Impact of age, clinical conditions, and lifestyle on routine semen parameters and sperm kinematics

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Objective: To assess the impact of aging on routine semen and computer-assisted sperm analysis (CASA) motility parameters according to the current World Health Organization guidelines; and to evaluate the effect of obesity and lifestyle (alcohol consumption, cigarette smoking) in older men's semen.
Design: Blind cross-sectional study.
Setting: Research laboratory and andrology and reproduction laboratory.
Patient(s): A population of 11,706 men.
Intervention(s): None.
Main Outcome Measure(s): Semen analysis: routine (semen volume, sperm concentration and count, motility, vitality, morphology, hypo-osmotic swelling test, round and peroxidase-positive cell concentration) and CASA (straight-line velocity, curvilinear velocity, average path velocity, linearity, straightness, beat cross frequency, wobble, amplitude of lateral head displacement, and mean angular displacement) parameters; and body mass index.
Result(s): A negative correlation was found between age and routine semen parameters: volume, sperm count, motility, vitality, total motile spermatozoa and normal-motile spermatozoa, round cell concentration, and hypo-osmotic swelling test values. Several CASA variables (straight-line velocity, curvilinear velocity, average path velocity, beat cross frequency, amplitude of lateral head displacement, and mean angular displacement) were also negatively affected. Using 40 years as a cut-off value, significant differences in most parameters correlated to age. In a selected subpopulation of men unexposed to known fertility-compromising factors, the same evaluations were performed, finding some parameters still decreased. Although obesity exerted a significant deleterious effect on older patients' semen quality, alcohol consumption and cigarette smoking mildly affected it.
Conclusion(s): Male aging, with the contribution of unhealthy conditions, are paramount effectors of sperm quality deterioration. (Fertil Steril® 2018;110:68–75. ©2018 by American Society for Reproductive Medicine.)
This abstract is available in Spanish at the end of the article.

Key Words: Age, alcohol consumption, CASA, cigarette smoking, obesity

From financial stability to second families' establishment, childbearing has been increasingly delayed in recent decades (1–3). In developed countries, parenthood is being widely deferred by new generations striving for socioeconomic security (4), in contrast to the mid-1900s baby-boom paradigm, when family constitution was promoted by the government (4, 5). In the last few years, college education has been linked to better financial and professional stability (6) and marriage rates (7), and the time needed to fulfill these goals consequently resulted in late parenthood (8).

Knowing that women have a reduced fertility potential as they approach menopause, maternal aging has been thoroughly studied. In this

Received December 2, 2017; revised January 31, 2018; accepted March 9, 2018.
G.L.V. has nothing to disclose. A.D.T. has nothing to disclose. R.B. has nothing to disclose. F.B. has nothing to disclose. G.R. has nothing to disclose. R.I.M. has nothing to disclose. M.H.V.-L. has nothing to disclose.
Supported by funds from the Laboratorio de Andrología y Reproducción (to R.I.M.) and from the National Research Council of Argentina (PIP 2015-2017#887 to M.H.V.-L.).
R.I.M. and M.H.V.-L. should be considered similar in author order.
Accepted for poster presentation at the American Society for Reproductive Medicine Scientific Congress, October 28–November 1, 2017, San Antonio, Texas.
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Fertility and Sterility® Vol. 110, No. 1, July 2018 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2018.03.016

regard, an association between aging and follicle depletion, diminished oocyte quality, and impaired DNA repair, among others, has been reported (9–11). Concerning male age and fertility, some evidence has linked a decrease in fertility potential with increased age, as shown in assisted reproductive technology outcomes (12–14). Nevertheless, further consensus remains to be achieved regarding male aging impact on sperm quality. Whereas some studies have shown an association between male aging and semen quality, others have reported no relationship (15–18). Specifically, regarding objective assessment of sperm kinematics using computer-assisted sperm analysis (CASA) technology, the information is rather scarce (19).

