Sperm concentration and normal sperm morphology decrease and follicle-stimulating hormone level increases with age

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OBJECTIVE

To assess hormone levels, testicular volume, and semen characteristics of fertile men of various age groups.

PATIENTS AND METHODS

The records of 889 men who sought a vasectomy between September 1999 and March 2003 were reviewed. Patients were divided into five groups by age; we evaluated semen volume, sperm concentration, motility, morphology and complex sperm motion variables. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone levels and both testicular volumes were compared.

RESULTS

There were no differences among the groups in the levels of LH, testosterone, or right and left testicular volumes. There were differences among the five groups in FSH levels, semen volume, sperm concentration and motility. Normal morphology according to the World Health Organisation criteria was significantly lower in patients aged >45 years. From a linear regression analysis, semen volume, sperm concentration and motility decreased by 0.01 mL, 2.1%, and 0.27%, respectively, per year, and the FSH level increased by 0.27%.

CONCLUSIONS

Sperm concentration and motility decrease and FSH levels increase with age. Normal sperm morphology decreases from 45 years old. Thus, the ageing effect should be considered when proposing standard values for semen characteristics in routine semen analysis.

KEYWORDS

age, sperm, fertility, andrology, semen, FSH

INTRODUCTION

Ageing in women significantly reduces the potential to produce oocytes and achieve conception [1]. Female fecundity declines slowly after 30 years old and more rapidly after 40 years old, and is considered the main limiting factor in the treatment of infertility [2]. However, very few data show similar trends in men, certainly because spermatogenesis can continue throughout life, although slight adverse changes in semen quality with age have been reported [3–5].

Endocrine function and morphological changes occur in the testes over time, and the risks of miscarriage and genetic abnormalities in offspring increase with the age of the male [6–9]. Recently, Ng *et al.* [10] detected that older men had lower semen volume and total sperm output than a population of younger men. In addition, older men had more abnormal sperm morphology, with fewer normal forms and reduced vitality, as well as more cytoplasmic droplets. However, Ng *et al.* [10] did not evaluate hormone levels and testicular volume in their study population.

The goal of the present study was to assess hormone levels, testicular volume and semen characteristics of fertile men of various age groups, to establish the existence of a possible ageing effect on those characteristics.

PATIENTS AND METHODS

The study was approved by the Internal Review Board of the Hospital das Clínicas and all the patients provided written informed consent. In all, 1445 men sought vasectomy for voluntary sterilization between September 1999 and March 2003. Patients whose youngest children were aged >2 years and/or men who smoked were excluded from this evaluation. In addition, patients with a history of an undescended testicle, prostate cancer, and had had either chemotherapy or radiation treatment and who had current reproductive problems, were also excluded from the study. Thus, 889 patients were included in the series; all were evaluated with a complete medical history, physical examination and semen analysis. The mean (SD, range) age of these men was 33.23 (6.42, 24–67) years; they were divided into five groups according to age: group I (24–30 years; 216 men), group II (31–35 years; 274), group III (36–40 years; 220), group IV (41–45 years; 110) and group V (>45 years; 69). FSH, LH, testosterone levels, both testicular volumes, semen volume, sperm concentration, motility, and morphology were evaluated according to the WHO criteria [11]. Also, sperm morphology was assessed using the Tygerberg strict criteria [12], and complex motion variables were evaluated by computer-assisted semen analysis (CASA).

Semen samples were obtained by masturbation after at least 48 h of abstinence, collected into sterile containers and allowed to liquefy at 37 °C for 30 min; they were then analysed for sperm concentration, motility and morphology according to WHO criteria [11]. CASA was used for all specimens, with a VP 50 semen

TABLE 1 The serum FSH, LH and testosterone levels, testicular volume, semen volume, sperm concentration, progressive sperm motility and morphology (WHO/Tygerberg criteria), and the CASA variables, according to age

