

# Decreases in Human Semen Quality with Age Among Healthy Men

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Decreases in human semen quality with age among healthy men

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**Capsule:** In 97 healthy, active men (aged 22 - 80 years), semen volume and sperm concentration, count, and motility significantly decreased with age, with no evidence of a threshold.

## ABSTRACT

**Objective:** To characterize the associations between age and semen quality among healthy active men after controlling for identified covariates.

**Design:** Cross-sectional study

**Setting:** University of California, Berkeley, and Lawrence Livermore National Laboratory, Livermore, California.

**Patients:** Ninety-seven healthy, nonsmoking men between 22 and 80 years without known fertility problems who worked for or retired from a large research laboratory.

**Main outcome measure(s):** Semen volume (mL), sperm concentration (millions/mL), total count (millions), motility (%), progressive motility (%), and total progressively motile sperm (millions).

**Results:** There was a gradual decrease in all semen parameters from 22-80 years of age. After adjusting for covariates, volume decreased 0.03 ml per year ( $p=0.001$ ); sperm concentration decreased 2.5% per year ( $p=0.005$ ); total count decreased 3.6% per year of age ( $p<0.001$ ); motility decreased 0.7% per year ( $p<0.001$ ); progressive motility decreased 3.1% per year ( $p<0.001$ ); and total progressively motile sperm decreased 4.8% per year ( $p<0.001$ ).

**Conclusions:** In a group of healthy active men, semen volume, sperm concentration, total sperm count, and sperm motility decrease continuously between 22-80 years of age, with no evidence of a threshold.

**Keywords:** human male; age; semen; spermatozoa; semen volume; sperm concentration; sperm motility; sperm count; fertility

## INTRODUCTION

Approximately 15% of couples of reproductive age experience infertility, and more than a quarter of infertility cases may be attributed to male factors (1). It is well-known that maternal age is a significant contributor to human infertility (2), due primarily to the precipitous loss of functional oocytes in women by their late thirties (3, 4). Effects of male age on fertility are expected to be more subtle and prolonged because human spermatogenesis continues well into advanced ages allowing men, at least theoretically, to reproduce during senescence.

Understanding the effect of male age on fertility has become increasingly important in public health, because there is a growing number of men who are fathering children at older ages (3). In the United States, for example, there has been a 24% increase in the birth rate for fathers over age 35 since 1980. Yet advanced male age is also associated with an increased risk of infertility as measured by significant reductions in pregnancy rates, increases in time-to-pregnancy, and increased subfecundity [see review by Kidd et al 2001(5)]. In a recent study of 8515 planned pregnancies, men older than 35 years had half the chance of fathering a child within 12 months compared to men less than 25 years after controlling for women's age and other factors (6).

Semen quality is generally considered to be a proxy measure of male fertility. It has also been broadly applied in male reproductive toxicology to assess the potential risks associated with male exposures to chemical and pharmaceutical agents (7, 8) 9, 10). There is also a growing number of reports about regional and temporal differences in semen quality from centers around the world (11-15). In European countries, poorer semen quality has been associated with greater

incidences of testicular cancer and hydrospadia (15), further raising the importance of understanding the factors that control semen quality.

Our recent review (5) of the relationship between age and semen quality showed that the vast majority of studies on age involved men in clinical settings, including sperm donors or men attending infertility clinics. The weight of evidence from these clinical studies suggests that age was associated with diminished semen volume, sperm motility, and/or sperm morphology, but that sperm concentration was little affected by age (5). 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 old) were underrepresented in these clinical studies limiting statistical power and preventing 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.

The purpose of the current research was to re-examine the magnitude and the shape of the relationships between age and semen quality in a non-clinical group of healthy active men selected to span across a wide range of age from the 20's to the 80's. These men also provided extensive information on medical and exposure factors that could affect semen quality.

## **SUBJECTS AND METHODS**

The study population of the Age and Genetic Effects on Sperm (AGES) Study consisted of 97 healthy male volunteers employed or retired from the Lawrence Livermore National Laboratory (LLNL) campus of the University of California, located in Livermore, California. The AGES Study was approved by the Institutional Review Boards of the participating

institutions and all subjects gave written consent. LLNL was used as the recruitment site because the workforce is relatively homogeneous and because it was the site of the semen analysis laboratory. Healthy males were recruited from advertisements posted at the Laboratory, e-mail listings, and in newsletters. Preliminary screening over the telephone was used to exclude men who had current fertility or reproductive problems; had smoked cigarettes in the last six months; had a vasectomy or a history of an undescended testicle or prostate cancer; had received chemotherapy or radiation treatments for cancer; or had a previous semen analysis with zero sperm count. If men at screening had a fever over 101°F in the last three months, they were recruited three months from the date of the fever. We recruited men until we had adequate numbers (n~15 to 20) in each age decade.