The effect of some clinical and lifestyle components—in particular obesity, alcohol consumption, and cigarette smoking—on semen parameters, has been addressed in some studies. The obesity epidemic is a growing public health concern. In this regard, the American Medical Association recently classified obesity as a disease (20). Even though most of the attention on the impairments caused by obesity is drawn to general health, recent data suggest that reproductive health seems to be compromised as well. Some studies have revealed that elevated male body mass index (BMI) can lead to impaired sperm production (21, 22). In contrast, other studies have found no relationship between male BMI and semen parameters (23, 24). Additionally, the relationship between body size and semen production upon weight loss puzzles our understanding even further. Some studies have demonstrated an impairment of sperm production related to a dramatic weight loss, whereas others have shown an improvement in semen parameters (22, 25, 26). On the other hand, both alcohol consumption and cigarette smoking have been proposed to negatively impact male fertility and subsequent reproductive outcome (27). However, their effect on routine semen parameters remains controversial (28–31). Moreover, the impact of an abnormally high BMI, alcohol consumption, and cigarette smoking on age-decreased sperm quality has yet not been thoroughly investigated.

The present study aimed to evaluate the impact of aging on both routine semen and CASA motility parameters in a large cohort of patients attending the Laboratorio de Andrología y Reproducción (LAR) andrology laboratory, according to the current World Health Organization (WHO) guidelines (32), providing a controlled study performed by the same operators, equipment, and laboratory procedures subjected to strict high-quality standards.

MATERIALS AND METHODS

Patients

Between July 2010 and December 2016, a population of 11,706 men (age [mean ± SD] 35.87 ± 6.34 years; range, 18–76 years) was subjected to routine semen analysis. Within this population, CASA evaluation was also carried out in a total of 5,146 samples. Studies were performed at the LAR (Córdoba, Argentina). The LAR's quality assurance comprises internal and external procedures. The former include monthly monitoring of semen analysis results to identify systematic errors as a statistical control, using a computerized algorithm; intra- and interoperator reproducibility evaluations; and validation of results taking into account each operator coefficient of variation. The external quality assurance monitoring is performed by the External Quality Evaluation Program organized by the Argentinean Biochemistry Foundation for sperm concentration, motility, and morphology. This study was approved by the Instituto de Biología y Medicina Experimental (IBYME) institutional ethics committee, and all data were included with the patients' written consent.

Semen Analysis

Samples were obtained by masturbation after a 2–7 days' abstinence. After liquefaction at 37°C, semen samples were analyzed according to WHO 2010 standards (32), with some modifications. Basically, semen volume was assessed directly from samples in graduated conical tubes. Sperm concentration and progressive motility were evaluated soon after liquefaction of the sample in a Makler counting chamber (Sefi-Medical Instrument), and sperm vitality was determined after cell eosin Y staining. Normal sperm morphology was evaluated in samples stained under the Papanicolaou technique and analyzed according to strict criteria. Peroxidase-positive cells were quantified among round cells using a colorimetric assay. The hypo-osmotic swelling (HOS) test was carried out by incubating spermatozoa in a hypo-osmotic solution (25 mM sodium citrate, 75 mM D-fructose) to detect osmotic-competent spermatozoa (percentage of those with an intact membrane). Routine sperm parameters were assessed in at least 200 spermatozoa per sample. In our study, sperm concentration, motility, and morphology evaluation was done by two operators, rendering a total of 400 scored sperm cells.

Semen parameter cut-off values (lower reference limits; LRL) established by the WHO manual (fifth edition) (32) were as follows: semen volume (1.5 mL), sperm concentration (15 $\times$ 10^6 spermatozoa/mL), motility (32% progressive motile), vitality (58% alive), morphology (4% normal forms), peroxidase-positive cells (1 $\times$ 10^6/mL), and HOS test score (58%) (Supplemental Table 1). In addition, the total motile sperm count [TM = motility (%) $\times$ sperm count] and normal-motile sperm count [NM = normal morphology (%) $\times$ motility (%) $\times$ sperm count] were included in the analysis in each case.

Computer-assisted Sperm Analysis

Computer-assisted sperm analysis was carried out using the Integrated Sperm Analysis System (ISAS v1; Proiser R&D), which analyzes 30 frames per second. With the aid of a temperature-controlled stage (Proiser R&D), spermatozoa are maintained at 37°C constant temperature during motility assessment. In each sample, at least six microscopic fields were analyzed in two or more replicates, and more than 300 spermatozoa were evaluated. The system provides objective sperm motility parameters: straight-line velocity (VSL), curvilinear velocity (VCL), average path velocity (VAP), linearity (LIN), straightness (STR), beat cross frequency (BCF), wobble (WOB), amplitude of lateral head displacement (ALH), and mean angular displacement (MAD). To date, cut-off values have not been reported for these parameters.