Mean (SD) variable*	Age, years 24–30	31–35	36–40	41–45	>45	P†
FSH, mIU/mL	3.5 (2.76)	3.8 (6.5)	3.7 (2.5)	4.4 (2.4)	4.9 (2.4)	<0.001
LH, mIU/mL	3.28 (1.7)	3.3 (2.4)	3.1 (1.6)	3.6 (1.9)	3.4 (1.2)	0.241
Testosterone, ng/dL	584.3 (191.6)	570.1 (175.1)	563.7 (179.4)	545.9 (175.5)	528.4 (170.3)	0.236
Testicular volume, mL						
right	23.2 (5.3)	24.8 (6.3)	24.8 (6.2)	24.2 (6.6)	23.9 (5.8)	0.324
left	22.08 (4.8)	23.9 (6.3)	24.11 (6.6)	21.9 (5.4)	21.4 (4.9)	0.182
Sperm:						
volume, mL	2.74 (1.32)	2.92 (1.51)	2.68 (1.51)	2.43 (1.31)	2.34 (1.52)	0.015
concentration, 10^6/mL	116.5 (75.72)	117.9 (78.26)	119.8 (87.84)	116.6 (81.01)	112.3 (95.37)	0.047
progressive motility, %	61.8 (15.6)	62.32 (14.63)	61.57 (15.62)	58.15 (15.07)	51.82 (17.09)	0.006
Morphology:						
WHO, %	28.8 (9.6)	28.56 (9.5)	27.84 (9.56)	29.29 (10.01)	26.89 (10.94)	0.074
Tygerberg, %	8.09 (3.99)	8.96 (4.33)	8.86 (4.34)	9.17 (4.47)	7.28 (3.61)	0.235
CASA variables:						
CLV	71.19 (17.4)	73.21 (15.9)	73.05 (16.1)	70.7 (17.5)	70.51 (18.2)	0.673
LHD	3.07 (0.93)	3.13 (0.92)	3.06 (0.93)	3.10 (0.98)	2.98 (1.24)	0.824
SLV	39.4 (11.2)	40.6 (9.8)	41.3 (10.4)	39.7 (9.8)	40.1 (10.2)	0.611
BCF	24.3 (6.0)	24.2 (6.4)	24.5 (5.9)	23.7 (6.2)	22.1 (7.1)	0.307
Linearity	54.58 (7.60)	54.91 (8.25)	55.81 (8.38)	55.75 (8.15)	56.15 (8.39)	0.307

**not log transformed; †P using ANOVA, (log transformed). CLV, curvilinear velocity; LHD, lateral head displacement; SLV, straight line velocity; BCF, beat cross frequency.*

analyser (Motion Analysis Corp., Santa Rosa, CA, USA). For each measurement, a 5-μL aliquot was loaded into a counting chamber (Makler, Sefi, Israel); four to eight representative fields containing ≥200 spermatozoa were examined. Samples were analysed for concentration, motility, and complex motion characteristics. The samples were also assessed manually to ensure the accuracy of the CASA results. Testicular size was measured in all patients, using callipers.

The log of the variables was used in calculations to normalize to the same distribution. The variables assessed were compared among the five groups using ANOVA and a nonparametric test (Kruskal–Wallis). As there were no differences between the results produced by the two different methods of statistical evaluation, the differences across the groups could not have been caused by the log transformation of the variables. Whenever there was a significant difference across the groups, the effect of age on the semen characteristics was assessed by linear regression analysis. Further, the analysis was controlled for the period of abstinence, which was limited at 2–5 days. For the analysis, $P < 0.05$ was considered to indicate statistical significance.

RESULTS

There were no differences among the five groups in LH and testosterone levels, or right and left testicular volume (Table 1), but there were differences in FSH levels, sperm concentration, sperm motility, and semen volume among the five groups (Table 1; Fig. 1). Sperm morphology according to the WHO and Tygerberg criteria, and the complex motion characteristics evaluated, were no different among the age groups ($P > 0.05$; Table 1), but normal sperm morphology according to WHO was significantly lower in patients aged >45 years than in the other four groups ($P = 0.03$; Table 1).