Eligible men were mailed a questionnaire and a semen collection container with instructions. The questionnaire gathered information on medical history, reproductive history (e.g. fatherhood history), sociodemographic characteristics (age, race, education), and lifestyle habits (e.g. alcohol, cigarette, and caffeine consumption). Completed questionnaires were mailed to the University of California at Berkeley or brought to LLNL with the semen specimen. Both semen collection procedure and questionnaires were reviewed with the participant over the telephone.

Numerically coded semen specimens were delivered within two hours of collection to the laboratory at LLNL for analysis. Laboratory technicians were blinded to the age and identity of the man. Semen analysis was performed immediately upon receipt of the sample, averaging about 45 minutes after production of the sample (range=5 to 120 minutes). Semen volume was

measured to the nearest 0.1 ml and aliquoted into cryogenic vials after being thoroughly stirred. Sperm concentration was determined by triplicate counting on a Neubauer hemocytometer. We counted a minimum of five squares per grid under phase contrast using a Zeiss Photomicroscope III, and recorded the concentration in millions of sperm per milliliter. A visual assessment of the percentage of motile sperm (any type of movement) and the percentage of progressive sperm (rapid and forward-moving) were performed following World Health Organization (WHO) guidelines (1992). Duplicate samples were requested from donors who did not meet specified criteria: volume < 1ml, 0% motility, samples contained red or white blood cells in semen, fever reported, loss of sample reported, or unscheduled delivery of samples. All samples were evaluated under the microscope. If there were no sperm, the sample was recorded as azoospermic. In total, 115 fresh semen specimens were provided from 97 men.

For sperm motility, 50  $\mu$ l of semen was diluted to a standardized sperm concentration of approximately 35 million sperm per ml using Dulbecco's phosphate-buffered saline solution (DBPS) with glucose (1g/L) and bovine serum albumin (BSA) added and 3-4ul pipetted into a 2X-CEL 20-um depth chambered microscope slide (Hamilton Thorne Research) maintained at 37°C by a MiniTherm slide warmer (Hamilton Thorne Research). Motile and immotile sperm were counted in separate microscope fields under 400x phase-contrast magnification using a Zeiss Photomicroscope III equipped with a 5x5 ocular grid until at least 150 sperm were counted. Percent motility was recorded as the number of motile sperm divided by the total number of sperm counted and multiplied by 100. The progression of at least 100 sperm were graded in different fields of the same drop of semen. Percent progressive motility was recorded as the number of forward-moving sperm traveling at least 25  $\mu$ m per second (five times the length of

the sperm head/sec) divided by the total number of sperm and multiplied by 100. Sperm motility and progression were evaluated in at least two separate drops of diluted semen from different aliquots. If more than a 10% difference in motility was found between analyses, then a third or fourth drop of semen was analyzed and the average calculated. Using the same procedure, undiluted semen was also evaluated from each donor sample when possible to ensure no effect of the diluent on sperm motility. All cell counting was performed on a Macintosh computer using a modified version of CytoScore™ developed at LLNL.

### **Statistical Analyses**

All analyses were conducted using STATA 7.0 (16). Total sperm count was calculated by multiplying volume and sperm concentration. The total progressively motile sperm was calculated as the product of total sperm count and percent progressive motility. Volume and motility were normally distributed. Sperm progressive motility, concentration, total count, and total progressively motile sperm were transformed to achieve normality. The square root transformation proved to be the best; however, log transformation was acceptable and is used for comparison with other studies and for ease of understanding. Zero values for semen parameters (e.g. count, concentration, progressive motility and total progressively motile sperm) were re-coded to half of the lowest measured value before log transformation. For analyses on motility measures, men with azoospermia were excluded (n=4).