Clinical and Lifestyle Conditions

Obese patients were defined as those with BMI $\geq$ 30.0 kg/m^2, whereas normal weight patients were those with a BMI between 18.5 and 25.0 kg/m^2. Those regarded as drinkers consumed at least one glass of an alcoholic beverage (beer, wine, spirits) daily, whereas nondrinkers did not. Smokers consumed at least 1 pack of cigarettes per day, whereas nonsmokers did not smoke.

Statistical Analysis

Subpopulation analyses were performed in randomized groups whose number was adjusted to those of narrower size through Microsoft Office Excel randomization. Results on routine semen parameters and CASA evaluations in each group are presented as mean ± SD. Spearman correlation coefficients were determined for male age and all semen parameters, as well as for BMI. Receiver operating characteristics (ROC) curves were constructed for assessment of the specific age predicting an abnormal semen parameter, using the WHO 2010 LRL as cut-off value. An acceptable threshold value was determined at maximum sensitivity and specificity in each case. Results between groups were compared by the nonparametric Mann-Whitney test or the Kruskal-Wallis test, as specifically indicated, followed by Dunn's multiple comparison tests in the latter. Comparison analyses of abnormality rates were evaluated by χ^2 test. All evaluations were performed using the GraphPad InStat program (GraphPad Software). Differences between groups were considered statistically significant at $P<.05$.

RESULTS

Age, Routine Semen Analysis, and Sperm Kinematics

In a large population of men attending the LAR andrology laboratory, age was found to exert a deleterious effect on routine semen variables. Semen volume, as well as sperm count, motility (percentage of progressive motile, TM, and NM), vitality, round cells, and HOS test values were negatively ($P<.05$) correlated to age. Contrastingly, a positive correlation ($P<.05$) was found between sperm concentration and peroxidase-positive cells with age (Table 1). Nevertheless, morphology did not show a significant correlation with age, and a large dispersion was observed among the results plotted for all variables (Supplemental Fig. 1A, available online).

Regarding CASA sperm kinematics, the analysis performed in a subset of more than 5,000 cases revealed the same trend observed for most routine parameters: VSL, VCL, VAP, BCF, ALH, and MAD negatively ($P<.05$) correlated with age (Table 1, Supplemental Fig. 1B). On the other hand, no significant correlation was found for LIN (VSL/VCL), STR (VSL/VAP), and WOB (VAP/VCL) (data not shown).

Age cut-off selection was addressed by using ROC curves. An age threshold was calculated for each parameter regarding its sensitivity and specificity for a better distinction between normal and abnormal values. As a result, age-sensitive and -specific cut-off values were found between 30.5 (sperm concentration) and 41.5 years (sperm count and vitality) (Table 2).

TABLE 1

Semen parameters and correlation to age.

Semen parameter	Mean ± SD	Correlation coefficient	*P* value
Volumel (mL)	3.02 ± 1.51	−0.09955	***
Concentration (million/mL)	67.49 ± 60.99	0.01903	*
Count (million)	185.50 ± 176.40	−0.03318	***
Motility (%)	45.85 ± 18.52	−0.11500	***
Total motile spermatozoa (million)	97.05 ± 110.20	−0.06939	***
Vitality (%)	83.31 ± 9.25	−0.11580	***
Normal-motile spermatozoa (million)	6.77 ± 10.35	−0.05903	***
HOS test (%)	79.30 ± 8.56	−0.08821	***
Round cells (million/mL)	1.24 ± 1.71	−0.05185	***
Peroxidase-positive cells (million/mL)	0.45 ± 1.43	0.01080	*
VSL (μm/sec)	26.19 ± 13.76	−0.05526	***
VCL (μm/sec)	46.55 ± 20.90	−0.08846	***
VAP (μm/sec)	32.17 ± 16.07	−0.07351	***
BCF (Hz)	8.54 ± 2.31	−0.03354	*
ALH (μm/sec)	2.43 ± 0.76	−0.08274	***
MAD (degrees)	42.13 ± 14.14	−0.05810	***

Note: Total number of samples subjected to routine semen analysis = 11,706. Total number of samples subjected to CASA analysis = 5,146.
*$P<.05$; **$P<.005$; ***$P<.0005$ (Spearman correlation analysis).

Verón. Aging, semen analysis, CASA, BMI. Fertil Steril 2018.

