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Semen and Sperm Reference Ranges for Men 45 Years of Age and Older

WAYNE J. G. HELLSTROM,* JAMES W. OVERSTREET,† SURESH C. SIKKA,† JONATHAN DENNE,‡ SANJEEV AHUJA,‡ AMY M. HOOVER,‡ GREGORY D. SIDES,‡ WILLIAM H. CORDELL,‡ L. MARK HARRISON,‡ AND J. STEVEN WHITAKER§

*From the *Tulane University School of Medicine, New Orleans, Louisiana; the †University of California-Davis, Davis, California; the ‡Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana; and the §ICOS Corporation, Bothell, Washington.*

ABSTRACT: The most widely used reference values for human semen and sperm variables were developed by the World Health Organization (WHO) to help assess the fertility status of men interested in reproduction (typically a younger population). In this retrospective analysis, data from a large population of men aged 45 years or older were analyzed to derive semen and sperm reference ranges for an older population. Baseline semen samples were obtained from 1174 men with no or mild erectile dysfunction (ED) during the screening phase of two clinical trials evaluating the effects of a drug on human spermatogenesis. The median values and 95% reference ranges for 4 measured semen and sperm parameters (semen volume, sperm concentration, sperm motility, and sperm morphology) and 1 derived parameter (total sperm count) were calculated for the population and by age quartile. These references ranges were compared to established WHO reference values. Associations between the semen and sperm parameters and smoking status, alcohol use, and serum hormone concentrations were also analyzed. The mean age was 52.9 years (range: 45–80). Median semen volume, sperm motility, and sperm morphology parameters declined significantly with age. Only 46% of study subjects had baseline values for semen and sperm parameters that met or surpassed all the WHO reference values. This is the first study to statistically derive semen reference ranges from a large population of men aged 45 years or older. The observation that less than half the men in this study met all 4 WHO reference values for measured semen and sperm parameters underscores the need for age-specific reference ranges.

Key words: WHO reference values, semen and sperm parameters, reproductive hormones.

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Semen and sperm analyses are frequently used to evaluate fertility and to investigate the effects of environmental agents or drugs. Pioneering research in the 1950s on male fertility provided initial standards for normal semen and sperm parameters (MacLeod and Gold, 1951). As research and clinical needs for semen and sperm analyses have grown, so has the need for standardized procedures for these analyses. In response to this need, in 1980 the World Health Organization (WHO) published the first of several laboratory manuals on the analysis of semen and sperm (World Health Organization, 1980). Current WHO guidelines for the analysis of semen samples are intended to provide standardized laboratory techniques for examination of human semen and sperm parameters and provide reference values for fertility clinics, reproductive toxicology studies, and laboratories investigating male contraception (World Health Organization, 1999).

The WHO reference values for semen and sperm parameters were included in the protocol entry inclusion/exclusion criteria for 2 clinical studies conducted in the United States. These multicenter studies were designed to evaluate the effects of tadalafil, which was being investigated for treatment of erectile dysfunction, on human spermatogenesis (Hellstrom et al, 2003). During the screening phase many semen samples did not meet the WHO reference standards, leading to a high exclusion rate for these studies. The observed high screen failure rate prompted this retrospective analysis, which re-examined the baseline screening data from these studies and had 3 objectives. The first objective was to calculate semen reference ranges for a population of men aged 45 years or older without suspected fertility impairment. The second objective was to determine whether these reference ranges should be stratified based on subject age, smoking history, alcohol consumption, or serum hormone concentrations. The third objective was to compare the calculated reference ranges with the WHO reference values.

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Correspondence to: Dr Wayne J. G. Hellstrom, Tulane University School of Medicine, Department of Urology, SL-42, 1430 Tulane Ave, New Orleans, Louisiana, 70112 (e-mail: whellst@tulane.edu).