Bivariate analyses examined the differences in semen parameters and age with the following potential confounders: duration of sexual abstinence before semen collection; average ejaculation frequency over the past three months; time from sample collection to sample

processing; ever exposure to occupational chemicals and radiation; history working with radioisotopes as measured from LLNL dosimetry records; history of tobacco use; alcohol and caffeine intake; prescription and non-prescription medication use; hot tub use during the last three months; history of chronic disease such as high blood pressure, heart problems, or diabetes; history of genitourinary disease including urinary tract or other genitourinary infection, sexually transmitted disease, varicocele, or past history of infertility (anyone with a history had to have fathered a child subsequently); fatherhood history; percent time of an average day currently spent sitting; body mass index; and ethnicity.

The unadjusted relationship of age and each semen parameter is graphically presented using locally weighted scatterplot smoothing (LOWESS) regression with a bandwidth of 0.8. Lowess regression predicts a smoothed estimated value for each point in the data. Separate multiple linear regression models were used to examine the relationship of age with each semen parameter controlling for other covariates. Covariates were included in the multivariate models if they changed the regression coefficient for age by >10% or if they were statistically significant at  $p < 0.1$ . For semen parameters that are log-transformed, we present the percent change per year as converted from the antilog of the logarithmic value of the regression coefficient. For semen parameters that remained un-transformed (volume and motility), we present the absolute change per year of age as well as the relative change in the outcome in men who were age 30 compared to age 50. We present the multiple coefficient of determination ( $R^2$ ) for each model and the partial correlation for age adjusted for the other covariates in the model.

The cumulative incidence of men with abnormal semen parameters at each year of age was calculated by summing the men who had an abnormal semen parameter, and by dividing by the total number of men in the study. Abnormal semen parameters are defined by WHO guidelines as volume  $\leq 2$  mL, concentration  $< 20$  million/mL, count  $\leq 40$  million, motility  $< 50\%$ , progressive motility  $< 25\%$ , and total progressively motile sperm  $< 10$  million (17).

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 square root or log transformations based on a likelihood test, ruling out any clear "threshold" effect for any of the semen parameters. Thus, we present the results of the regression analyses only.

## RESULTS

### Characteristics of Study Population

The 97 men were on average 46.4 years old (SD= 15.8 years, range=22 to 80 years). As shown in Table 1, these men were well-distributed across the six age decades. Median semen volume was 2.7 mL; median sperm concentration was 93 million per mL; and median total sperm count was 259 million sperm. The median motility parameters were 42% motility, 20% progressive motility, and 55 million progressively motile sperm. Seven men who were more than 60 years old were azoospermic (63, 77, 77, and 78 years old) or had no progressively motile sperm (60, 64, and 67 years old).

As shown in Table 2, older age was associated with prior tobacco use (none smoked cigarettes during the six months prior to providing their specimen), increased duration of sexual abstinence, and ever having had high blood pressure. Semen volume was lower in men who ever used tobacco and in those who ever drank alcohol. Sperm concentration was higher in those who had abstained for more than five days. Sperm count was also higher in men who had abstained, 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 were lower in men who had ever had high blood pressure.

### ***Semen volume***

Men in their 20's (aged 22 through 29) had a median semen volume of 3.0, 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. Semen volume decreased with age by 0.03 mL per year (95% C.I. = -0.05, -0.02) after adjusting for length of abstinence, prior use of tobacco and alcohol use (Table 3). A 50-year-old man was calculated to have a 20% relative decrease in semen volume compared to a 30-year-old man. Age explained 14.4% (partial  $r = -0.38$ ,  $p < 0.001$ ) of the total variance in semen volume. As shown in Figure 2, by age 50, ~10% of men had abnormal semen volumes (<2 mL) which increased to ~30% by age 80.

### ***Sperm Concentration and Total Count per specimen***

Men in the 20's age group had median sperm concentrations and total sperm counts of 92 million/mL and 345 million, respectively (Table 1). Of all the parameters, sperm concentration was least affected by age (Table 1, Figure 1B), but total count decreased significantly across age decades (p value for trend = 0.01). Linear regression analyses showed a decrease of 2.5% (95% CI = -4.2, -0.8) per year of age in sperm concentration and 3.6% (95% CI = -5.4, -1.7) per year of age in count after adjustment for covariates (Table 3). Age explained 8.4% (partial  $r = -0.29$ ,  $p = 0.005$ ) of the total variance in log concentration and 13.7% (partial  $r = -0.37$ ,  $p < 0.001$ ) in log count. When the four azoospermic men were excluded from the analyses, the decrease in count with age remained statistically significant (-1.7% change/year, 95% CI = -3.4, -0.06), but the decrease in concentration became nonsignificant (-0.6% change/year, 95% CI = -2.0, 0.9). These findings were not altered when we used a squared root transformation, the best transformation

for these semen parameters. Concentration and count were also least affected by age based on the proportion that reached clinically abnormal levels. About 5% of the men in our cohort had abnormal values for sperm concentrations and total counts by age 50, which more than tripled by age 80 (see Figure 2).