On the basis of these findings, a subset of samples from the whole population was distributed in four groups based on patient age, as follows: 18–29 years (group 1), 30–39 years (group 2), 40–49 years (group 3), and $\geq$50 years (group 4) (n = 307 for each group). As expected, samples from the youngest men (group 1) were found to have higher ($P<.05$) semen volume, sperm count, motility, TM, vitality, NM, and round cells, as well as HOS test scores, when compared with the oldest patients (group 4). Moreover, a decrease was also found in most routine semen parameters between samples from groups 3 and 4 (Supplemental Fig. 2A). Likewise, increased values of VSL, VCL, VAP, and ALH sperm kinematics were also found in the youngest group (Supplemental Fig. 2B).

Interestingly, a noticeable breach was observed between samples from men of group 2 and those from men at aged $\geq$40 years (groups 3 and 4) in most of these parameters (volume, concentration, count, motility, vitality, morphology, NM, and HOS test) (Supplemental Fig. 2A) and in CASA sperm kinematics (VSL, VCL, VAP, ALH, and MAD) (Supplemental Fig. 2B). Therefore, a cut-off value of 40 years was established

TABLE 2

ROC curves.

Semen parameter	Cut-off	Sensitivity	Specificity
Semen volume	35.5	0.51	0.58
Sperm concentration	30.5	0.83	0.18
Sperm count	41.5	0.84	0.19
Progressive sperm motility	36.5	0.59	0.49
Sperm morphology	35.5	0.51	0.50
Sperm vitality	41.5	0.84	0.35
HOS test	39.5	0.75	0.42
Round cells	33.5	0.38	0.66
Peroxidase-positive cells	35.5	0.51	0.50

Verón. Aging, semen analysis, CASA, BMI. Fertil Steril 2018.

for this study, taking into account results from both ROC curves and decades analyses. Groups of age <40 years (average group age 33.1 ± 4.2 years; range, 18–39 years; n = 2,859) and age ≥40 years (44.1 ± 4.6 years; range, 40–76 years; n = 2,859) were defined. Semen volume, sperm count, motility (percentage of progressive motile and TM), NM, vitality, HOS test, and round cells values were decreased ($P<.05$) in the older population when compared with the younger one (Table 3). Moreover, lower ($P<.05$) values were also found in older men for VSL, VCL, VAP, BCF, ALH, and MAD parameters (Table 3).

Taking into account the WHO (2010) established LRL for semen volume, sperm concentration, motility, vitality, morphology, peroxidase-positive cells, and HOS test score (Supplemental Table 1), abnormality rates were also calculated. Thus, increased age (≥40 years) was associated with a higher abnormality rate in semen volume and in sperm motility, vitality, and HOS test score (Supplemental Table 2).

TABLE 3

Semen parameters in <40-year and ≥40-year age groups.

Semen parameter	<40 y	≥40 y	*P* value
Unselected population			
n	2,859	2,859	
Volume (mL)	3.09 ± 1.50	2.82 ± 1.51	***
Concentration (million/mL)	67.52 ± 62.21	69.58 ± 62.77	NS
Count (million)	192.80 ± 183.40	177.60 ± 176.90	***
Motility (%)	47.31 ± 18.17	42.40 ± 18.96	***
Total motile spermatozoa (million)	103.20 ± 114.70	86.52 ± 105.10	***
Morphology (%)	5.45 ± 3.41	5.31 ± 3.33	NS
Normal-motile spermatozoa (million)	7.19 ± 10.94	5.96 ± 9.39	***
Vitality (%)	83.75 ± 8.78	81.49 ± 10.60	***
HOS test (%)	79.79 ± 8.45	78.08 ± 9.06	***
Round cells (million/mL)	1.26 ± 1.68	1.18 ± 1.54	*
Peroxidase-positive cells (million/mL)	0.45 ± 1.49	0.46 ± 1.33	NS
VSL (μm/sec)	26.61 ± 13.92	25.17 ± 12.57	***
VCL (μm/sec)	47.05 ± 21.75	44.89 ± 18.60	***
VAP (μm/sec)	32.53 ± 16.50	30.96 ± 14.38	***
LIN (%)	59.21 ± 23.85	58.99 ± 26.01	NS
STR (%)	84.60 ± 27.15	84.06 ± 29.22	NS
BCF (Hz)	8.65 ± 2.21	8.40 ± 2.29	***
WOB (%)	72.82 ± 27.88	72.97 ± 29.73	NS
ALH (μm/sec)	2.46 ± 0.77	2.35 ± 0.70	***
MAD (degrees)	42.10 ± 8.32	41.75 ± 10.72	**
Selected population			
n	50	50	
Volume (mL)	2.81 ± 1.40	2.26 ± 1.42	*
Motility (%)	44.92 ± 17.75	34.70 ± 19.44	**
Total motile spermatozoa (million)	126.00 ± 131.30	80.69 ± 102.90	*
Vitality (%)	85.02 ± 6.28	79.72 ± 10.80	**
VSL (μm/sec)	24.67 ± 6.96	21.36 ± 6.10	*
VCL (μm/sec)	44.78 ± 7.21	39.34 ± 8.49	**
VAP (μm/sec)	30.45 ± 6.20	26.62 ± 5.84	**
ALH (μm/sec)	2.42 ± 0.40	2.13 ± 0.42	**