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Materials and Methods

Subjects

Men who were at least 45 years of age, with no or mild ED, were eligible for study inclusion. Subjects were excluded from entry into the clinical trials if, on the screening visit, the medical history, physical examination, or laboratory tests revealed health concerns such as significant liver or kidney disease or unstable cardiovascular conditions. Subjects were also excluded based on current use of chemotherapy, nitrates, or antiandrogens. Additional exclusion criteria included conditions known to affect spermatogenesis, such as recent genitourinary infections, current occupational exposures to environmental toxins (lead, dibromochloropropane, ethylene glycol, cadmium, and sulfur mustard), hemochromatosis, or pituitary endocrinopathies. Detailed inclusion/exclusion criteria for subjects in these clinical trials have been previously published (Hellstrom et al, 2003).

Study Protocols

This retrospective analysis used baseline data from subjects screened for 2 multicenter studies (labeled Study A and Study B). These studies were conducted in 2000–2001 to evaluate the effect of tadalafil on semen and sperm characteristics. During the screening phase and before random assignment to clinical treatments, subjects in Study A (n = 559; 9 centers) provided 3 baseline semen samples. Subjects in Study B (n = 615; 18 centers) provided 2 baseline semen samples. Subjects were instructed to abstain from ejaculation for approximately 48 hours (Study A) or at least 48 hours (Study B) and not more than 5 days (both studies) before each semen collection. All semen samples for each subject were collected within approximately 2 weeks (Study A) or 1 week (Study B). Five semen and sperm parameters were studied, 4 measured and 1 derived. The measured parameters were semen volume, sperm concentration, percentage of normal sperm motility, and percentage of normal sperm morphology. Total sperm count was derived by multiplying semen volume times sperm concentration for each sample.

Semen evaluations were performed using standard methods (Overstreet and Brazil, 1997). To ensure standardization in the interpretations of semen and sperm parameters and to minimize interobserver and intraobserver variability in semen and sperm analyses, the same protocols and supplies were used by all centers. All technicians from the different study centers were trained, certified and recertified at the Tulane Andrology training facilities, Tulane University Health Sciences Center, New Orleans. Quality control criteria were set at less than 15% coefficient of variation for each technician throughout the training and study duration. Quality assurance was maintained by proficiency challenge evaluation at 6-month intervals. A single technician at the University of California-Davis read all sperm morphology slides using criteria established by the second WHO Laboratory Manual for the Examination of Human Semen (World Health Organization, 1987). A single blood sample was obtained during the screening process and used to measure serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), and total testosterone.

Statistical Analysis

Single values for each of the 5 semen or sperm parameters were calculated for each subject by averaging the available sample measurements. Analysis of each semen and sperm parameter included all subjects with any data on that parameter.

Semen and sperm parameters were analyzed as continuous variables. Ninety-five percent reference ranges were derived nonparametrically and were defined as the interval spanned by the 2.5 and 97.5 percentiles. The associations both among semen and sperm parameters and between semen and sperm parameters and continuous covariates were assessed with Spearman's rank correlation. The continuous covariates analyzed by age were body mass index (BMI), FSH, LH, and testosterone. For purposes of estimation and display of the relationship between the semen and sperm parameters and these covariates, subjects were ranked and divided into 4 equal size groups by quartile for each of these covariates. The median value and 95% reference ranges for each parameter were calculated within each quartile.

The associations between the semen and sperm parameters and each of the categorical covariates were assessed with the Wilcoxon test (for 2 groups) or the Kruskal-Wallis test (>2 groups). The categorical covariates analyzed were ethnicity, smoking, and alcohol use. Given the large sample size and the multiple comparisons being made, to avoid overinterpretation, only P values less than .001 were considered to be significant.

Results

Study Population

A total of 1499 subjects were screened for entry into Study A or Study B (Figure 1). Of these, 325 were excluded from the reference range calculations for the following reasons: health problems or issues unrelated to semen or sperm (n = 189), withdrawal from the study for personal reasons before providing a semen sample (n = 41), missing semen or sperm data analyses (n = 58), and other (n = 37). The remaining 1174 subjects were included in this analysis. Of the 1174 subjects, 1075 (Study A, n = 461; Study B, n = 614) had at least 1 sample for all 5 semen and sperm parameters available for analysis, and 885 of these provided the number of semen samples specified by the respective study protocol (Study A, 3 samples, n = 349; Study B, 2 samples; n = 536). Ninety-nine of the 1174 subjects had 1 or more semen or sperm parameters not available for analysis.

