Steril 2004.

compared to mice 2.4 months, with the greatest effect on mice more than 28 months of age. Lowe et al. also detected higher frequencies of sperm with sex chromosomal aneuploidies in the old mice. The age effect was limited to MII disjunction errors (i.e., disomy X or Y sperm) (Fig. 1A; compare with Fig. 1B, age response for MI errors in humans). No male age effect was detected for sperm diploidy. A separate sperm FISH study of autosomes 2 and 8 in untreated transgenic mice revealed about fourfold higher rate of disomy 8 and about threefold higher rate of disomy 2 in mice aged 26 months vs. 2.5 months (89). In addition, about threefold increase in diploid sperm was detected in the same mice.

These mouse sperm FISH data were confirmed by a finding using the hamster spermatid micronucleus assay. Allen et al. (90) observed higher frequencies of round spermatids with kinetochore positive micronuclei in 18-month-old vs. 6-month-old hamsters (Fig. 2), indicating increased chromosome loss with age in the hamster. In contrast, Lowe et al. (86) did not detect an age-related increase in mouse round spermatids with kinetochore-positive micronuclei.

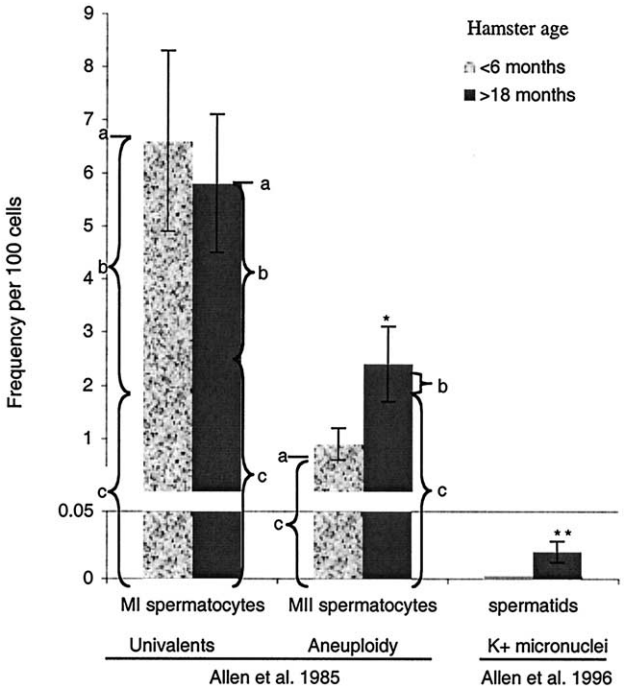
In a study of MI spermatocytes (i.e., meiotic cells), Pacchierotti et al. (92) observed higher frequencies of cells with chromosomal univalents (i.e., chromosome with no synaptic mate) in mice aged 29–32 months compared to mice 3–6 months (Fig. 3). In hamsters, Allen and Gwaltney (91) observed no difference in the frequency of univalents in MI spermatocytes between young and old mice, yet they did find a significant increase in MII nondisjunction (see Fig. 2).

Human Offspring Studies of Paternal Age-Related Structural Chromosomal Abnormalities

Structural chromosomal abnormalities are slightly less common than aneuploidy at birth (0.25% vs. 0.33%) (93), but it is estimated that ~80% of de novo cases are paternally derived (94). The frequency of structural chromosomal abnormalities among stillbirths and spontaneous abortions was

FIGURE 2

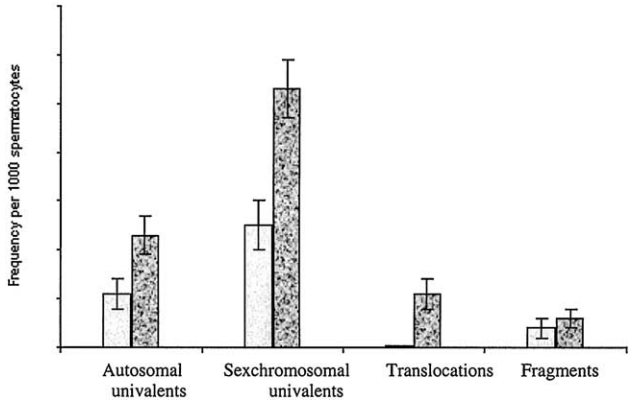
Effects of age on frequencies of chromosomal nondisjunction in hamster spermatocytes and spermatids (90, 91). *Bars* (±SE) represent frequencies of univalents (MI spermatocytes), aneuploidy (MII spermatocytes), or kinetochore positive (K+) micronuclei in male hamster spermatids less than 6 months of age (*light shade bars*) vs. old hamsters more than 18 months of age (*darker shade bars*). *Brackets* indicate the fraction of the bar represented by a particular chromosomal group (a = large metacentric chromosomes, b = medium submetacentric, c = small subtelocentric). *$P<.01$, **$P<.001$ vs. young hamsters.