Note: Values are mean ± SD. NS = nonsignificant.
*$P<.05$; **$P<.005$; ***$P<.0005$ (Mann-Whitney test).

Verón. Aging, semen analysis, CASA, BMI. Fertil Steril 2018.

Considering the negative impact of certain factors on semen quality (mostly environmental exposure and lifestyle/clinical conditions), samples were classified and selected. Patients who had disclosed the conditions cryptorchidism, genital infections, epidemic parotitis, surgeries, chronical treatments, cigarette smoking, alcohol, anabolic supplement or steroid consumption, and exposure to toxics were identified and removed from the sample database. The previous set of studies was repeated in two groups of this selected population: age <40 years (33.8 ± 4.6 years; range, 19–39 years; n = 50) and age ≥40 years (46.3 ± 7.5 years; range, 40–76 years; n = 50). As a result semen volume, as well as sperm motility, TM, and vitality parameters from the routine analysis, were found to be diminished ($P<.05$) in the older group from the unselected population. In addition, lower ($P<.05$) average values of VSL, VCL, VAP, and ALH sperm kinematic variables were also found in older men from the selected group (Table 3).

Altogether, these findings revealed the negative impact of age on several routine semen parameters and sperm motility kinematics. Specifically, a deleterious effect of age was observed when samples from groups aged <40 years and ≥40 years were compared, in both unselected and selected populations. To further address the differences observed between younger and older men, the contribution of some clinical and lifestyle conditions was assessed. Results are shown in the next sections.

Age and BMI

In a dataset of 4,061 samples from our study group, 22.6% had a BMI ≥30 kg/m^2 (obese class I, II, and III). In addition, BMI was positively correlated to age ($r = 0.1311$; $P< .0001$) (Supplemental Fig. 3). On the basis of these findings, an analysis was performed to determine the impact of abnormal BMI on semen parameters by comparing samples from obese and normal BMI in the age ≥40 years subgroup. As a result, a subset of older men with BMI ≥30 kg/m^2 (n = 277) was found to have a lower ($P<.05$) sperm quality when compared with normal-BMI older controls (18.5 kg/m^2 < BMI < 25 kg/m^2; n = 277). This quality decrease was evidenced by a lower semen volume, sperm concentration, count, motility (percentage and TM), and morphology (percentage and NM). In addition, lower ($P<.05$) VCL, VAP, and ALH kinematic variables were also observed in older obese men compared with the non-obese group (Table 4). Among patients aged ≥40 years, increased BMI was found to result in higher abnormality rates for semen volume, sperm concentration, count, motility, vitality, and morphology (Supplemental Table 3).

On the basis of these findings, having a BMI ≥30 kg/m^2 negatively affected the age-associated semen quality reduction, showing abnormal values for several routine and CASA sperm parameters.

Age, Alcohol Consumption, and Cigarette Smoking

The potential effect of alcohol consumption and cigarette smoking on semen parameters was assessed in men aged ≥40 years. In a first set of analyses, those who consumed

TABLE 4

Sperm parameters in control and obese populations.

Sperm parameter	18.5 kg/m^2 < BMI < 25 kg/m^2	BMI ≥ 30 kg/m^2	*P* value
n	277	277	
Volume (mL)	2.98 ± 1.46	2.69 ± 1.56	**
Concentration (million/mL)	98.77 ± 68.42	82.65 ± 69.65	**
Count (million)	273.70 ± 218.20	201.10 ± 187.00	***
Motility (%)	44.74 ± 16.67	38.57 ± 18.93	***
Total motile spermatozoa (million)	136.10 ± 135.00	88.40 ± 104.10	***
Morphology (%)	5.73 ± 3.32	4.83 ± 3.12	***
Normal-motile spermatozoa (million)	9.61 ± 11.99	5.70 ± 9.03	***
VCL (μm/sec)	44.07 ± 9.16	42.50 ± 10.11	*
VAP (μm/sec)	29.96 ± 5.95	28.85 ± 6.82	*
ALH (μm/sec)	2.37 ± 0.46	2.30 ± 0.50	*

Note: Values are mean ± SD.
*P< .05; **P< .005; ***P< .0005 (Mann-Whitney test).