Demographic data for subjects enrolled in Study A and Study B were comparable (Table 1). Based on the similarity of demographics, subjects with any semen or sperm data were considered to be representative of all entered subjects. The mean age of the study population was 52.9 (range: 45 to 80).

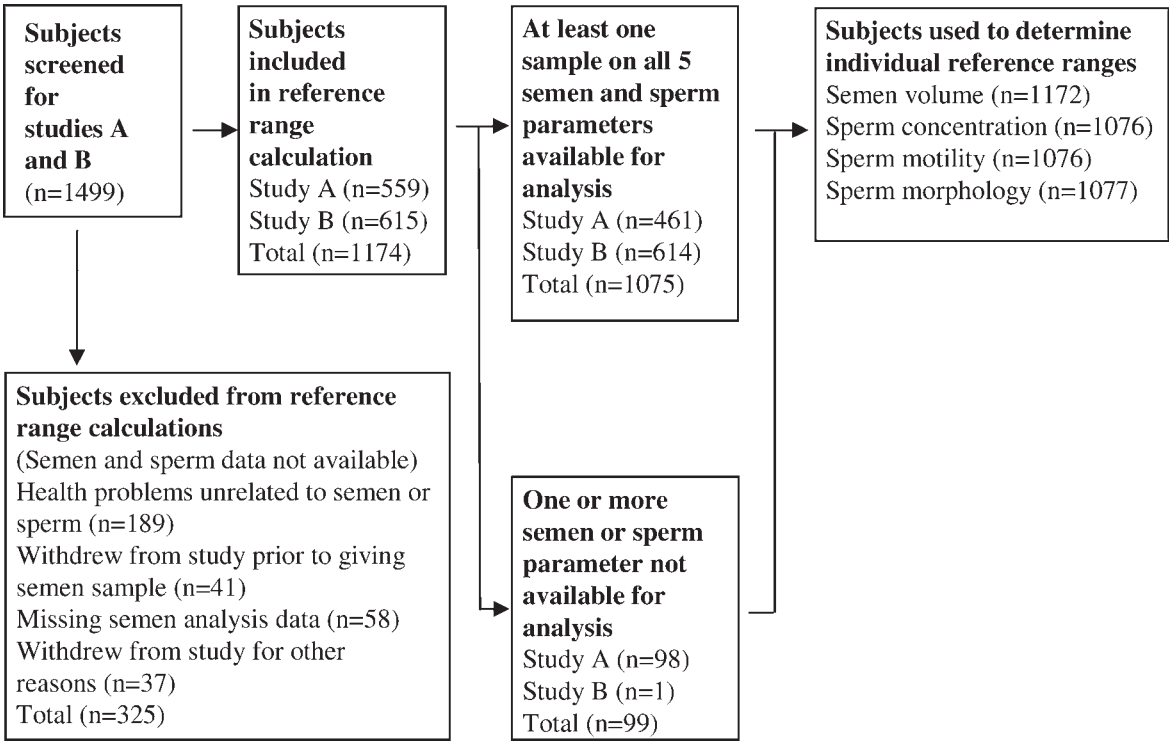

Figure 1. Derivation of study population.

Semen and Sperm Characteristics

Median semen and sperm parameters within each of the quartiles were determined (Table 2). Semen volume, sperm motility, and sperm morphology parameters declined with increasing age ($P < .001$). Semen volume decreased with age, from a median volume of 2.80 mL in the youngest age group to 1.95 mL in the oldest age group. The median value for normal sperm motility decreased from 55% in the youngest age group to 50% in the oldest age group. The median value for normal sperm morphology decreased from 59% in the youngest group to 55% in the oldest group. Total sperm count appeared to decrease with age ($P = .007$). This decrease was similar in magnitude to the decrease in semen volume, so that sperm concentration (count/volume) did not significantly change across the age range.

The pairwise correlation coefficients (Table 3) for the semen and sperm parameters showed that neither sperm motility nor sperm morphology was related to semen volume. However, sperm motility and sperm morphology were associated with each other and with higher sperm concentrations. Total sperm count was positively correlated with semen volume, sperm concentration, sperm motility, and sperm morphology.