Sloter. Effects of male age on chromosomal abnormalities. Fertil Steril 2004.

FIGURE 3

Structural and numerical chromosomal abnormalities in the primary spermatocytes of mice (92). *Bars* (±SE) represent frequencies of MI mouse spermatocytes with autosomal or sex chromosomal univalents in C57BL/Cne × C3H/Cne male mice 82–157 days old (*light bars*) vs. mice 804–891 days old (*darker shade bars*). *Bars* (±SE) also represent frequencies of MI mouse spermatocytes containing translocations or acentric fragments for male mice 82–157 days old (*light shaded bar*) vs. mice 804–891 days old (*dark bars*). *$P<.005$ for old vs. young mice.



Sloter. Effects of male age on chromosomal abnormalities. Fertil Steril 2004.

estimated to be 0.4% and 2%, respectively (95). Among live offspring, structural chromosomal abnormalities are associated with mental retardation, developmental and morphological defects, and genetic disease such as cancer (96).

The pre- and postnatal health effects of structural chromosomal abnormalities on offspring vary tremendously depending on the size of the imbalance and specific genes involved in the breakage or rearrangement event. Unbalanced rearrangements and unstable aberrations are expected to be cell lethal and, thus, are strongly selected against during development. In contrast, balanced rearrangements and small partial duplications or deletions are more compatible with postnatal life.

Several case studies have noted spontaneously occurring structural chromosomal rearrangements in children of old fathers (see for example, Ref. 97). Population-based studies, however, have not found evidence for an increase in structural chromosomal abnormalities with advancing paternal age. Among 63,000 fetuses evaluated by amniocentesis, Hook and Cross (98) reported no age-related increase in the frequency of those with structural chromosomal abnormalities of paternal origin (n = 38); however, there was a positive association with maternal age for maternally derived cases. Similarly, Jacobs (99) found no evidence for a paternal age effect on de novo rearrangements in spontaneous abortions, yet the sample size of paternal cases was very small (n = 8). Furthermore, Olson and Magenis (94) did not find an effect of paternal age in a larger study of 27 paternal cases.

There was conflicting evidence from the literature concerning a paternal age effect for isochromosome X Turner syndrome, a type of structural aberration involving the X chromosome. Carothers et al. (100) observed a significant difference in the mean paternal age of Scottish cases vs. controls but not among English cases. Lorda-Sanchez et al. (101) reported no age effect for 14 paternally derived cases of isochromosome X, consistent with the lack of a general paternal age effect for this condition.

Given the overall lower occurrence of structural chromosomal abnormalities among live births compared to the more readily detectable aneuploidy syndromes, determining the effects of paternal age on structural aberrations from epidemiological studies of human offspring have been enormously difficult. However, the ever-increasing availability of assays for detecting structural chromosomal aberrations

directly within male gametes are helping to evaluate potential factors such as age that may increase the fraction of a man's sperm with structural chromosomal abnormalities.

Human Sperm Studies of Structural Chromosomal Aberrations

Effects of Male Age on Unstable Aberrations Including Breaks in Sperm

The hamster–egg method provided the first glimpse of the incidence and types of structural chromosomal abnormalities carried in human sperm. Surprisingly, high baseline frequencies of unrejoined breaks and acentric fragments were found with this method (64–66). About 75% of the total fraction of structural abnormalities was unstable aberrations. Martin and Rademaker (64) examined 1,582 sperm chromosomal complements from 30 fertile men in six age groups ranging in age from 20–24 years to ≥45 years and found about a fourfold increase in total structural chromosomal abnormalities by age 45 years (i.e., 2.8% in men 20–24 years vs. 13.6% in men ≥45 years). As shown in Figure 4A, a reanalysis of these data by our laboratory showed that the effect was due almost entirely to a significant increase in chromosomal breaks in sperm (P=.004), with a nonsignificant increase in acentric fragments (P=.2) (Fig. 4B), suggesting that the postmeiotic cell types of spermatogenesis, which are known to be DNA-repair deficient, may be particularly vulnerable to the effects of aging.