***Percent Motility, Percent Progressive Motility, and Total Number of Progressively Motile Sperm***

Compared to the other semen parameters in our study, measures of motility were most affected by age. The semen specimens provided by men in their 20's (Table 1) had medians of 50.0% motility, 29.0% progressive motility, and 96.6 million progressively motile sperm. There were significant trends towards reduced sperm motility across age decades for all three parameters ( $p$  for trend  $<0.01$ , for all three) (Table 1, Figure 1D, 1E, 1F). 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 3). A 50-year-old man was calculated to have a relative decrease of 28% motile sperm compared to a 30-year-old man. Similarly, there was 3.1% (95% CI=-4.6, -1.6) decrease in the percent of progressively motile sperm per year and a 4.8% adjusted decrease per year (95% CI=-7.5, -2.2) for total progressively motile sperm after adjusting for covariates (Table 3). 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 progressive motility ( $r=-0.36$ ,  $p<0.001$ ). After excluding the three men with no progressively motile sperm, we still observed a statistically significant association between age and each of the three measures of sperm

motility. By age 50 years, ~30% of men surpassed clinical thresholds for abnormal motility or progressive motility, which increased to ~ 60% of men by age 80 (Figure 2).

## DISCUSSION

This investigation found significant age-dependent reductions in semen quality among healthy active workers and retirees. The largest effects of age were on the motility parameters and the smallest effects were on the sperm-number parameters. Semen volume showed intermediate effects. In our sample, among men up to 50 years old, ~30% had clinically abnormal motility, ~10% had abnormally low semen volume, and ~5% had abnormally low sperm numbers. These proportions increased to ~60%, ~30%, and ~15%, respectively for all men by age 80. There was no evidence of an age 'threshold' for any of the semen parameters, but rather a gradual change over time.

Our findings are consistent with previous studies demonstrating decreased pregnancy rate and longer time to pregnancy in older men (18-27). Our findings also support and extend the findings of prior clinical studies of infertile patients and sperm donors (5). However, the percent changes with age in our study are generally at the high end of changes observed in the clinical studies. For example, we observed a 20% decrease 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 (5, 11, 12, 19). Similarly, our 28% relative decrease in motility is near the high end of the 3 to 37% range of decreases reported in clinical studies (11, 19, 28, 29). Sperm concentration was least associated with age among the semen quality parameters we studied, and was not significant when the four azoospermic men were excluded from analysis. This is consistent with studies from the clinical literature, which in general, did not find that sperm concentration decreased with age (5, 13, 18, 21, 30-32). Although our age-related findings for total sperm

count were more robust, this was likely driven by the strong association of age with semen volume.

Our study design has several notable strengths. Recruitment was limited to healthy men so that persons of ill health or with known infertility-related problems were excluded prior to analyses. The cohort was relatively homogeneous, within a similar socio-economic class and with similar access to medical care. In addition, unlike almost all previous studies (11, 13, 18-21, 29-41), we obtained detailed information on a wide range of potential confounding factors (abstinence, smoking habits, etc.) and adjusted the regression analyses using identified covariates. Our ability to detect an association between age and semen parameters was enhanced by our inclusion of relatively large numbers of older men than in previous studies (18, 20, 21, 30, 38, 39). Also, by including sufficient numbers of men over a wide age range, we were able to examine the shape of the relationship between age and semen quality.