Median and 95% reference ranges for each of the semen and sperm parameters were compared to the WHO reference criteria for semen volume, sperm concentration, sperm motility, and sperm morphology (Table 4) (WHO criteria for minimum sperm count not available). The semen volume or one of the three sperm parameters fell below the WHO reference values for 14% to 38% of study subjects. Overall, only 46% of subjects met or surpassed WHO reference values for all

Table 1. *Subject demographics*

Characteristic	Subjects Screened			Subjects Included in Reference Range Calculation		
	Study A (n = 701)	Study B (n = 798)	Combined (n = 1499)	Study A (n = 559)	Study B (n = 615)	Combined (n = 1174)
Age,* y	53.5 (6.8)	53.1 (6.9)	53.3 (6.9)	53.2 (6.6)	52.7 (6.5)	52.9 (6.5)
Weight,* kg	90.8 (16.4)	89.5 (17.0)	90.1 (16.7)	90.6 (16.3)	89.7 (15.9)	90.1 (16.1)
Height,* cm	178.5 (7.3)	178.4 (19.5)	178.4 (15.0)	178.4 (7.2)	178.5 (18.5)	178.4 (14.2)
Smokers (%)	21.8	19.8	20.8	21.8	19.2	20.4
Alcohol use (%)	61.8	61.5	61.6	63.2	61.8	62.4

* Mean (standard deviation).

Table 2. *Median semen and sperm parameters by age quartile*

Parameter	Age Groups					
	>45 to ≤47.8	>47.8 to ≤51.5	>51.5 to ≤56.6	>56.6 to ≤80.1	Correlation Coefficient	*P*
Volume (mL)	2.80	2.37	2.45	1.95	−0.26	<.001
Sperm concentration (M/mL)	52.9	53.5	51.1	60.5	0.03	.25
Total sperm count (M)*	145	137	133	114	−0.08	.007
Sperm motility (%)†	55	55	53	50	−0.21	<.001
Sperm morphology (%)‡	59	59	57	55	−0.17	<.001

* Total sperm count derived by multiplying volume times sperm concentration.
† Percentage of normal sperm motility.
‡ Percentage of sperm with normal morphologic features.

4 measured semen or sperm parameters. The 95% reference ranges by age quartile also reflect the wide variability in the semen and sperm parameters (Figure 2).

The effects of geographic area, smoking, alcohol use, ethnicity, and BMI on semen volume or sperm parameters were also investigated. Geographic region affected only sperm motility ($P < .001$), as the West Coast, Southeast, Central, and Northeast portions of the United States had median sperm motility values of 49%, 50%, 54%, and 57%, respectively (data not shown). There were no differences in semen or sperm parameters in smokers compared with nonsmokers (Table 5). Similarly, men who consumed moderate amounts of alcohol did not have semen and sperm parameters that differed from men who did not consume alcohol (Table 5). Neither ethnicity nor BMI had a significant effect on semen volume or other measured sperm parameters (data not shown).

Reproductive Hormone Characteristics

Increases in LH were correlated ($P < .001$) with decreases in sperm concentration but not with semen volume, sperm motility, or sperm morphology (Table 6a). Increased FSH levels were correlated ($P < .001$) with decreases in sperm concentration, sperm motility, and sperm morphology but not with semen volume (Table 6b). Total testosterone levels were not significantly correlated with any semen or sperm parameters (Table 6c).

Discussion

Previous studies have documented that semen and sperm parameters assessed in this study are lower in older men compared with younger men (Schwartz et al, 1983; Kidd et al, 2001). A recent meta-analysis of male fertility research published between 1980 and 1999 concluded that increasing age is associated with decreased semen volume, decreased sperm motility, and decreased number of morphologically normal sperm (Kidd et al, 2001). Most of these studies used data from men visiting infertility or assisted conception clinics. A limitation of such clinic-based studies is that they use patients who may be subfertile compared to the general population.