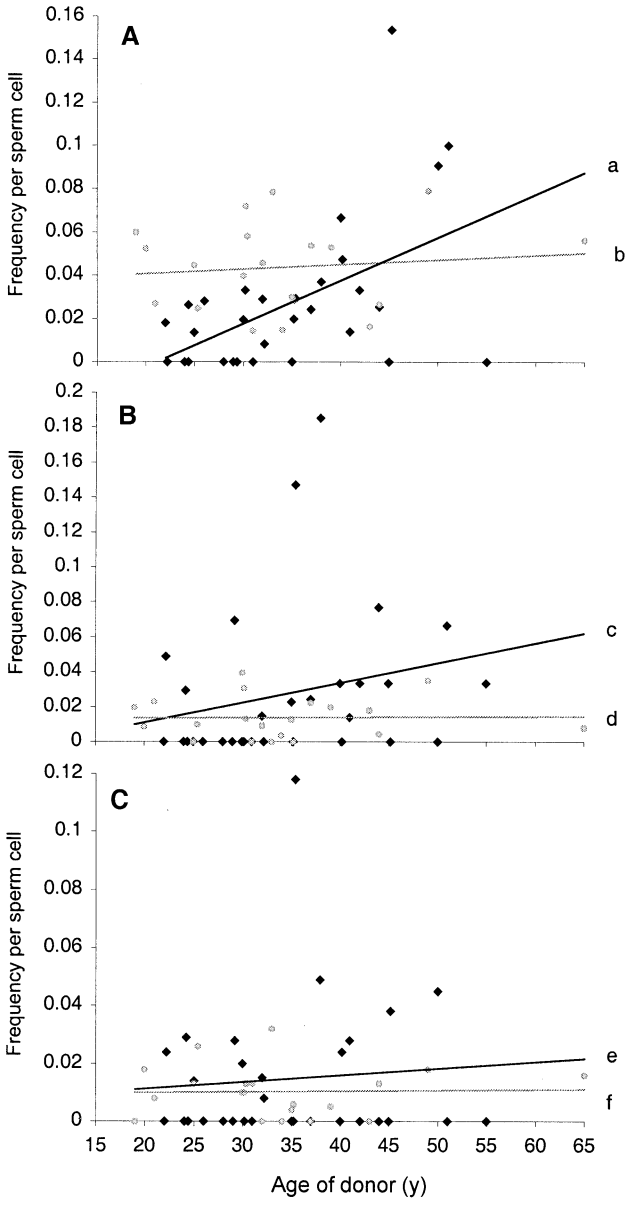
Estop et al. (65) and Rosenbusch et al. (102) also observed increased frequencies of sperm with unstable breaks and fragments with age from an analysis of 19 and 15 donors, respectively, yet the effects did not reach statistical significance. Contrary to these findings, our own reanalysis of published data on ~5,000 sperm complements from 20 donors aged 19–65 years (66) showed no increase in chromosomal breaks or fragments in human sperm (Fig. 4A,B), yet there was a suggestive increase in the frequency of sperm carrying dicentric chromosomes (P=.05).

In a human–sperm/hamster–egg study that included several older men aged 59–74 years, Sartorelli et al. (67) observed a significantly higher frequency of sperm complements with acentric fragments compared to donors aged 23–39 years. Higher frequencies of complex radial figures, which form during DNA synthesis or later after penetration of the hamster egg as a result of chromatin damage, were also found in the older group.

In a study using single cell gel electrophoresis (i.e., Comet assay) (103), the amount of DNA breakage was measured in sperm from 60 men aged 29–44 years undergoing IVF treatment. Sperm DNA damage positively correlated with donor age and with impairment of postfertilization embryo cleavage after fluorescence in situ hybridization (ICSI), further indicating an overall decline in the integrity of sperm DNA in older men.