Verón. Aging, semen analysis, CASA, BMI. Fertil Steril 2018.

at least one glass of alcohol (beer, wine, spirits) daily were regarded as "drinkers." Thus, a new subset of two groups was established: older nondrinkers and older drinkers (n = 571 each). No significant differences were observed between groups when comparing both routine and CASA parameters by means of the Mann-Whitney test (data not shown). Nevertheless, vitality abnormality rates were significantly increased in the older drinkers group when compared with the older nondrinkers group (2.5% vs. 5.4%; $P<.05$).

In the second set of studies, patients who smoked at least 1 cigarette pack per day were defined as "smokers." Accordingly, data were dichotomized (older nonsmokers and older smokers; n = 304 each) to assess the contribution of cigarette smoking to age-dependent sperm distress. As a result, older smokers had higher sperm concentration (19.2% vs. 12.5%) and count (21.9% vs. 14.8%) abnormality rates when compared with nonsmokers ($P<.05$; χ^2 test). No significant differences were observed in CASA kinematics in these subsets of patients.

DISCUSSION

As paternal age increases in the developed world, more attention has been drawn to the effects of aging on male reproductive and sexual functions. Although the reproductive biological potential seems to remain for most of a man's life, changes in sperm production were already reported in the early 1980s (33) and later confirmed (16, 34).

Over the past several decades paternal age has been reported to increase in many countries (35, 36). In Denmark, whereas the average age of women and men becoming parents in 1986 was 25.6 and 30.9 years, respectively, it increased to 29.1 and 33.4 years by 2016 (37). Similar findings were reported in England and Wales, with more than one-third of children currently born to fathers older than 35 years (38). The etiology for delayed procreation has been proposed to result mainly from social reasons (35, 39). Regardless of the cause, concern arises pertaining to the effects of such demographic shifts on pregnancy success and the health of new generations. Parental age at conception seems to impact on male offspring's reproductive health, as shown in studies describing a correlation between increased maternal age and higher risks of cryptorchidism and testicular cancer (40, 41). Specifically regarding the father, researchers have linked paternal age to higher risks of congenital abnormalities, impaired cognitive abilities, childhood and breast cancer, acute lymphoblastic leukemia, autism, schizophrenia, bipolar disorder and "paternal age effect" disorders (42). A genome-wide mutation analysis revealed the dominant impact of the increased paternal age on de novo mutations in the child, estimated at two mutations per year (43).

In the present study the effect of aging has been extensively evaluated for a broad set of semen parameters on the largest cohort analyzed from a single center to date. Most of the semen parameters analyzed were found to negatively correlate with age. Semen volume, sperm count, motility (percentage and TM), NM, vitality, HOS test, and round cells values were negatively correlated to increased age. Our findings are in line with previous studies reporting lower sperm motility (15, 17, 19) and count (17, 18) in older men. Moreover, we do not find significant changes in sperm concentration, in agreement with previous reports (15, 16, 19). To our understanding, this is the first report describing the negative impact of male aging on sperm vitality and peroxidase-positive cells and HOS test results. The significant negative correlation found between HOS test values and male age may be of great relevance, taking into account the negative impact of abnormal HOS test recently reported on embryo implantation (44).

Within the population studied, a breaking point was found around 40 years of age, whereby samples of younger (<40 years) and older (≥40 years) patients depicted significant differences in almost all parameters correlated to age. This trend was closely followed by an increase in abnormality rates (according to the LRL established by the WHO 2010 guidelines) for semen volume, sperm motility, vitality, and HOS test. Even though there is no current agreement concerning advanced paternal age, 40 years was used as a cut-off in 44% (14 of 32) of the reports included in a recent review (45). Offspring from men aged ≥40 years showed higher chances of breast cancer (46), childhood leukemia, and central nervous system tumors (47). Moreover, there have been reports advising to limit the sperm donor age to 40 years (48).