At least two broad modes of action may explain the age-dependent changes we observed in semen quality. First, there may be cellular or physiologic changes in the genitourinary tract with aging. 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 (42, 43). Decreased semen volume with age may be caused by seminal vesicle insufficiency, since seminal vesicle fluid contributes most of the ejaculate volume (27, 44). Changes in the prostate that occur with aging, such as smooth muscle atrophy and a decrease in protein and water content, may contribute to decrease semen volume and sperm motility (45). There may also be age-related changes in the epididymis where sperm acquire the capacity for vigorous forward

motility during transit . Also, the epididymis is a hormonally sensitive tissue and it plays an important role in sperm maturation (44). 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. 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. ~~Affected men may have been born predisposed to these changes perhaps due to *in utero* exposures that limited the number of Sertoli or gonocyte cells at birth.~~ More generally, male age may 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 (5, 32). A cross-sectional investigation such as ours is not able to separate the effects of the aging process from “cohort effects”.

The semen parameters evaluated in this report are not expected to be the only sperm endpoints that will show age-related damage. Other parameters which may be affected by age include sperm morphology, which has been shown to be a sensitive indicator of the status of the germinal epithelium (7, 46). 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 (47) and in mice (48-51) and with increases in the incidence of de novo germinal mutations (52-54).

In summary, the observed age-dependent decreases in semen quality suggests that men may become progressively less fertile as they age. However, unlike for women, there appears to be no evidence of an age threshold. Thus, men who choose to delay fatherhood may reduce their chances of success.

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## REFERENCES

1. Templeton A. Infertility-epidemiology, aetiology and effective management. *Health Bull (Edinb)* 1995;53:294-8.
2. Joffe M, Li Z. Male and female factors in fertility. *Am J Epidemiol* 1994;140:921-9.
3. Ventura S, Martin J, Curtin S, Mathews T. Report of Final Natality Statistics, 1995. *Monthly Vital Statistics Report* 1997;45.
4. Tietze C. Reproductive span and rate of conception among Hutterite women. *Fertil Steril* 1957;8:89-97.
5. Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. *Fertil Steril* 2001;75:237-48.
6. Ford WC, North K, Taylor H, Farrow A, Hull MG, Golding J. Increasing paternal age is associated with delayed conception in a large population of fertile couples: evidence for declining fecundity in older men. The ALSPAC Study Team (Avon Longitudinal Study of Pregnancy and Childhood). *Hum Reprod* 2000;15:1703-8.
7. Wyrobek AJ. Methods for evaluating the effects of environmental chemicals on human sperm production. *Environ Health Perspect* 1983;48:53-9.
8. Wyrobek AJ, Alhborn T, Balhorn R, Stanker L, Pinkel D. Fluorescence in situ hybridization to Y chromosomes in decondensed human sperm nuclei. *Mol Reprod Dev* 1990;27:200-8.

9. Wyrobek AJ, Gordon LA, Burkhardt JG, et al. An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic function. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat Res* 1983;115:73-148.
10. Chapin RE, Sloane RA. Reproductive assessment by continuous breeding: evolving study design and summaries of ninety studies. *Environ Health Perspect* 1997;105 Suppl 1:199-205.
11. Fisch H, Goluboff E, Olson J, Feldshuh J, Broder S, Barad D. Semen analyses in 1283 men from the United State over a 25-year period: no decline in quality. *Fertility and Sterility* 1996;65:1009-14.
12. Andolz P, Bielsa MA, Vila J. Evolution of semen quality in North-eastern Spain: a study in 22,759 infertile men over a 36 year period. *Hum Reprod* 1999;14:731-5.
13. Berling S, Wolner-Hanssen P. No evidence of deteriorating semen quality among men in infertile relationships during the last decade: a study of males from Southern Sweden. *Hum Reprod* 1997;12:1002-5.
14. Itoh N, Kayama F, Tatsuki TJ, Tsukamoto T. Have sperm counts deteriorated over the past 20 years in healthy, young Japanese men? Results from the Sapporo area. *J Androl* 2001;22:40-4.
15. Jorgensen N, Andersen AG, Eustache F, et al. Regional differences in semen quality in Europe. *Hum Reprod* 2001;16:1012-9.
16. Stata Statistical Software: Release 7.0. College Station, TX: Stata Press, 1999.
17. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge: World Health Organization, 1992.