FIGURE 4

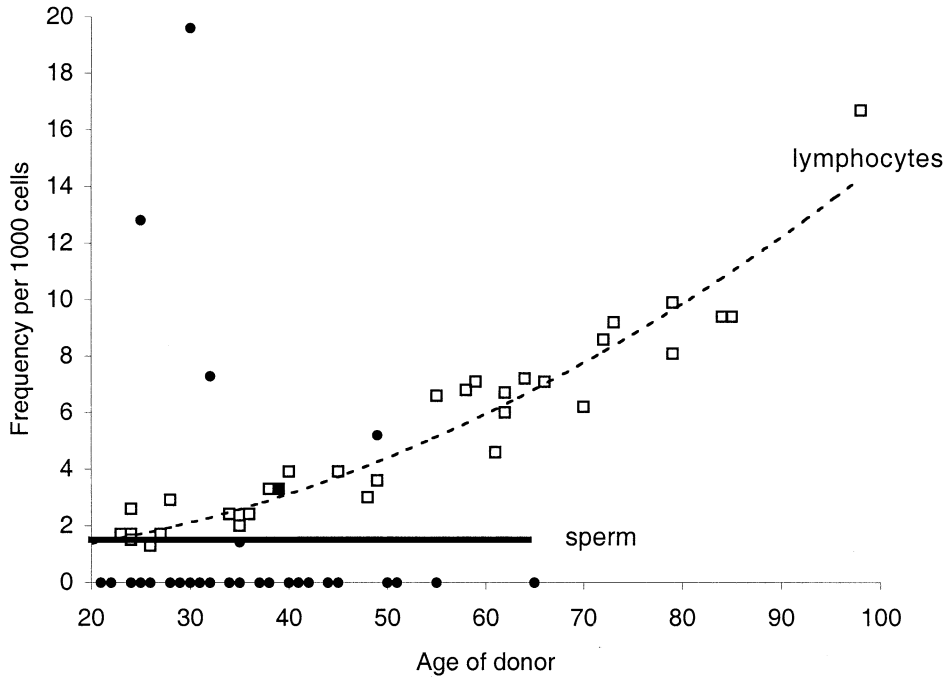
Effects of donor age on frequencies of human sperm with structural chromosomal abnormalities by type of damage. Linear regression analyses were performed using published hamster–egg data (66, 157). Data were separated into chromosomal breaks (**A**), acentric fragments (**B**), and rearrangements (**C**). Rearrangements included translocations, inversions, duplications, deletions, markers, and rings. For comparison, the data points (*diamond shape*) and regression lines for Martin et al. are shown in *black*, and the data points (*circles*) and regression lines for Brandriff et al. are shown in *gray*. β-Coefficients and P values are shown next to each regression line. a: β = 0.002, P=.004; b: β = 0.0002, P=.6; c: β = 0.001, P=.2; d: β = 0.000009, P=.9; e: β = 0.0002, P=.7; f: β = 0.00004, P=.9.



Sloter. Effects of male age on chromosomal abnormalities. Fertil Steril 2004.

FIGURE 5

Translocation frequencies in somatic vs. sperm cells by age of donor. Data points represent frequencies of translocations for human lymphocytes (*open squares*) and human sperm (*solid circles*) for each healthy unexposed individual. The *dotted line* represents a quadratic fit for lymphocyte data obtained from 35 healthy men and women (15). The *solid black line* represents the baseline frequency (total abnormal per 6,582 sperm) for 50 donors evaluated using the hamster–egg method (66, 157). Some data points overlap giving the appearance of only one data point.



Sloter. Effects of male age on chromosomal abnormalities. Fertil Steril 2004.

Effects of Male Age on Chromosomal Rearrangements in Sperm

As shown in Figure 5, the frequency of lymphocytes containing translocations is known to increase exponentially with age in both men and women (15, 16). However, as shown in Figures 4C and 5, there is no evidence for a similar age-related increase in rearrangements in human sperm (64, 66). The discrepancy between somatic and sperm cells may be because of the small number of sperm able to be analyzed per donor using the hamster–egg method (~100 haploid sperm complements per donor vs. 1,000 lymphocytes per donor). Alternatively, male germ cells may be more protected against the deleterious effects of aging than somatic cells, as has been suggested in gene mutation studies (104).

Our laboratory developed a sperm FISH method for detecting partial chromosomal duplications and deletions in sperm (105, 106) and showed that a t(1;10)(p22.1;q22.3) reciprocal translocation carrier produced high frequencies of sperm containing duplications and deletions of the probed chromosomal segment (107). McInnes et al. (70) did not detect an age-related increase in the frequency of sperm with terminal duplications and deletions using this assay on 18 healthy donors aged 23–58 years; however, technical deficiencies were noted because the same subtelomeric probe was not used throughout the study and significant differences in hybridization efficiency were detected among the different labeling strategies. They did report an increase in centromeric deletions with age and suggested that these represent acentric fragments. Van Hummelen et al. (107) showed these defects could be produced by chromosomal rearrangements.

Additional FISH studies are needed to determine the effects of age on various other categories of chromosomal aberrations in human germ cells, especially postmeiotic chromosomal damage in human sperm.

