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The association of age and semen quality in healthy men

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BACKGROUND: Although the effect of maternal age on fertility is well known, it is unclear whether paternal age also affects fertility. This cross-sectional study sought to characterize the association between age and semen quality, a well-known proxy of fertility status. METHODS: A convenience sample of 97 non-smoking men (aged 22–80 years) without known fertility problems was recruited from a national government laboratory. The men provided semen samples and information relating to lifestyle, diet, medical and occupational details. Semen volume (ml), sperm concentration ($\times 10^6$/ml), total sperm count ($\times 10^6$), motility (%), progressive motility (%) and total progressively motile sperm count ($\times 10^6$) were measured. RESULTS: After adjusting for covariates, semen volume decreased by 0.03 ml per year of age (95% CI: –0.05, –0.01); motility decreased by 0.7% per year (95% CI: –0.92, –0.43); progressive motility decreased by 3.1% per year (95% CI: –4.5, –1.6); and total progressively motile sperm count decreased by 4.7% per year (95% CI: –7.2, –2.2). There was a suggested decrease in sperm concentration and count. The proportion of men with abnormal volume, concentration and motility was significantly increased across the age decades. CONCLUSIONS: In a convenience sample of healthy men from a non-clinical setting, semen volume and sperm motility decreased continuously between 22–80 years of age, with no evidence of a threshold.

Key words: age/semen volume/sperm concentration/sperm motility/total sperm count

Introduction

Approximately 15% of couples of reproductive age experience infertility, and more than a quarter of infertility cases may be attributed to male factors (Templeton, 1995). It is well known that maternal age is a significant contributor to human infertility (Joffe and Li, 1994), due primarily to the precipitous loss of functional oocytes in women by their late thirties (Tietze, 1957; Lansac, 1995). Human spermatogenesis, on the other hand, continues well into advanced ages, allowing men to reproduce during senescence. Although very little is known about the topic, paternal age may also contribute to human infertility.

Understanding the effect of male age on fertility has become increasingly important in public health because a growing number of men are choosing to father children at older ages (Ventura *et al*., 1997). In the United States, for example, there has been a 24% increase in the birth rate for fathers aged 35 to 54 years since 1980. However, advanced male age has been associated with significant reductions in pregnancy rates, increased time-to-pregnancy and increased subfecundity (Kidd *et al*., 2001). In a recent study of 8515 planned pregnancies (of greater than 24 weeks gestation), men older than 35 years had half the chance of fathering a child within 12 months compared with men aged less than 25 years, after controlling for women's age and other factors (Ford *et al*., 2000).

Semen quality is generally considered to be a proxy measure of male fertility, and changes in semen quality can occur after exposure to toxic agents (Wyrobek *et al*., 1983; Wyrobek, 1993) or from host factor effects such as age (Kidd *et al*., 2001). The weight of evidence primarily from clinical studies (see Kidd *et al*., 2001 for a review) suggests that age is associated with diminished semen volume, sperm motility and/or sperm morphology, but that sperm concentration is affected little by age (Schwartz *et al*., 1983; Wang *et al*., 1985; Abramsson, 1988; Carlsen *et al*., 1992; Berling and Wolner-Hanssen, 1997; Lemcke *et al*., 1997; Spandorfer *et al*., 1998). However, it is unclear whether these observations are applicable to the general population of healthy men. Also, men at older ages (e.g. >50 years) were under-represented in many of these clinical studies, which limited statistical power and prevented the determination of the shape of the relationship between age and semen quality. In addition, potential confounders that might explain changes with age, such as smoking history or duration of abstinence, were seldom controlled.

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The purpose of the current research was to examine the magnitude and the shape of the relationships between age and semen volume, sperm concentration and sperm motility in a non-clinical group of men selected to span across a wide range of age, from their 20s to their 80s. These men provided extensive information on medical, lifestyle and occupational exposure factors that could affect semen quality.

Materials and methods

Subjects

The study population of the Age and Genetic Effects on Sperm (AGES) Study consisted of a convenience sample of 97 male volunteers employed or retired from the Lawrence Livermore National Laboratory (LLNL) located in Livermore, California. LLNL was used as the recruitment site because the workforce is relatively homogeneous and because it was the site of the semen analysis laboratory. The AGES Study was approved by the Institutional Review Boards of the participating institutions and all subjects gave written informed consent.

Male workers and retirees were recruited between October 1997 and July 1998 from advertisements posted at the LLNL, e-mail listings, and in newsletters. Preliminary screening over the telephone excluded men who had smoked cigarettes in the last 6 months; had a vasectomy or a history of an undescended testicle or prostate cancer; had ever received chemotherapy or radiation treatments; had current fertility or reproductive problems (men were included who claimed their wives had a fertility problem); or had a previous semen analysis with zero sperm count. Twenty men were excluded after the preliminary screening due to current smoking (n = 11), varicocele (n = 3), one testicle (n = 2), undescended testicles (n = 1), valium use (n = 1), chemotherapy (n = 1) and hepatitis B infection (n = 1). At least 15 men were recruited from each age decade from age 20 to age 70 years; additional men above age 70 were also enrolled.