18. Spandorfer SD, Avrech OM, Colombero LT, Palermo GD, Rosenwaks Z. Effect of parental age on fertilization and pregnancy characteristics in couples treated by intracytoplasmic sperm injection. *Hum Reprod* 1998;13:334-8.
19. Rolf C, Behre H, Nieschlag E. Reproductive parameters of older compared to younger men of infertile couples. *international journal of andrology* 1996;19:135-42.
20. Gallardo E, Simon C, Levy M, Guanes P, Remohi J, Pellicer A. Effect of age on sperm fertility potential: oocyte donation as a model. *Fertility and Sterility* 1996;66:260-4.
21. Abramsson L. On the Investigation of Men from Infertile Relations: A clinical study with special regard to anamnesis, physical examination, semen-, hormone-, and chromosome analyses, from men with non-"normal" semen. *Scandinavian Journal of Urology and Nephrology* 1988;Suppl 113:7-47.
22. Mathieu C, Ecochard R, Bied V, Lornage J, Czyba J. Cumulative conception rate following intrauterine artificial insemination with husband's spermatozoa: influence of husband's age. *Human Reproduction* 1995;10:1090-7.
23. Stanwell-Smith R, Hendry W. The prognosis of male subfertility: A survey of 1025 men referred to a fertility clinic. *British Journal of Urology* 1984;56:422-8.
24. Ducot B, Spira A, Feneux D, Jouannet P. Male factors and the likelihood of pregnancy in infertile couples. II. Study of clinical characteristics - practical consequences. *International Journal of Andrology* 1988;11:395-404.
25. Brzechffa PR, Daneshmand S, Buyalos RP. Sequential clomiphene citrate and human menopausal gonadotrophin with intrauterine insemination: the effect of patient age on clinical outcome. *Hum Reprod* 1998;13:2110-4.

26. Olsen J. Subfertility according to the age of the mother and the father. *Danish Medical Bulletin* 1990;37:281-2.
27. Goldman N, Montgomery M. Fecundability and husband's age. *Soc Biol* 1989;36:146-66.
28. Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years [see comments]. *N Engl J Med* 1995;332:281-5.
29. Schwartz D, Mayaux M-J, Spira A, et al. Semen characteristics as a function of age in 833 fertile men. *Fertility and Sterility* 1983;39:530-5.
30. Wang C, Chan S, Leung A, et al. Cross-sectional study of semen parameters in a large group of normal Chinese men. *International Journal of Andrology* 1985;8:257-74.
31. Lemcke B, Behre HM, Nieschlag E. Frequently subnormal semen profiles of normal volunteers recruited over 17 years. *Int J Androl* 1997;20:144-52.
32. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years [see comments]. *Bmj* 1992;305:609-13.
33. Homonnai ZT, Fainman N, David MP, Paz GF. Semen quality and sex hormone pattern of 29 middle aged men. *Andrologia* 1982;14:164-70.
34. Nieschlag E, Lammers U, Freischem C, Langer K, Wickings E. Reproductive functions in young fathers and grandfathers. *Journal of Clinical Endocrinology and Metabolism* 1982;55:676-81.
35. Dondero F, Mazzilli F, Giovenco P, Lenzi A, Cerasaro M. Fertility in older men. *J. Endocrinol. Invest* 1985;8(2):87-91.
36. Singer R, Sagiv M, Levinsky H, Allalouf D. Andrological parameters in men with high sperm counts and possible correlation with age. *Archives of Andrology* 1990;24:107-11.
37. Haidl G, Jung A, Schill WB. Ageing and sperm function. *Hum Reprod* 1996;11:558-60.

38. Irvine S, Carwood E, Richardson D, MacDonald E, Aitken J. Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 in Scotland over 11 years. *British Medical Journal* 1996;312:467-71.
39. Check JH, Shanis B, Bollendorf A, Adelson H, Breen E. Semen characteristics and infertility in aging. *Arch Androl* 1989;23:275-7.
40. Bujan L, Mansat A, Pontonnier F, Mieusset R. Time series analysis of sperm concentration in fertile men in Toulouse, France between 1977 and 1992. *British Medical Journal* 1996;312:471-2.
41. Mladenovic I. Sperm morphology and motility in different age populations. *Archives of andrology* 1994;32:197-205.
42. Bishop MW. Ageing and reproduction in the male. *J Reprod Fertil Suppl* 1970;12:65-87.
43. Johnson L. Spermatogenesis and aging in the human. *J Androl* 1986;7:331-54.
44. Hamilton D, Naftolin F. Basic reproductive medicine. Cambridge: MIT press, 1981.
45. Schneider EL. The aging reproductive system. New York: Raven Press, 1978.
46. MacLeod J. Human seminal cytology as a sensitive indicator of the germinal epithelium. *International Journal of Fertility* 1964;9.
47. Lowe X, Eskenazi B, Nelson DO, Kidd S, Alme A, Wyrobek AJ. Frequency of xy sperm increases with age in fathers of boys with klinefelter syndrome. *Am J Hum Genet* 2001;69:1046-54.
48. Robbins W, Baulch J, Moore DI, Weire H-U, Blakey D, Wyrobek A. Three-probe fluorescence in situ hybridization to assess chromosome X, Y, and 8 aneuploidy in sperm of 14 men from two healthy groups: Evidence for a paternal age effect on sperm aneuploidy. *Reprod Fertil Dev* 1995;7:799-809.