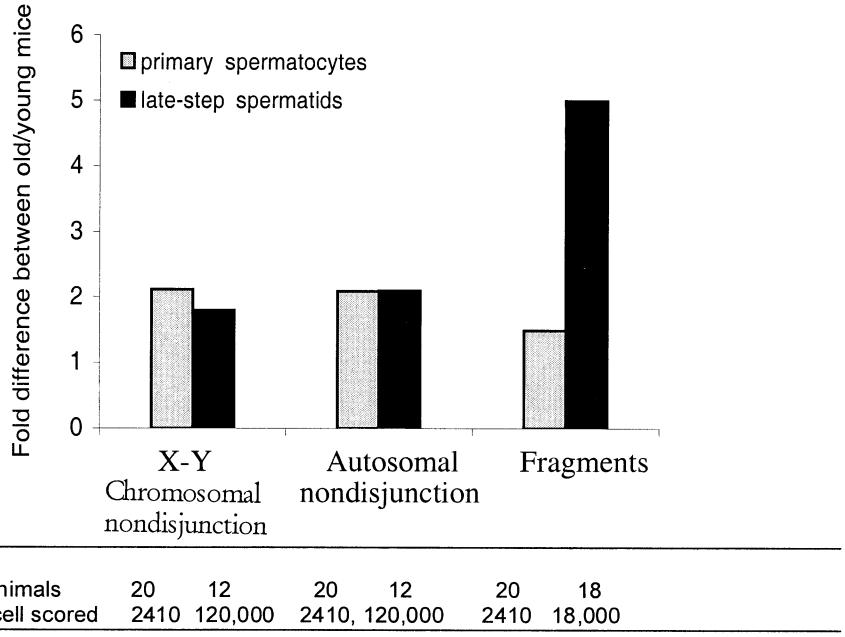
Rodent Studies of Male Age Effects on Structural Aberrations

Age Effects on Unstable Aberrations in Male Germ Cells

There is evidence from rodent studies for an age-dependent increase in unstable structural aberrations in male germ cells, but the pre- and postmeiotic compartments of spermatogenesis appear to be affected differently (see Fig. 6 for comparison of spermatocytes vs. spermatids). Pacchierotti et al. (92) found that mice of advanced age (<29 months) carried about the same frequencies of unstable chromosome

FIGURE 6

Fold increase in the frequency of chromosomal abnormalities for mouse MI spermatocytes vs. spermatids shown by type of abnormality. Data for spermatocytes (*light bars*) adapted from (92). Data for spermatids (*dark bars*) adapted from (86). The number of animals and cells analyzed per study are indicated below the histogram.



Sloter. Effects of male age on chromosomal abnormalities. Fertil Steril 2004.

aberrations in their spermatocytes as mice less than 6 months of age (see Fig. 3 for spermatocyte data).

However, studies of postmeiotic cell types using the micronucleus assay showed significantly increased frequencies of unstable aberrations in the spermatids of old mice compared to young (Fig. 7). Lowe et al. (86) reported a fivefold increase in the average frequency of kinetochore negative micronucleated round spermatids in mice aged 22–31 months when compared to mice aged 2.4 months (Fig. 7). Similar results were observed in micronuclear analyses of round spermatids of aged hamsters. Allen et al. (90) detected twofold higher frequencies of kinetochore negative micronuclei among 15 hamsters aged 24 months vs. 15 hamsters aged 3 months (Fig. 7). Unstable chromosomal aberrations transmitted by the sperm are expected to be embryo lethal. Alternatively, unstable sperm lesions may be converted into rearrangements in the zygote. Chemicals such as acrylamide, which induce unstable lesions in sperm, have also been found to increase the incidence of rearrangements in zygotes and offspring by 20%–30% (108). An age-related increase in unstable lesions in sperm from older males may increase the risk for rearrangements in live offspring.

Age Effects on Stable Aberrations in Male Germ Cells

The spontaneous or baseline frequency of germ cell rearrangements in unexposed male rodents is extremely low in mice less than a year old. In fact, not a single rearrangement was found among 41,000 primary spermatocytes evaluated from three different strains of mice (Fig. 8) (92, 109, 110). By 2 years of age, however, almost 1% of mouse spermatocytes contained rearrangements (Fig. 8), and there were indications of strain differences. Translocation frequencies in C57BL spermatocytes increased exponentially beginning at 1 year of age, whereas the increase was postponed until about age 2 years in C57BL/CnexC3H/Cne mice (110).

DISCUSSION

Potential Mechanisms Associated With Age Effects on Chromosomal Abnormalities in Male Germ Cells