Eligible men were mailed a questionnaire, a semen collection container, and a small foam-insulated vacuum container (Aladdin Industries Inc., Nashville, TN, USA). The men were instructed to provide a semen sample by masturbation into the collection container after abstaining from ejaculating for 2–7 days, and to deliver it inside the insulated container to a drop box at LLNL. The questionnaire gathered information on medical history, reproductive history (e.g. fatherhood history), sociodemographic characteristics (age, race, education) and lifestyle habits (e.g. diet, alcohol, cigarette and caffeine consumption). Completed questionnaires were mailed to the University of California at Berkeley. The completed questionnaires were reviewed with the participant over the telephone.

Semen analysis

All semen samples were delivered within 2 h of collection. One laboratory technician performed all the analyses, and was blinded to the age and identity of the donors. Semen analysis was performed immediately upon receipt of the sample, averaging about 45 min after its production (range 5–120 min). Duplicate samples were requested from donors if the sample exhibited any of the following: volume <1 ml, zero motility, abnormal numbers of red or white blood cells, or potential loss of sample reported by donor. In total, 115 fresh semen specimens were provided from 97 men. The study results did not change regardless of whether a man's first or second sample was used for the analyses.

The semen analysis was performed using slightly modified protocols provided by P. Turek of the University of California, San Francisco, Department of Urology and by Hamilton Thorne Research (Beverly, MA, USA), following published guidelines (World Health Organization, 1992). Each specimen was liquefied at room temperature and the volume measured to the nearest 0.1 ml. Sperm concentration of gently mixed samples was determined for three independent loadings on a Neubauer haemocytometer. For sperm motility analysis, 50 µl of semen were diluted to a standardized sperm concentration of ~35×10^6 sperm per ml using Dulbecco's phosphate-buffered saline solution supplemented with glucose (1 g/l) and bovine serum albumin (World Health Organization, 1992). At least 150 motile and immotile sperm were evaluated at 400× phase-contrast magnification using a 2X-CEL 20 µm depth chambered microscope slide (Hamilton Thorne Research) maintained at 37°C by a MiniTherm slide warmer (Hamilton Thorne Research). If >10% difference was found between the two analyses of the same sample, the sample was re-analysed. All cell counting was performed on a Macintosh computer using a modified version of CytoScore© developed at LLNL.

Statistical analysis

All analyses were conducted using STATA 7.0 (Stata Statistical Software, 1999). Total sperm count was calculated by multiplying semen volume by sperm concentration. Total progressively motile sperm was defined as the product of total sperm count and percent progressive motility. Volume and motility appeared to be normally distributed, while sperm progressive motility, concentration, total count, and total progressively motile sperm were log-transformed to achieve normality. Zero values for semen parameters were re-coded to half of the lowest measured value before transformation. All count and concentration analyses were performed with and without four subjects with azoospermia; all motility analyses were performed without these men.

Separate multiple linear regression models were used to examine the relationship of age with each semen parameter controlling for the following potential confounding factors: duration of abstinence; average ejaculation frequency (last 3 months); time from sample collection to sample processing; season; time worked at LLNL; body mass index; ethnicity; years of education; potential occupational exposures (last 3 months); dosimetry records; time spent sitting; medication use; alcohol and caffeine intake; history of tobacco use; history of chronic disease (e.g. high blood pressure, heart problems or diabetes); history of genitourinary disease (e.g. urinary tract or other genitourinary infection, sexually transmitted disease or past history of infertility); and fatherhood history. Covariates were included in the multivariate models if they changed the regression coefficient for age by >10% or if they were statistically significant at $P < 0.1$.

Results for log-transformed semen parameters were presented as the relative percent change per year as converted from the antilog of the regression coefficient. Results for untransformed semen parameters were presented as the absolute change per year of age as well as the relative change in the outcome in men who were age 50 years compared with age 30 years. The partial correlation for age, adjusted for the other covariates in the model, is presented.

A hockey-stick model was fitted to the adjusted data to determine if there was a change in slope at any age (Bacon and Watts, 1971). Hockey-stick analysis of the adjusted regression models did not fit the data better than the log transformations based on a likelihood test, ruling out any clear 'threshold' effect for any of the semen parameters. Thus, only the results of the regression analyses are presented.