Although population-based studies frequently have a large sample size, they generally do not screen the subjects for health problems that might affect semen quality. For example, reproductive disorders such as hypogonadism or prostatic hyperplasia could affect semen and sperm parameters (Wu, 1985; Ablin et al, 1988). Health problems that are not reproductive-specific, such as diabetes or cancer, have systemic effects that could also impact testicular function. One study (Schwartz et al, 1983) evaluated semen characteristics in 833 fertile men but did not screen the subjects for any health-related issues. Likewise, another study examined semen characteristics in over 22 000 men in Spain but only excluded subjects who were aspermic (Andolz et al, 1999).

Table 3. *Pairwise correlation coefficients for semen and sperm parameters*

Parameter	Sperm Concentration (M/mL)	Sperm Motility (%)†	Sperm Morphology (%)‡	Total Sperm Count (M)
Volume (mL)	−0.21*	0.05	−0.07	0.32*
Sperm concentration (M/mL)	...	0.32*	0.29*	0.81*
Sperm motility (%)†	...	...	0.38*	0.35*
Sperm morphology (%)‡	...	...	...	0.25*

* $P < .001$.
† Percentage of normal sperm motility.
‡ Percentage of sperm with normal morphologic features.

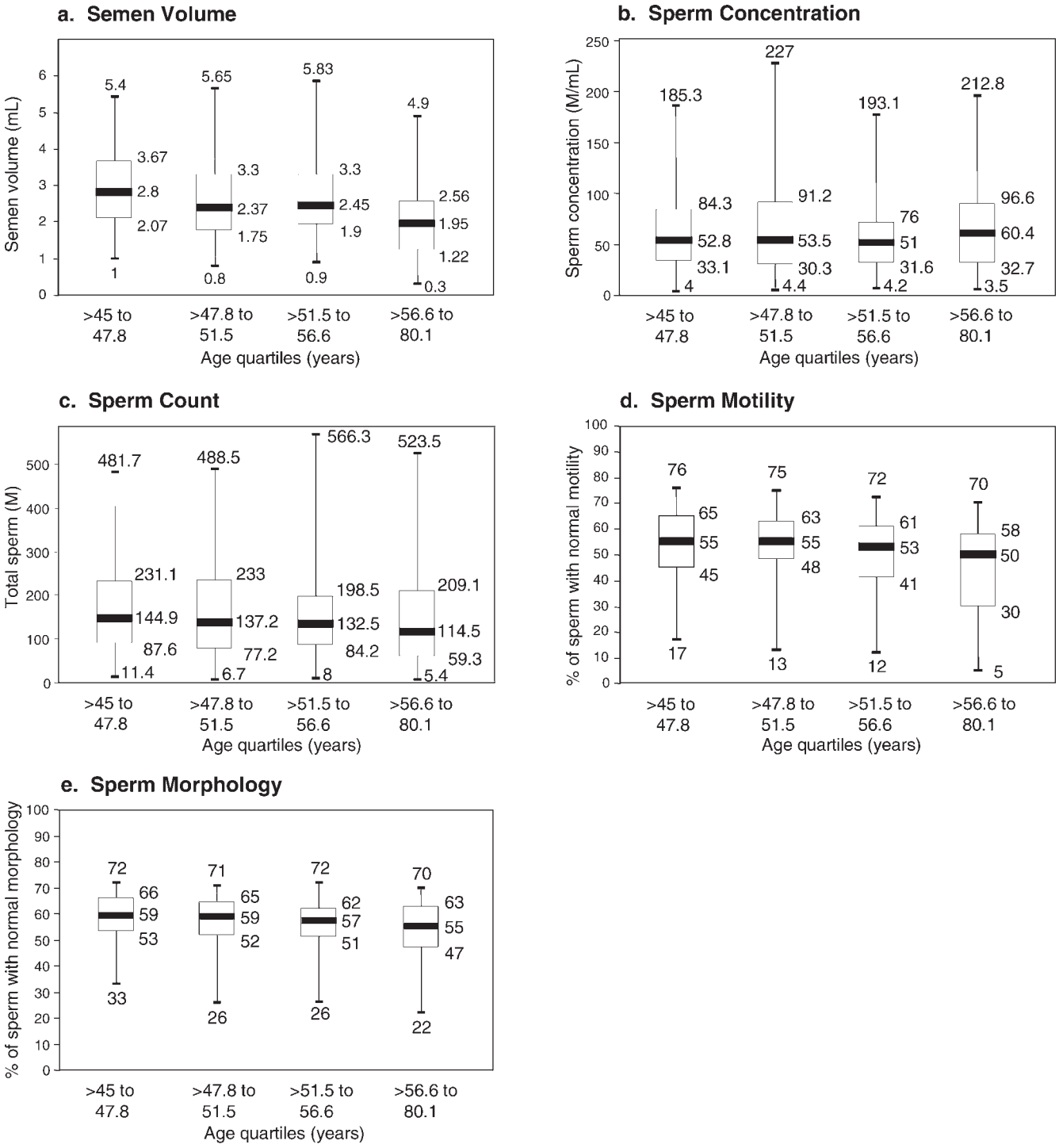

Figure 2. Reference ranges by age quartile for **(a)** semen volume (mL), **(b)** sperm concentration (M/mL), **(c)** total sperm count (M), **(d)** sperm motility (percentage of normal sperm motility), and **(e)** sperm morphology (percentage of sperm with normal morphologic features). Each box represents the distribution of data between the 25th and 75th percentile. The median is indicated by a band across each box. Whiskers on each box represent the 95% reference range for the data set. There were 247 to 294 subjects per age quartile.

Table 4. *Median and 95% reference ranges for semen and sperm parameters in men aged 45 years or older*

	Semen	Sperm Parameters			
	Volume (mL)	Concentration (M/mL)	Total Sperm (M)	Motility (% Normal)	Morphology (% Normal)
Median	2.4	53.7	132	54%	58%