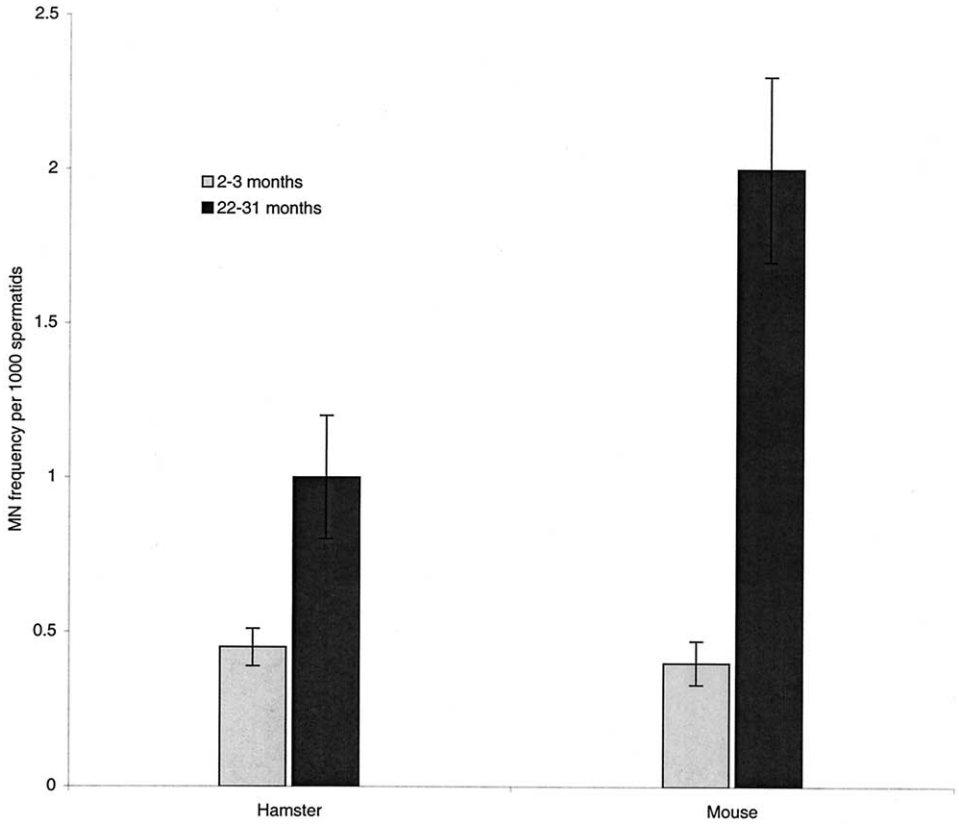
Age-related effects may be because of various genetic, physiologic, or environmental factors, but the underlying mechanisms are not well understood. The following section discusses the available evidence for various potential mechanisms that have been associated with age effects on chromosomal abnormalities in male germ cells.

Environmental Factors

Age provides increased opportunity for germ cells to suffer genetic damage from exogenous exposures or dis-

FIGURE 7

Effects of age on the frequency of micronuleated round spermatids in rodents. *Bars* (±SE) represent frequencies of micronuclei in hamster (90) or mouse (86) spermatids of male rodents 2–3 months old (*light bars*) vs. 22–31 months (*dark bars*). All mouse micronuclei were kinetochore negative (K−). Kinetochore positive (K+) micronuclei in the spermatids of old hamsters (*hatched portion of bar*) were significantly increased over the young hamsters at $P<.001$.



Sloter. Effects of male age on chromosomal abnormalities. Fertil Steril 2004.

eases. For example, older men are more likely to have smoked and to have smoked for a longer period than younger men, or to have had illnesses including genitourinary infections. Male age may also be a proxy for a "cohort effect," that is, a common specific exposure experienced by men in the same birth cohort. For example, men who were born before 1972 were more likely to have been exposed to DDT, an endocrine disruptor, which was later banned. It is also possible that men of specific age groups (e.g., very young or old men) are particularly sensitive to the effects of germ cell mutagens. For example, Rubes et al. (85) found significantly elevated rates of sex chromosomal aneuploidy in the sperm of 18-year-old smokers vs. 18-year-old nonsmokers, whereas a study of men aged 19–35 years did not detect an effect (79).

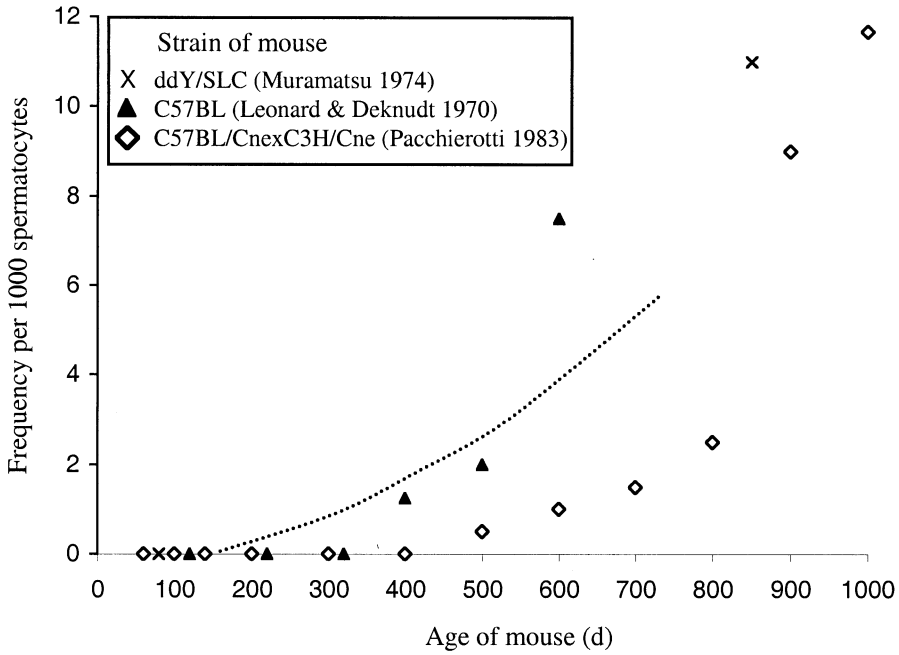
A number of environmental factors such as smoking and medications have been identified that increase frequencies of chromosomally abnormal sperm independent of age (75, 79, 85, 106, 111–118). An environmental mechanism for chromosomal abnormalities in male germ cells would predict an accumulation of genetic damage based on chronological and not biological age. However, the age response for translocation frequencies in mice is nearly the same as that in humans (compare Figs. 6 and 7), despite the fact that the lifespan of a mouse is only about 2 years. If the amount of chromosomal damage was influenced by the amount of time exposed to the environment, then the lifetime amount of genetic damage should be less in mice. This suggests that the age-related increase in translocation frequencies is not solely the result of environmental exposure but may be because of biological processes associated with aging.