Maximum-likelihood logit models were fit to the data for each semen parameter to estimate the probability of having an abnormal value at each year of age, adjusting for the same covariates that were in the linear regression models. Abnormal semen values were defined from WHO (1992) standards: volume ⩽2 ml, concentration $<20\times10^6$/ml,

Table I. Mean (SD) of age and semen parameters by selected characteristics of the participants

Variable	*n* (%)[a]	Age (years)	Volume (ml)	Concentration (10^6/ml)	Total count (10^6)	Motility (%)	Progressive motility (%)	Total progressively motile sperm count (10^6)
Abstinence								
2–5 days	73 (75)	44.5 (15.6)	2.6 (1.4)	120.4 121.2)	304.6 (336.4)	39.8 (20.3)	23.0 (15.9)	82.4 (102.5)
>5 days	24 (25)	52.4 (15.8)[c]	3.2 (1.6)	187.3 (156.8)[c]	603.6 (517.4)[d]	28.9 (20.7)[c]	17.6 (16.3)	126.7 (179.4)
Time to sample processing								
<45 min	51 (53)	45.9 (15.2)	2.9 (1.5)	129.8 (128.4)	384.8 (460.6)	41.0 (21.9)	24.5 (17.6)	108.8 (153.9)
>45 min	46 (47)	47.1 (16.7)	2.7 (1.4)	144.9 (139.4)	371.8 (343.4)	33.9 (18.6)	18.6 (13.7)	75.1 (80.8)
Tobacco use								
Never	70 (72)	43.8 (15.5)	3.2 (1.5)	124.5 (101.7)	417.2 (419.0)	38.9 (21.1)	23.7 (16.6)	107.6 (137.0)
Ever	27 (28)	53.2 (15.4)[d]	1.9 (1.1)[e]	169.4 (191.3)	278.5 (363.2)[c]	32.8 (19.9)	16.2 (13.4)	52.7 (74.3)[c]
Alcohol								
Never	34 (35)	49.2 (15.3)	3.3 (1.4)	115.0 (107.1)	391.5 (350.5)	33.4 (20.7)	20.3 (16.9)	75.1 (67.6)
Ever	63 (65)	45.0 (16.2)	2.5 (1.4)[d]	148.8 (144.9)	371.7 (437.3)	38.8 (20.3)	22.5 (15.7)	102.6 (147.4)
BMI (kg/m^2)								
20–25	47 (48)	45.4 (16.4)	2.6 (1.4)	128.8 (140.6)	342.0 (400.9)	35.2 (23.3)	20.3 (17.8)	74.2 (100.2)
>25	50 (52)	47.5 (15.6)	3.0 (1.6)	144.7 (108.6)	413.1 (414.1)	39.2 (18.1)	23.0 (14.4)	110.4 (143.8)
Hypertension								
Never	81 (84)	44.0 (15.6)	2.9 (1.5)	144.1 (132.8)	413.4 (425.4)	38.7 (20.6)	22.7 (16.1)	102.4 (132.5)
Ever	16 (16)	59.0 (10.9)[e]	2.4 (1.4)	100.9 (133.7)	202.6 (237.5)[d]	29.1 (20.8)	16.2 (15.3)	39.2 (45.4)[c]
UTI[b]								
Never	85 (88)	45.6 (16.2)	2.9 (1.5)	139.7 (130.9)	401.8 (423.2)	39.2 (20.4)	23.1 (16.4)	101.3 (131.0)
Ever	12 (12)	52.6 (12.9)	2.3 (1.3)	117.6 (154.0)	214.4 (215.1)	23.7 (19.5)[d]	12.5 (10.4)[c]	35.9 (48.9)[c]

BMI = body mass index; UTI = urinary tract infection.
[a]*n* = 97 for age, volume, concentration, total count; *n* = 93 for motility, progressive motility, and total progressively motile sperm.
[b]Includes infections of the bladder and kidney.
[c]$P < 0.05$ for *t*-tests (volume, motility) or Mann–Whitney tests (concentration, count, progressive motility, total progressively motile sperm).
[d]$P < 0.01$.
[e]$P < 0.001$.

Table II. Median and interquartile range (IQR, 25th–75th percentile) of semen parameters by age decade

Age group (years)	*n*	Days of abstinence (mean)	Volume (ml)	Concentration (10^6 /ml)	Total count (10^6)	Motility (%)	Progressive motility (%)	Total progressively motile sperm count (10^6)
22–29	19	3.7	3.0 (2.1–4.0)	92.0 (52–177)	345.0 (109–658)	50.0 (35–60)	29.0 (21–36)	96.6 (30–237)
30–39	20	4.0	3.5 (2.6–4.6)	74.5 (58–110)	268.0 (166–428)	51.0 (44–54)	24.5 (18–31)	65.6 (38–116)
40–49	16	4.6	3.5 (3.0–4.9)	156.5 (42–250)	432.0 (154–993)	41.5 (27–56)	18.5 (10–38)	95.6 (30–177)
50–59	17	5.4	2.2 (1.5–2.6)	101.0 (89–170)	250.8 (192–297)	38.0 (14–48)	16.0 (4–27)	47.9 (12–69)
60–69[a]	17	6.4	2.0 (1.1–3.0)	102.0 (42–168)	215.6 (55–287)	21.5 (10–40)	11.5 (4–25)	23.7 (4–62)
70+[a]	8	8.9	1.2 (0.6–2.0)	29.5 (0–141)	30.7 (0–582)	11.0 (11–12)	4.0 (1–5)	2.6 (0.4–55)
Total	97	5.1	2.7 (1.5–3.8)	93.0 (43–177)	258.5 (100–460)	42.0 (17–53)	20.0 (8–30)	55.0 (15–115)
Test for trend (*P*)		< 0.01	< 0.01	0.34	0.01	< 0.01	< 0.01	< 0.01