From the linear regression analysis there was a decrease of 0.01 mL in semen volume, 0.27% in sperm motility and 0.039% in normal sperm morphology for each year of age (Table 2); the FSH level increased by 0.27% each year. The decrease in semen volume was 18.5% between the 24–30-year age group and the men aged >45 years.

DISCUSSION

The present sample included only those men who requested a vasectomy and thus provided semen specimens before the surgical procedure, and may therefore not be representative of the general population of sexually active men. As the volunteers had active lives, a potential bias might have affected the results; the present results might therefore underestimate the age effect. Even with this possible bias, men aged >40 years tended to have poorer semen quality than younger men.

The effects of paternal age on a couple's fertility are real and may be greater than has previously been thought. Ford *et al.* [2] stated that, after adjusting for other factors, the probability that a fertile couple will take >1 year to conceive nearly doubles, from 8% when the man is <25 years old to 15% when he is >35 years old; thus paternal age is a further factor to be considered when deciding

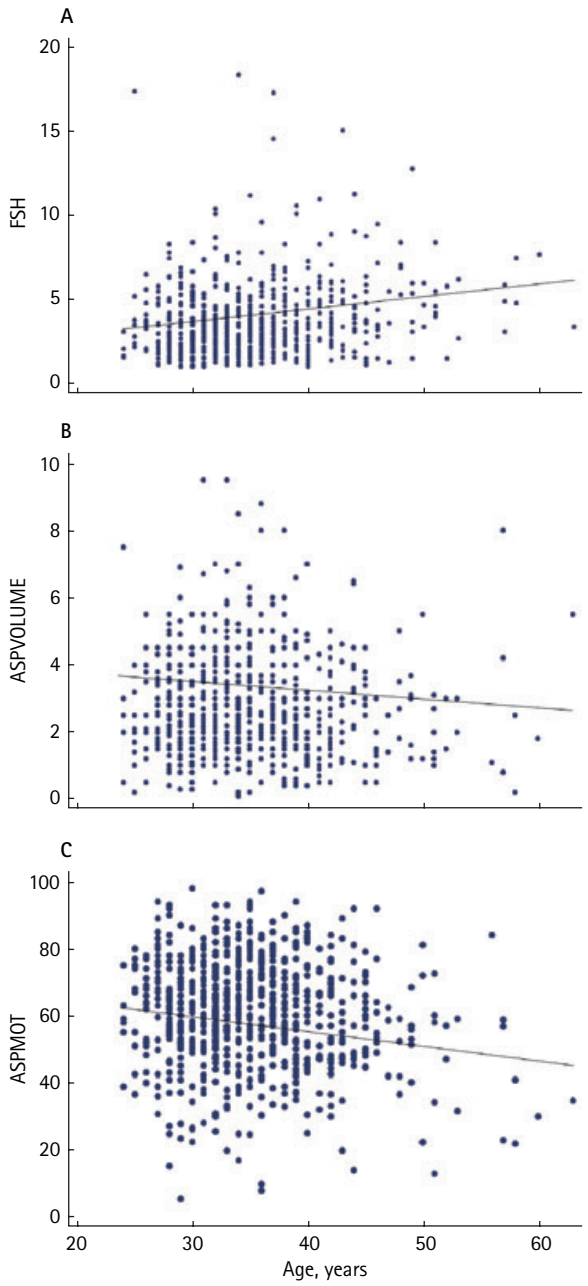

FIG. 1.
*The relationships between: **A**, FSH levels and age; **B**, semen volume and age; and **C**, sperm motility and age.*

Variable	R (Pearson)	β coefficient	P
Volume	–0.08	–0.01996	0.039
Progressive sperm motility	–0.11	–0.27143	0.016
Morphology (WHO)	–0.003	–0.03969	0.036
FSH	0.13	0.27279	0.009

Values are calculated using age as a continuous variable and the data were not log-transformed.

TABLE 2
The effect of age on seminal variables and FSH levels, in a linear regression model

the prognosis for infertile couples. Hassan and Killick [4] stated that increased male age is associated with a significant decline in fertility (five times longer to paternity when aged >45 years), which is independent of the woman's age, coital frequency, and life-style effect, as well as the effect of other subfertility risk factors. In addition, paternity at older ages may have significant effects on the viability and genetic health of human pregnancies and offspring, primarily as a result of structural chromosomal aberrations in sperm. The evidence for sex chromosomal aneuploidy suggests that there may be about a doubling of the risk at the age of 50 years [9].