Table 1. Median and interquartile range (25%-75%) of semen parameters by age decade.

| Age Group             | N         | Days of abstinence (mean) | Volume (mL) |                  | Concentration (10 <sup>6</sup> /mL) |                 | Total Count (10 <sup>6</sup> ) |                  | Motility (%) |                | Progressive Motility (%) |               | Total Progressively Motile Sperm (10 <sup>6</sup> ) |                 |
|-----------------------|-----------|---------------------------|-------------|------------------|-------------------------------------|-----------------|--------------------------------|------------------|--------------|----------------|--------------------------|---------------|-----------------------------------------------------|-----------------|
|                       |           |                           | Median      | (25-75)          | Median                              | (25-75)         | Median                         | (25-75)          | Median       | (25-75)        | Median                   | (25-75)       | Median                                              | (25-75)         |
| 20-29                 | 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                 | 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+                   | 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)        |
| <b>Total</b>          | <b>97</b> | <b>5.1</b>                | <b>2.7</b>  | <b>(1.5-3.8)</b> | <b>93.0</b>                         | <b>(43-177)</b> | <b>258.5</b>                   | <b>(100-460)</b> | <b>42.0</b>  | <b>(17-53)</b> | <b>20.0</b>              | <b>(8-30)</b> | <b>55.0</b>                                         | <b>(15-115)</b> |
| <b>Test for trend</b> |           |                           |             |                  |                                     |                 |                                |                  |              |                |                          |               |                                                     |                 |
| P value               | <0.01     | <0.01                     |             |                  | 0.34                                |                 | 0.01                           |                  | <0.01        |                | <0.01                    |               | <0.01                                               |                 |

\*Four azoospermic men were aged 63, 77, 77, and 78. 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)

Table 2. Age and semen parameters by characteristics of the participant.

| Variable                             | N* (%)  | Age<br>(years)<br>Mean (SD) | Volume<br>(mL)<br>Mean (SD) |                            | Concentration<br>(10 <sup>6</sup> /mL)<br>Mean (SD) |                          | Total Count<br>(10 <sup>6</sup> )<br>Mean (SD) |                          | Motility<br>(%)<br>Mean (SD) |  | Progressive<br>Motility (%)<br>Mean (SD) |  | Total Progressively<br>Motile Sperm (10 <sup>6</sup> )<br>Mean (SD) |  |
|--------------------------------------|---------|-----------------------------|-----------------------------|----------------------------|-----------------------------------------------------|--------------------------|------------------------------------------------|--------------------------|------------------------------|--|------------------------------------------|--|---------------------------------------------------------------------|--|
|                                      |         |                             |                             |                            |                                                     |                          |                                                |                          |                              |  |                                          |  |                                                                     |  |
| 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 <sup>†</sup> (15.8)    | 3.2 (1.6)                   | 187.3 <sup>‡</sup> (156.8) | 603.6 <sup>§</sup> (517.4)                          | 28.9 <sup>‡</sup> (20.7) | 17.6 (16.3)                                    | 126.7 (179.4)            |                              |  |                                          |  |                                                                     |  |
| 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 <sup>§</sup> (15.4)    | 1.9 <sup>§</sup> (1.1)      | 169.4 (191.3)              | 278.5 <sup>‡</sup> (363.2)                          | 32.8 (19.9)              | 16.2 (13.4)                                    | 52.7 <sup>‡</sup> (74.3) |                              |  |                                          |  |                                                                     |  |
| 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 <sup>‡</sup> (67.6) |                              |  |                                          |  |                                                                     |  |
| Ever                                 | 63 (65) | 45.0 (16.2)                 | 2.5 <sup>§</sup> (1.4)      | 148.8 (144.9)              | 371.7 (437.3)                                       | 38.8 (20.3)              | 22.5 (15.7)                                    | 102.6 (147.4)            |                              |  |                                          |  |                                                                     |  |
| Body Mass Index (kg/m <sup>2</sup> ) |         |                             |                             |                            |                                                     |                          |                                                |                          |                              |  |                                          |  |                                                                     |  |
| 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)            |                              |  |                                          |  |                                                                     |  |
| High Blood Pressure                  |         |                             |                             |                            |                                                     |                          |                                                |                          |                              |  |                                          |  |                                                                     |  |
| 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 <sup>§</sup> (10.9)    | 2.4 (1.4)                   | 100.9 (133.7)              | 202.6 <sup>§</sup> (237.5)                          | 29.1 (20.8)              | 16.2 (15.3)                                    | 39.2 <sup>‡</sup> (45.4) |                              |  |                                          |  |                                                                     |  |
| Urinary Tract Infection <sup>†</sup> |         |                             |                             |                            |                                                     |                          |                                                |                          |                              |  |                                          |  |                                                                     |  |
| 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 <sup>§</sup> (19.5) | 12.5 <sup>‡</sup> (10.4)                       | 35.9 <sup>‡</sup> (48.9) |                              |  |                                          |  |                                                                     |  |