[a]Four azoospermic men were aged 63, 77, 77 and 78 years. Thus, the sample sizes for the sperm motility parameters are reduced accordingly (*n* = 16 for the 60–69 group and *n* = 5 for the 70+ group).

count $\leqslant 40\times10^6$, motility <50%, progressive motility <25%, and total progressively motile sperm count $<10\times10^6$.

Results

Characteristics of study population

The 97 men were on average 46.4 (±15.8, SD) years old (range 22–80 years). The group was 91% Caucasian, 80% college graduates and 72% never-users of tobacco. Ethnicity and level of education did not vary by age of the man (data not shown). As shown in Table I, older age was associated with prior tobacco use (none smoked cigarettes during the previous 6 months), increased duration of sexual abstinence, and ever having had high blood pressure. Age was unrelated to time between collection and sample processing, alcohol use, body mass or history of urinary tract infection. Some 64% of the men had fathered children earlier in life; and as expected, men with proven fertility were older than men with unproven fertility (50.6 versus 39.0 years; $P = 0.001$; data not shown).

The median semen volume was 2.7 ml; median sperm concentration was 93×10^6/ml; and median total sperm count was 259×10^6 (Table II). The median motility parameters were 42% motility, 20% progressive motility and 55×10^6 progressively motile sperm. Seven men, all of whom were aged over 60 years, were either azoospermic (63, 77, 77 and 78 years old) or had no progressively motile sperm (60, 64 and 67 years old). Two of the four azoospermic men and all of the men with zero