To explain the age-dependent changes in semen quality, two issues should be considered [2–5]. First, cellular or physiological changes due to ageing have been described in testicles, seminal vesicles, prostate, and epididymis. Age-related narrowing and sclerosis of the testicular tubular lumen, decreases in spermatogenic activity, increased degeneration of germ cells, and fewer and less functional Leydig cells have been reported in autopsies of men who died from accidental causes [13]. Smooth muscle atrophy and a decrease in protein and water content, which occur in the prostate with ageing, may contribute to decreased semen volume and sperm motility. Also, the epididymis, a hormonally sensitive tissue, may undergo age-related changes. This hormonal or epididymal senescence may lead to decreased sperm motility in older men. Second, increasing age implies more frequent exposure to exogenous damage or disease [5]. Older men are more likely to have smoked for a longer period than younger men, or to have had such illnesses as urogenital infections.

In the present study we detected a decrease in semen volume across the groups evaluated. Indeed, published reports show a decrease in semen volume with ageing [2,5,7,10]. The longer period of sexual abstinence in men aged >50 years could explain these results. In the studies where the analyses were adjusted for the period of abstinence there was a decrease in semen volume of 3–22% [5].

In the present study, sperm concentration decreased with age. Even though some studies have reported a decrease in sperm concentration with increasing age, several others reported an increase in sperm concentration with age or found little or no

association between age and sperm concentration [2,4,7,14,15].

In the present study sperm motility tended to decrease with age; indeed most studies have found a decrease in sperm motility with increasing age [4,10,16–20]. Those studies that adjusted the results for the duration of abstinence reported statistically significant effects, such as negative linear relationships and decreases in motility of 0.17–0.6% for each year of age [5,15,21,22]. Thus, the present study supports the conclusion based on the data from most others, that there is strong and consistent evidence for a decrease in sperm motility with increasing age.

In the present study no correlations were detected between specific motion variables, as evaluated with CASA, and age. CASA was developed as a specific tool to make the assessment of semen quality more objective and detailed [23]. Several specific motility variables describing the movements of spermatozoa in more detail can be assessed using CASA. In addition, the classification into motile and immobile spermatozoa can be based on well-defined velocity thresholds.

Sperm morphology is a good indicator of the status of the germinal epithelium [20,24]. Degenerative changes in the germinal epithelium because of ageing may affect spermatogenesis and thus sperm morphology. In the present series, based on a linear regression analysis, normal sperm morphology tended to decrease by 0.039% for each year of age. Auger *et al.* [16], in a linear regression model, showed that the normal sperm morphology decreased by 0.9% yearly. Thus, compared with the average 30-year-old man, an average 50-year-old had an 18% decrease in normally shaped sperm. Ng *et al.* [10] showed that older men had more abnormal sperm morphology with fewer normal forms and reduced vitality, as well as more cytoplasmic droplets and sperm tail abnormalities (30% vs 17%) than younger men.

In the present study we evaluated two very important factors in older men not previously reported, i.e. hormone levels and testicular volume. There were no differences among the five groups in LH and testosterone levels, or right and left testicular volume. However, FSH levels tended to increase with age. A possible explanation for this is that, even with no decrease in testicular volume, the testicle begins to accumulate some damage (weakness sign) over time, as shown by the increase in FSH levels. Therefore, the increase in FSH levels may reflect that the testicles does not have the same capacity for normal spermatogenesis as earlier in life.

In conclusion, in 889 patients evaluated for a vasectomy, there tended to be a decrease in sperm concentration and normal motility, and a tendency for FSH levels to increase with age. Sperm morphology tended to decrease from 45 years old. We suggest that the ageing effect be considered when standard values for semen variables in routine semen analysis and normal values for hormone levels are proposed.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **CASA**, computer-assisted sperm analysis.