DNA Repair

Chromosomal breaks and point mutations are known to increase exponentially with male age in the somatic and germ cells of both rodents and humans (16, 17, 104, 119). This could be the result of a decrease in the overall efficiency

FIGURE 8

Translocation frequencies in MI spermatocytes for three different strains of mice (92, 109, 110). For comparison, the age curve for translocations in mouse lymphocytes is indicated by a *dotted line* (141).



Sloter. Effects of male age on chromosomal abnormalities. Fertil Steril 2004.

of DNA repair with age, although the mechanism for this decrease is not known. Several components of the DNA repair machinery also control aspects of meiotic chromosome pairing and recombination, processes known to be critical for proper chromosome segregation. For example, mutations in mismatch repair genes in yeast (e.g., pms1, msh2, mlh1) and mice (e.g., Mlh1, Pms2) (120–122) have been associated with abnormal chromosome segregation during meiosis. Men carrying mutations in the DNA mismatch repair gene, hMSH2, were found to carry significantly higher frequencies of sperm disomic for chromosomes 13, 21, and X and sperm diploidy (123). An age-related decline in DNA repair capabilities, therefore, would be expected to have adverse effects on both chromosomal integrity and segregation.

Altered Recombination

Meiotic recombination is marked by the presence of chiasmata, which are the physical links that hold together homologous chromosomes during MI until they are properly segregated. Absent or reduced recombination has been associated with nondisjunction in humans (25, 31), as well as in model organisms such as yeast (124, 125), *Drosophila* (126–128), and mice (129). An age-dependent decrease in recombination frequencies has been observed for both men and women (130, 131). It has been suggested that the sex chromosomes in men may be particularly susceptible to age-related disturbances in meiotic recombination due to potential pairing difficulties within the pseudoautosomal region of the X and Y. In further support of this, several sperm studies have detected increased frequencies of XY sperm disomy with increasing male age (73, 76, 80, 81). Recently, however, a study using single-sperm typing found no reduction in recombination events within the pseudoautosomal region with male age (132).

There is evidence that the position of recombination events along the bivalents may be critical to proper chromosome segregation. Studies of yeast (125, 133–135) and *Drosophila* (127, 136) have shown that bivalents containing a single distally located exchange were more likely to be involved in nondisjunction events than those with more proximally located exchanges. Maternal cases of trisomy 16 have been associated with a shift toward distally positioned exchanges rather than a reduction in recombination (25).

For other human trisomies, exchanges positioned too near the centromere were implicated in nondisjunction (135, 136). Tanzi et al. (137) observed a significant reduction in the frequency of crossover events in the most telomeric portion of chromosome 21 with increasing maternal age, with a less significant decrease in the pericentromeric region. Whether or not the positioning of recombination events is

altered with increasing paternal age has not been determined.

Other Predisposing Genetic Factors

There has been steady progress in the identification of genes that control meiosis and recombination. For example, studies of model organisms such as *Drosophila* have found several mutations (*nod*, *Axs*, *Dub*, and *ncd*) that increase the frequency of aneuploid germ cells (120, 138). Recently, Yuan et al. (139) found that the absence of synaptonemal complex protein 3 promotes aneuploidy in mouse oocytes and significantly increases embryo death with advancing maternal age. However, no specific mutations or polymorphisms have yet been identified that infer an age effect on the induction of chromosomal abnormalities (numerical or structural) in males of any species.