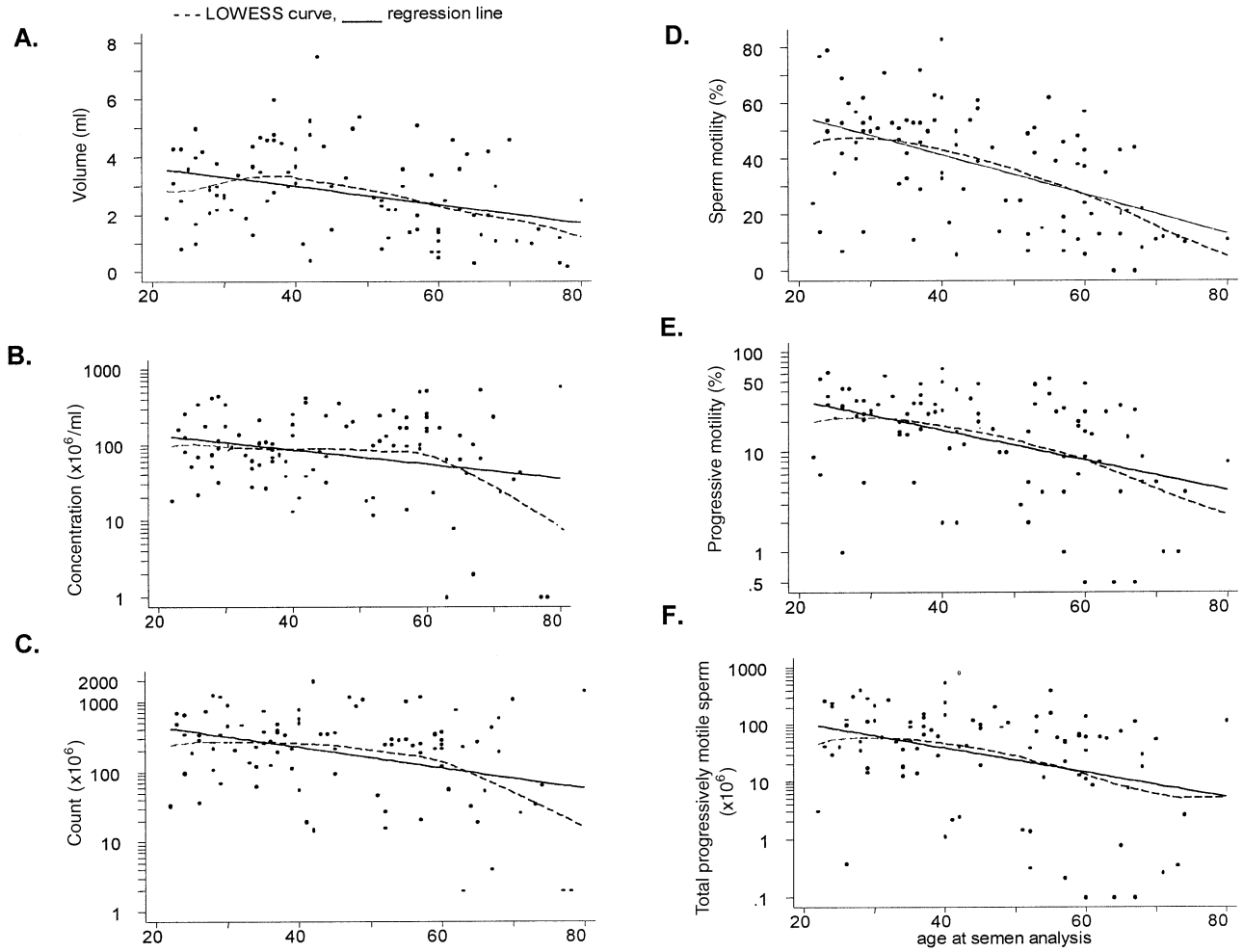

Figure 1. The relationship between age in years and semen volume (**A**), concentration (**B**), count (**C**), sperm motility (**D**), progressive motility (**E**) and total progressively motile sperm (**F**). Individual data points are shown as well as locally weighted smoothed fits (using a bandwidth of 0.8) (broken lines) and unadjusted linear regression lines (solid lines). Detailed results and additional fits adjusted for covariates are described in the text.

sperm motility had fathered children earlier in their life, and none had ever been diagnosed with their own fertility problem. The aetiology of the azoospermia was unknown.

Semen quality varied with some lifestyle and medical factors (Table I). Semen volume was higher in men who never used tobacco or who never drank alcohol. Sperm concentration was higher in those who had abstained from ejaculation for more than 5 days. Sperm count was also higher in men who had abstained for >5 days, but lower in men who had ever had high blood pressure. Both percent motile and percent progressively motile sperm were lower in men who had ever had a urinary tract infection. Total number of progressively motile sperm was lower in men who had ever had high blood pressure.

Semen volume

Men in their 20s had a median semen volume of 3.0 ml (Table II), and there was a significant trend toward lower volumes across age decades (*P*-value for trend, < 0.01). This decrease with age is shown graphically in Figure 1A. In the multiple linear regression analyses, semen volume decreased with age by 0.03 ml per year (95% CI = –0.05, –0.02) after adjusting for length of abstinence and prior use of tobacco and alcohol (Table III). From the multivariate regression models, a 50-year-old man was calculated to have a 20% relative decrease in semen volume compared with a 30-year-old man. Age explained 14.4% (partial $r = -0.38$, $P < 0.001$) of the total variance in semen volume. The number of men with abnormal semen volume (⩽2 ml) also significantly increased across the age decades (*P*- value for trend, < 0.001; Figure 2). As shown in the adjusted logit graph (Figure 3), at age 30 years, about 10% of the men would have an abnormal semen volume, and this proportion increased to approximately 35% at age 50 and 80% by age 80 ($P < 0.001$).

Sperm concentration and total sperm count per specimen

Men in their 20s had median sperm concentrations and total sperm counts of 92×10^6/ml and 345×10^6 respectively. Total count decreased significantly across age decades (*P*-value for trend, 0.01) (Table II; Figure 1B). The relationship between

Table III. Unadjusted and adjusted linear regression analyses of age on semen quality

	Volume (ml)[a]	Concentration (10^6 /ml)[b]	Count (10^6)[b]	Motility (%) (n = 93)[a]	Progressive motility (%) (n = 93)[b]	Total progressively motile sperm count (10^6) (n = 93)[b]
Unadjusted change/year	–0.03	–2.2	–3.4	–0.70	–3.4	–4.9
95% CI	(–0.05, –0.01)	(–3.8, –0.5)	(–5.1, –1.6)	(–0.94, –0.46)	(–4.8, –2.0)	(–7.4, –2.3)
P	0.001	0.01	<0.001	<0.001	<0.001	<0.001
Adjusted change/year	–0.03[c]	–2.5[d]	–3.5[e]	–0.67[f]	–3.1[g]	–4.7[h]
95% CI	(–0.05, –0.02)	(–4.2, –0.8)	(–5.3, –1.7)	(–0.92, –0.43)	(–4.5, –1.6)	(–7.2, –2.2)
P	< 0.001	0.005	< 0.001	< 0.001	< 0.001	< 0.001
Adjusted R-squared	0.31	0.07	0.17	0.29	0.24	0.22

[a]For volume and motility, result expressed as the absolute change per year of age (β coefficient).
[b]Result expressed as the relative percent change per year of age for sperm concentration, total sperm count, progressive motility and total progressively motile sperm. This number is converted from the antilog of the regression coefficient (β) of the log-linear model [(antilog(β) – 1)×100]. For example, if x_1% is the progressive motility at age 1 year, then the progressive motility at age years 2 (x_2%) = $x_1 - (x_1 \times 0.031)$.
[c]Adjusted for abstinence, any prior tobacco use, and any alcohol use.
[d]Adjusted for abstinence.
[e]Adjusted for abstinence and any prior tobacco use.
[f]Adjusted for abstinence and time to sample processing (in min).
[g]Adjusted for abstinence, time to sample processing, previous urinary tract infections, and body mass index.
[h]Adjusted for abstinence, body mass index, and previous urinary tract infections.