\* N = 97 for age, volume, concentration, total count; n=93 for motility, progressive motility, and total progressively motile sperm.

† Includes infections of the bladder and kidney

‡ p value < 0.05 for t-tests (volume, motility) or Mann-Whitney tests (concentration, count, progressive motility, total progressively motile sperm)

§ p value < 0.01

\* p value < 0.001

Figure 1

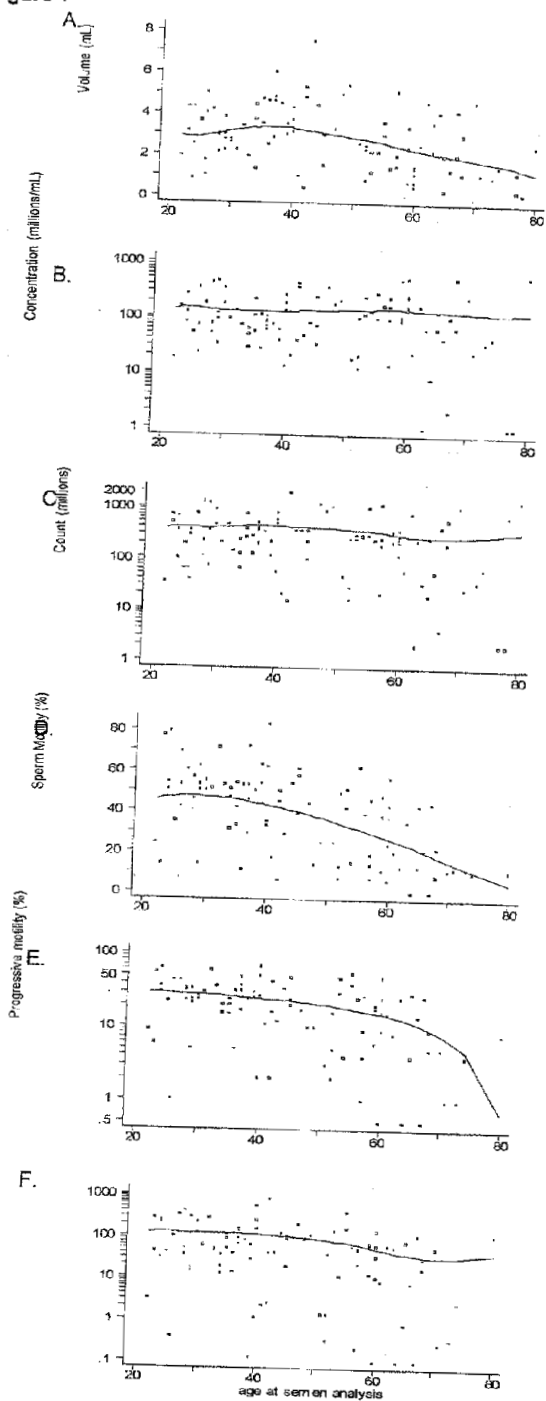


Figure 2

Cumulative Frequency of Healthy Men with Clinically Abnormal Semen Parameters by Age