Evidence for the genetic basis of age-related aneuploidy in males may be inferred from a recent study of strain differences in the age effect on chromosomal aberrations in the germline of short-lived (SAMP) and long-lived male mice (CBA and SHR) (140). Mutant SAMP mice exhibit increased rates of somatic cell mutations and accelerated aging. By 9 months of age, SAMP mice showed an approximate twofold increase in the frequency of primary spermatocytes with chromosomal aberrations vs. controls. Although frequencies were significantly lower than for SAMP mice, control mice also showed a twofold increase in chromosomal aberrations by 9 months of age. Chromosomal aberrations did not increase significantly with age in the long-lived CBA and SHR strains. Similar strain differences in the effects of age on chromosomal rearrangements were seen using mouse lymphocytes (141).

Further research is needed to identify genes involved in the paternal and maternal age effects and to understand why our species is so highly prone to meiotic errors compared to other organisms, despite the remarkable conservation of genes in the meiotic pathway among divergent species.

Hormonal Status of Men

Serum levels of LH and FSH increase slightly in men between 40 and 70 years of age and more precipitously after age 70 years (142). Associations between increased serum FSH concentrations and meiotic disturbances leading to germ cell degeneration have been observed in humans (143). Reduced Leydig cell function is also observed in aging men with a concurrent reduction in serum T levels (1). Bakshi et al. (144) recently found that T is required for the maintenance of rat DNA topoisomerase II alpha (topoII) expression during development of the postnatal testis and during spermatogenesis. TopoII is required in a wide range of biological functions including DNA replication, maintenance of genome stability, chromosome segregation, and chromosome condensation. Thus, reduced T levels with male age may increase frequencies of chromosomal nondisjunction or breakage resulting from diminished topoII activity. In support of this, Marchetti et al. (106) showed that treatment of male mice with the topoII inhibitor, etoposide, induced structural and numerical chromosomal abnormalities in spermatocytes and detected in zygotes after mating of treated males with unexposed females. Further research is warranted to address the relationship between chromosomal abnormalities in male germ cells and the hormonal status of older and peripubertal males.

Suppressed Apoptosis in Older Men

Apoptosis occurs in the testes of young and old men and is important for eliminating defective cells from the germ cell pool (145). Reduced apoptosis may result in the accumulation of chromosomally abnormal sperm. Long-term exposure of male mice to the germ cell mutagen, cyclophosphamide resulted in a reduced rate of apoptosis and an increase in abnormalities in offspring (146). In addition, an age-dependent decrease in the ability of damaged germ cells to undergo apoptosis after oxidative stress was observed in mice (147, 148). It is possible that genotoxic agents may be particularly detrimental for the germ cells of older men because of the inability of chromosomally damaged germ cells in aged males to respond to apoptotic stimuli resulting in a greater number of chromosomally defective sperm, but this will require further research.

Nutritional Status

In somatic cells, frequencies of chromosome breakage and loss, as measured using the micronucleus assay, are significantly affected by dietary factors (149). Caloric restriction has been shown to extend life span, delay spontaneous damage and tumorigenesis, and prolong reproductive life (150). There is evidence that deficiencies or excesses of specific micronutrients (e.g., folic acid, vitamin A) can increase the risk for certain birth defects (151). Of recent interest is the link between Down syndrome pregnancies and maternal polymorphisms in enzymes involved in folic acid metabolism, namely methylene-tetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) (152–155). However, other investigators found no obvious increase in MTHFR or MTRR mutations in case mothers of Downs' children compared with controls or to other trisomies involving chromosomes 2, 7, 10, 13, 14, 15, 16, 22, or the sex chromosomes (156). However, there was an association with mothers of trisomy 18 conceptuses (156). These results are exciting because they suggest the possibility of a preventive measure to reduce the risk of trisomy through dietary supplementation to the mother. Recent sperm data indicate beneficial effects of certain dietary micronutrients and antioxidants on physiological parameters of semen quality (i.e., sperm counts, motility, volume) (S. A. Kidd et al., unpublished data). It is unknown whether nutrition might also postpone the paternal age effect on chromosomal abnormalities in sperm.

CONCLUSION

Both human and animal evidence suggests that fathering children at older ages may come with a greater risk for pregnancy loss or genetic disorders due to sex chromosomal aneuploidies or structural aberrations transmitted by the sperm. There seems to be little evidence in support of a consistent effect of age on sperm aneuploidy involving autosomes. The evidence for sex chromosomal aneuploidy, however, suggests that there may be about a twofold increase in risk at age 50 years.

Animal studies suggest that the paternal age effect for breaks and rearrangements in sperm is much greater than for aneuploidy. In light of the fact that the majority of *de novo* structural chromosomal abnormalities in human offspring are of paternal origin, further research is needed to identify specific environmental or paternal host factors that are associated with paternally transmissible structural chromosomal abnormalities.

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