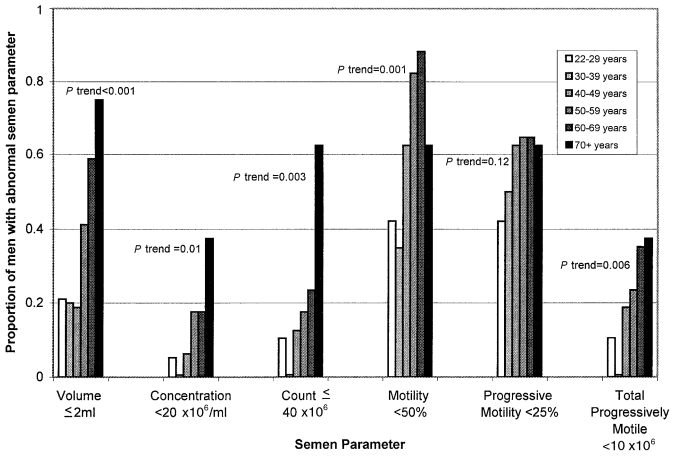

Figure 2. The frequency of men in each age decade with abnormal semen values as defined by WHO guidelines. The WHO guidelines used were: volume <2 ml, concentration $<20\times10^6$/ml, count $<40\times10^6$, motility <50%, progressive motility <25% and total progressively motile sperm count $<10\times10^6$.

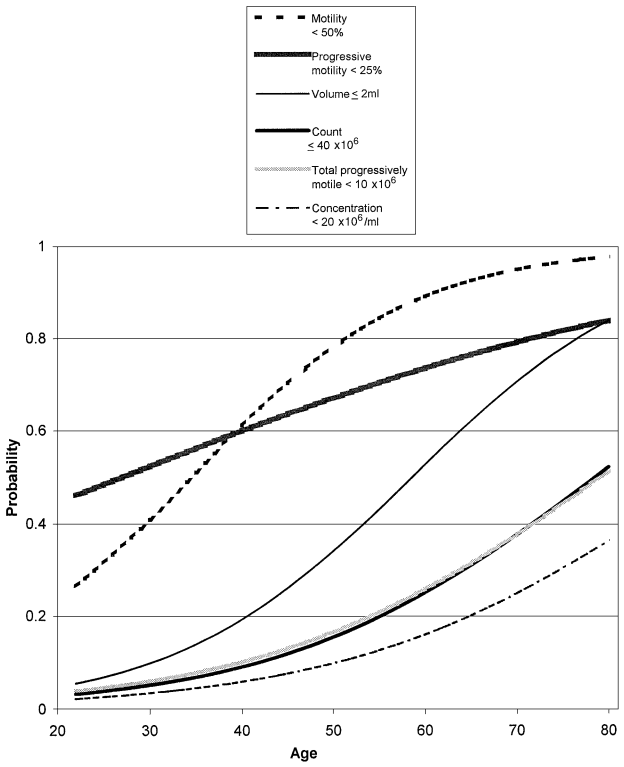

Figure 3. Estimated probability of having a clinically abnormal semen parameter at each year of age using maximum-likelihood adjusted logit graphs. Each logit model was adjusted for the same covariates as the linear regression models. For graphing purposes, all covariates were set to the mean value of the population.

age and concentration was statistically significant in the model including the four azoospermic men (slope = –2.5% per year; 95% CI = –4.2, –0.8) (Table III), but non-significant in the model with the four men excluded (slope = –0.6% per year; 95% CI = –2.0, 0.9). The relationship between total sperm count and age was statistically significant, both including the four azoospermic men (slope = –3.5% per year; 95% CI = –5.3, –1.7) and excluding them (slope = –1.7% per year; 95% CI = –3.4, –0.006), although the effect was greatly decreased for the latter.

As shown in Figure 2, the number of men with abnormal levels for sperm concentration and count significantly increased across age decades (P-values for trend 0.01 and 0.003 respectively); however, the number of men affected in each decade was small. Based on the adjusted logit model, the predicted probability of having an abnormal value for concentration was approximately 5% at age 30 years, 10% at age 50 and 35% by age 80 (P = 0.006). For sperm count, the predicted probability for an abnormal value was about 5% at age 30 years, 15% at age 50, and 50% by age 80 (P = 0.03) (Figure 3).