95% reference range	0.5 to 5.5	3.7 to 209	8 to 513	12 to 73	28 to 72
WHO criteria*	≥2	≥20	NA†	≥50	≥50
% below WHO criteria	32%	14%	NA†	38%	22%

* WHO criteria for semen and sperm parameters (WHO Laboratory Manual,1987).
† WHO criteria for minimum total sperm count not available (NA).

Table 5. *Median semen and sperm parameters by smoking or alcohol use*

	Smoking*		Alcohol†	
Parameter	Yes (n = 239)	No (n = 935)	Yes (n = 733)	No (n = 441)
Volume (mL)	2.3	2.4	2.4	2.4
Concentration (M/mL)	52.0	55.4	55.0	52.8
Total sperm count (M)	123	141	139	132
Sperm motility (%)†	55	53	54	54
Sperm morphology (%)§	57	58	58	58

* No statistical differences in semen/sperm parameter distribution between subjects who did/did not smoke.
† No statistical differences in semen/sperm parameter distribution between subjects using/not using alcohol.
‡ Percentage of normal sperm motility.
§ Percentage of sperm with normal morphologic features.

Table 6a. *Median semen and sperm parameters by luteinizing hormone quartiles**

	Luteinizing Hormone Groups (U/L)					
Parameter	<3.0	3.0 to ≤4.3	>4.3 to ≤5.7	>5.7	Correlation Coefficient	*P*
Volume (mL)	2.4	2.4	2.4	2.4	0	.877
Concentration (M/mL)	62.4	57.7	51.1	44.3	−0.19	<.001
Total sperm count (M)	173	139	128	102	−0.18	<.001
Sperm motility (%)†	55	53	52	53	−0.09	.003
Sperm morphology (%)†	59	58	56	57	−0.09	.003

* n = 255 to 278 per group.
† Percentage of normal sperm motility.
‡ Percentage of sperm with normal morphologic features.

Table 6b. *Median semen and sperm parameters by follicle stimulation hormone quartiles*

	Follicle Stimulating Hormone Groups* (U/L)					
Parameter	<3.6	3.6 to ≤5.0	>5.0 to ≤7.1	>7.1	Correlation Coefficient	*P*
Volume (mL)	2.4	2.5	2.3	2.4	−0.05	.074
Concentration (M/mL)	71.9	54.8	53.8	40.4	−0.26	<.001
Total sperm count (M)	163	145	121	114	−0.27	<.001
Sperm motility (%)†	55	54	53	52	−0.13	<.001
Sperm morphology (%)†	59	58	58	56	−0.13	<.001

* n = 255 to 278 per group.
† Percentage of normal sperm motility.
‡ Percentage of sperm with normal morphologic features.