Percent motility, percent progressive motility and total number of progressively motile sperm

Semen specimens provided by men in their 20s had medians of 50.0% motility, 29.0% progressive motility and 96.6×10^6 total progressively motile sperm (Table II). There were significant trends towards reduced sperm motility across age decades for all three parameters (*P*-values for trend < 0.01, for all three) (Table I; Figure 1D, E and F). In the regression analyses, percent motility decreased by 0.7% per year of age (95% CI = –0.92, –0.43) after controlling for length of abstinence and time before the sample was processed (Table III). A 50-year-old man was calculated to have a relative decrease of 28% motile sperm compared with a 30-year-old man. Similarly, after adjusting for covariates, there was 3.1% (95% CI = –4.5, –1.6) relative decrease in the percent of progressive motility per year, and a 4.7% decrease per year (95% CI = –7.2, –2.2) for total progressively motile sperm. The proportion of the total variance explained by age was 27.0% for motility ($r = –0.52$, $P < 0.001$), 17.6% for log progressive motility ($r = –0.42$, $P < 0.001$) and 13.0% for log total progressively motile ($r = –0.36$, $P < 0.001$).

The number of men with abnormal percent motile and total progressively motile sperm also increased significantly across age decades (*P*-value for trend 0.001 and 0.006 respectively). The frequency of percent abnormal progressive motility did not significantly increase across age decades (*P*-value for trend 0.12), although the frequency appeared to increase up to the 40s and then plateau (Figure 2). The adjusted logit model predicted that about 40% of the men by age 30 years would have abnormal percent motility. This increased to approximately 80% by age 50, and to 100% by age 80 ($P < 0.001$) (Figure 3). The probability for having abnormal progressive motility was approximately 52% at age 30 years, 67% at age 50, and 84% by age 80 ($P = 0.07$). The probability for having abnormal total progressively motile sperm was approximately 5% at age 30 years, 15% at age 50, and 50% by age 80 ($P = 0.01$).

Discussion

This investigation found evidence of significant age-dependent reductions in several aspects of semen quality among a cohort of healthy workers and retirees: the largest effects of age were on sperm motility, with intermediate effects on semen volume and the smallest effects on sperm numbers. The age effects on sperm concentration and total count appeared to be driven by four azoospermic men over age 60 years. For men aged 50 years, an approximate 80% probability of clinically abnormal motility, 35% probability of abnormally low semen volume and 15% probability of abnormally low sperm count was predicted. These probabilities increased to approximately 100, 80 and 50% respectively for men aged 80 years. There was no evidence of an age ‘threshold’’ for any of the semen parameters, but rather a gradual change over time.

These findings support and extend those for semen quality in prior clinical studies of infertile patients and sperm donors (Kidd *et al*., 2001). However, the percent changes with age in the present study were generally at the high end of changes observed in the clinical studies. For example, a 20% decrease was observed in semen volume in 50-year-old compared to 30-year-old men, which is near the high end of the 3 to 22% range reported in prior clinical studies (Fisch *et al*., 1996; Rolf *et al*., 1996; Andolz *et al*., 1999; Kidd *et al*., 2001). Similarly, the 28% relative decrease in motility identified was near the high end of the 3 to 37% range of decreases reported in clinical studies (Schwartz *et al*., 1983; Auger *et al*., 1995; Fisch *et al*., 1996; Rolf *et al*., 1996). Sperm concentration was least affected by age among the semen quality parameters studied, although there was a significant trend toward increased numbers of men with abnormal concentration across age decades. This was not inconsistent with reports from the clinical literature which, in general, did not find that sperm concentration decreased with age (Schwartz *et al*., 1983; Wang *et al*., 1985; Abramsson, 1988; Carlsen *et al*., 1992; Berling and Wolner-Hanssen, 1997; Lemcke *et al*., 1997; Spandorfer *et al*., 1998; Kidd *et al*., 2001). Although the present age-related findings for total sperm count were more robust, this was likely driven by the strong association of age with semen volume.

The present findings are generally consistent with some previous studies which demonstrated a decreased pregnancy rate and longer time to pregnancy in partners of older men (Stanwell-Smith and Hendry, 1984; Abramsson, 1988; Ducot *et al*., 1988; Goldman and Montgomery, 1989; Olsen, 1990; Mathieu *et al*., 1995; Rolf *et al*., 1996; Brzechffa *et al*., 1998; Spandorfer *et al*., 1998), but not others (Abramsson, 1988; Gallardo *et al*., 1996; van der Westerlaken *et al*., 1998; Paulson *et al*., 2001). Many of the pregnancy-based studies may be limited because they do not control for maternal age (Stanwell-Smith and Hendry, 1984; Bostofte, 1987; Abramsson, 1988; Ducot *et al*., 1988; Ford *et al*., 1994; Joffe and Li, 1994; van der Westerlaken *et al*., 1998) and/or they may not include sufficient numbers of men in the older age ranges (Bostofte, 1987; Abramsson, 1988; Ducot *et al*., 1988; Olsen, 1990; Ford *et al*., 1994; Mathieu *et al*., 1995; Gallardo *et al*., 1996; Rolf *et al*., 1996; Spandorfer *et al*., 1998; Paulson *et al*., 2001).

The design of the present study has several notable strengths. The ability to detect an association between age and semen parameters was enhanced by the inclusion of relatively larger numbers of older men compared with most previous studies (Wang *et al*., 1985; Abramsson, 1988; Check *et al*., 1989; Gallardo *et al*., 1996; Irvine *et al*., 1996; Spandorfer *et al*., 1998). The finding of decreased sperm motility with age was consistent with the findings of others (Nieschlag *et al*., 1982), whose study also included a large number of men aged over 60 years. Also, by including men over a wide age range, the shape of the relationship between age and semen quality could be examined. In addition, the men in this study were relatively homogeneous in sociodemographic characteristics across age groups. The sample consisted of employed and retired workers from a single occupational setting, of middle to high socioeconomic class, and with employer-paid access to medical care. Unlike most previous studies, control was introduced for other demographic or lifestyle differences in the statistical analysis (Homonnai *et al*., 1982; Nieschlag *et al*., 1982; Schwartz *et al*., 1983; Dondero *et al*., 1985; Wang *et al*., 1985;

Abramsson, 1988; Check *et al*., 1989; Singer *et al*., 1990; Carlsen *et al*., 1992; Mladenovic, 1994; Bujan *et al*., 1996; Gallardo *et al*., 1996; Haidl *et al*., 1996; Irvine *et al*., 1996; Rolf *et al*., 1996; Berling and Wolner-Hanssen, 1997; Lemcke *et al*., 1997; Spandorfer *et al*., 1998). However, as in all semen quality studies, the present sample included only those men who were willing to provide semen specimens and thus, may not have been representative of the general population of sexually active men. Several different sources of bias can be introduced by a non-representative sample. Although no men with known fertility problems volunteered to participate, men who suspected a potential fertility problem may have been more likely to volunteer. If this differed by age, bias could have been introduced, although there was no evidence for this. In addition, since the volunteers were all workers or retirees, a 'healthy worker' bias could have been introduced into the study, resulting in an underestimate of the age effect.

At least two broad modes of action may explain the age-dependent changes observed in semen quality. First, there may be cellular or physiological changes in the genitourinary tract with ageing. In autopsies of men who died from accidental causes, there have been age-related narrowing and sclerosis of the testicular tubular lumen, decreases in spermatogenic activity, increased degeneration of germ cells, and decreased numbers and function of Leydig cells (Bishop, 1970; Johnson, 1986). Decreased semen volume with age may be caused by seminal vesicle insufficiency, since seminal vesicle fluid contributes most of the ejaculate volume (Hamilton and Naftolin, 1981; Goldman and Montgomery, 1989). Changes in the prostate that occur with ageing, such as smooth muscle atrophy and a decrease in protein and water content, may contribute to decreased semen volume and sperm motility (Schneider, 1978). In addition, there may be age-related changes in the epididymis where sperm acquire the capacity for vigorous forward motility during transit. The epididymis is a hormonally sensitive tissue, which plays an important role in sperm maturation (Hamilton and Naftolin, 1981). Thus, hormonal or epididymal senescence may lead to decreased motility in older men. Also, older men may have decreased capacity to repair cellular and tissue damage from toxicant or disease exposure.

Second, age provides increased opportunities to suffer reproductive damage from exogenous exposures or diseases (Wyrobek *et al*., 1983; Wyrobek, 1993). 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 prior to 1972 were more likely to have been exposed to DDT, an endocrine disruptor, which was later banned (Carlsen *et al*., 1992; Kidd *et al*., 2001). In the present study, the decline in semen quality could be due to some unknown occupational exposure that was related to age, or time worked at LLNL. However, the analyses provided no evidence that time worked at LLNL or any occupational exposure explained the age-related decline in either sperm count or motility.

The semen parameters evaluated herein are not expected to be the only sperm end-points that will show age-related damage. Other parameters that may be affected by age include sperm morphology, which has been shown to be a sensitive indicator of the status of the germinal epithelium (MacLeod, 1964; Wyrobek, 1983). Several studies have suggested age-related defects in the genetic integrity of the sperm. For example, age has been associated with increased sperm aneuploidy in humans (Griffin *et al*., 1995; Martin *et al*., 1995; Robbins *et al*., 1995; Lowe *et al*., 2001) and in mice (Lowe *et al*., 1995), and with increases in the incidence of de-novo germinal mutations (Friedman, 1981; Martinez-Frias *et al*., 1988; Modell and Kuliev, 1990).

In summary, significant age-related decreases in semen quality were observed, most notably for semen volume and sperm motility. Because semen quality is a proxy for fertility, these data suggest that men may become progressively less fertile as they age. However, unlike women, there appears to be no evidence of an age threshold for men. The present findings have important implications for men who choose to delay fatherhood, since they may reduce their chance of success the longer they delay.

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