Table 6c. *Median semen and sperm parameters by total testosterone quartiles*

Parameter	Total Testosterone Groups* (nmol/L)					
	<11.4	11.4 to ≤14.4	>14.4 to ≤18.0	>18.0	Correlation Coefficient	*P*
Volume (mL)	2.4	2.3	2.4	2.5	−0.04	.145
Concentration (M/mL)	52.7	60.5	49.4	51.1	−0.06	.056
Total sperm count (M)	129	148	128	130	−0.01	.75
Sperm motility (%)†	55	53	54	53	−0.05	.073
Sperm morphology (%)‡	59	58	57	57	−0.05	.075

* n = 255 to 278 per group. Ranges in ng/dL are <328.5; 328.5 to ≤415; >415 to ≤518.7; >518.7.
† Percentage of normal sperm motility.
‡ Percentage of sperm with normal morphologic features.

The current analysis suggests that semen quality in healthy males will continue to decline as men progress in age from their late 40s to mid-50s. Although the difference in subject median age between the youngest and oldest quartiles was less than 9 years, there were significant decreases in median values of semen volume, sperm motility, and proportion of sperm with normal morphology. The wide 95% reference ranges for each of these criteria, as well as the large number of sample values that fell below the WHO reference values, suggest that there is considerable variability in each age quartile with regard to these measures of semen quality.

These data indicate that spermatogenesis decreased in the oldest men compared with the youngest men in this study. However, decreased spermatogenesis does not necessarily imply a decrease in fertility potential. Several recent studies have found that there is little influence of age on male fertility (Spandorfer et al, 1998; Paulson et al, 2001). Determination of fertility in older men may also be confounded by the decrease in sexual activity with increasing age (Handelsman, 2002).

This study emphasizes the need for reference ranges for semen volume, sperm concentration, total sperm count, sperm motility, and sperm morphology for men aged 45 years or older. Although WHO reference values for normal semen and sperm parameters are derived using samples from normal donors with a wide range of ages, they lack a subset of semen and sperm reference values that would apply only to older men.

The use of age-specific reference values for semen and sperm parameters could be beneficial in several types of studies. Investigations of environmental agents and their possible toxic effects on humans often focus on detecting changes in reproductive function. Semen and sperm reference values that are age-specific could help determine more accurately whether certain compounds of environmental concern attenuate spermatogenesis. Likewise, clinical studies designed to assess the effects of therapeutic drug treatment may use changes in semen and sperm parameters to monitor drug side effects. The reference ranges for semen and sperm parameters in the current study may be useful when the patient population in such clinical trials is composed of older men or compares the drug response of younger men with older men.

Because capturing endocrine data was not a primary objective of this study, only single serum FSH, LH, and total testosterone measurements were obtained during the screening process. Despite this limitation, several trends are present. Higher levels of FSH correlated with decreased sperm concentration, decreased sperm motility, and a decrease in sperm with normal morphology, while higher levels of LH correlated with decreased sperm concentration. An increase in gonadotrophin secretion relative to declines in spermatogenesis in older men has been previously described (Hermann et al, 2000).

A decrease in the function and number of Leydig cells (Neaves et al, 1984), reduced testicular perfusion (Suoranta, 1971), decreased Sertoli cell function (Tenover et al, 1988), and increased testicular connective tissue deposition (Plas et al, 2000) have been suggested as age-related changes that might impair spermatogenesis and diminish feedback from the testes to the pituitary, resulting in elevated LH and FSH. However, the lack of correlation between semen and sperm parameters and serum total testosterone levels suggests that any changes in gonadotrophin secretion or spermatogenesis in this study are not due to a loss of testicular steroidogenic potential.

This study identified significant decreases in semen volume, sperm motility, and sperm morphology parameters in older men. Sperm count tended to decrease and sperm concentration remained unchanged. Less than half the men in this study met all 4 WHO reference values for semen and sperm parameters. In summary, this is the first study to provide semen and sperm reference ranges that were statistically derived from a large population of men aged 45 years or older. These reference ranges may be useful in future reproductive toxicity studies or drug development research that include men in this older age group.

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