The initial large, unbiased population analyzed in our study was exposed to known fertility-compromising factors, such as cryptorchidism, genital infections, epidemic parotitis, surgeries, chronical treatments, cigarette smoking, alcohol, anabolic supplements or steroid consumption, and exposure to toxics (49–51). A selected subpopulation of men not exposed to any of these conditions was defined and reanalyzed. Herein, some of the altered parameters found in the whole population displayed an age-related decrease (volume, motility, TM, vitality, VSL, VCL, VAP and ALH). These findings led us to analyze the contribution to this gap of lifestyles and disease between exposed and unexposed populations.

In the evaluations assessing the impact of obesity on age-dependent semen abnormalities, older men with BMI ≥ 30 kg/m^2 displayed lower semen volume, sperm concentration, count, motility, TM, morphology, and NM when compared with older men with normal BMI. In addition, semen volume, sperm concentration, count, motility, vitality, and morphology abnormality rates were significantly higher in older obese individuals when compared with older non-obese men. These findings are in agreement with previous research reporting that increasing BMI correlates with a decreasing sperm concentration and count (21), motility, and morphology, as recently reported (52). To our understanding, this is the first study that reports the negative impact of obesity in older men on objective sperm kinematics.

Alcohol intake has been linked to cardiovascular disease, premature death, and obesity (53, 54) and negatively associated with semen volume, sperm count, motility, and morphology (28, 55). Herein, alcohol consumption was found as an added factor to increase vitality abnormality rates in the older population studied. Previous studies have shown the negative impact of alcohol consumption on semen quality in a healthy young men cohort (56).

Cigarette smoking leads to well-known diseases, including cancer as well as cardiovascular, respiratory, and perinatal conditions (57, 58). Previous studies done in men of all ages have found contradictory results, reporting a deleterious effect of cigarette smoking on sperm concentration and count (59), or no relation (21, 60). In our study, increased sperm concentration and count abnormality rates were determined in older smokers.

In the present study CASA sperm kinematics (VSL, VCL, VAP, BCF, ALH, and MAD parameters) analysis done in a large group of more than 5,000 samples revealed decreased values in several parameters with increasing age, suggesting the presence of alterations in the sperm motility molecular machinery in association with patient's higher age. An age-dependent reduction in VSL, VAP, and LIN was previously shown in a cohort of approximately 100 patients, whereas no association was found for VCL, ALH, and BCF (19). Disparities between this last study and ours may be due to differences in population size. In our study, age was still associated with reduced VSL, VCL, VAP, and ALH kinematic sperm variables in a selected subset of samples from men not exposed to common diseases and exposures that impact on fertility. An abnormally high BMI negatively impacted sperm motility kinematics in obese older men, as revealed by the lower VCL, VAP, and ALH values registered in these samples when compared with those from non-obese patients. This is the first study that reports alterations in sperm kinematics in older men in a large cohort of patients and the negative impact of obesity on some of these variables. These parameters (17) were reported to predict IUI (61, 62) and IVF (63, 64) success in men of different age from couples under infertility treatment.

In summary, age is a factor that, per se, affects semen volume, sperm motility (percentage and TM) and vitality, and sperm kinematic variables, suggesting that parenthood withholding is a worldwide fashion that hijacks fertility potential. Additionally, several clinical conditions and lifestyles contribute to a further impoverishment in several routine and CASA sperm parameters. Specifically, obesity leads to a decrease in sperm quality, as evidenced in several parameters, whereas cigarette smoking and alcohol consumption barely affect the already diminished age-dependent sperm deterioration. Henceforth, male aging and exposure to unhealthy conditions are paramount effectors of sperm quality deterioration, making it essential for physicians to bear it in mind when counseling couples consulting for infertility. Supplemental Figure 4 presents a summary of findings reported in this investigation.

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Impacto de la edad, estados clínicos y estilo de vida en los parámetros seminales rutinarios y cinemática del espermatozoide

Objetivo: Valorar el impacto de la edad en los parámetros de motilidad según los criterios de la organización mundial de la salud (OMS) tras análisis del semen manual o informatizado (computer-assisted sperm analysis-CASA); y evaluar el efecto de la obesidad y los hábitos de vida (consumo de alcohol y tabaquismo) en los parámetros seminales de varones de edad avanzada.

Diseño: Estudio transversal, ciego.

Ámbito: Laboratorio de Investigación y laboratorio de Andrología y Reproducción humana.

Paciente(s): Población de 11.706 varones

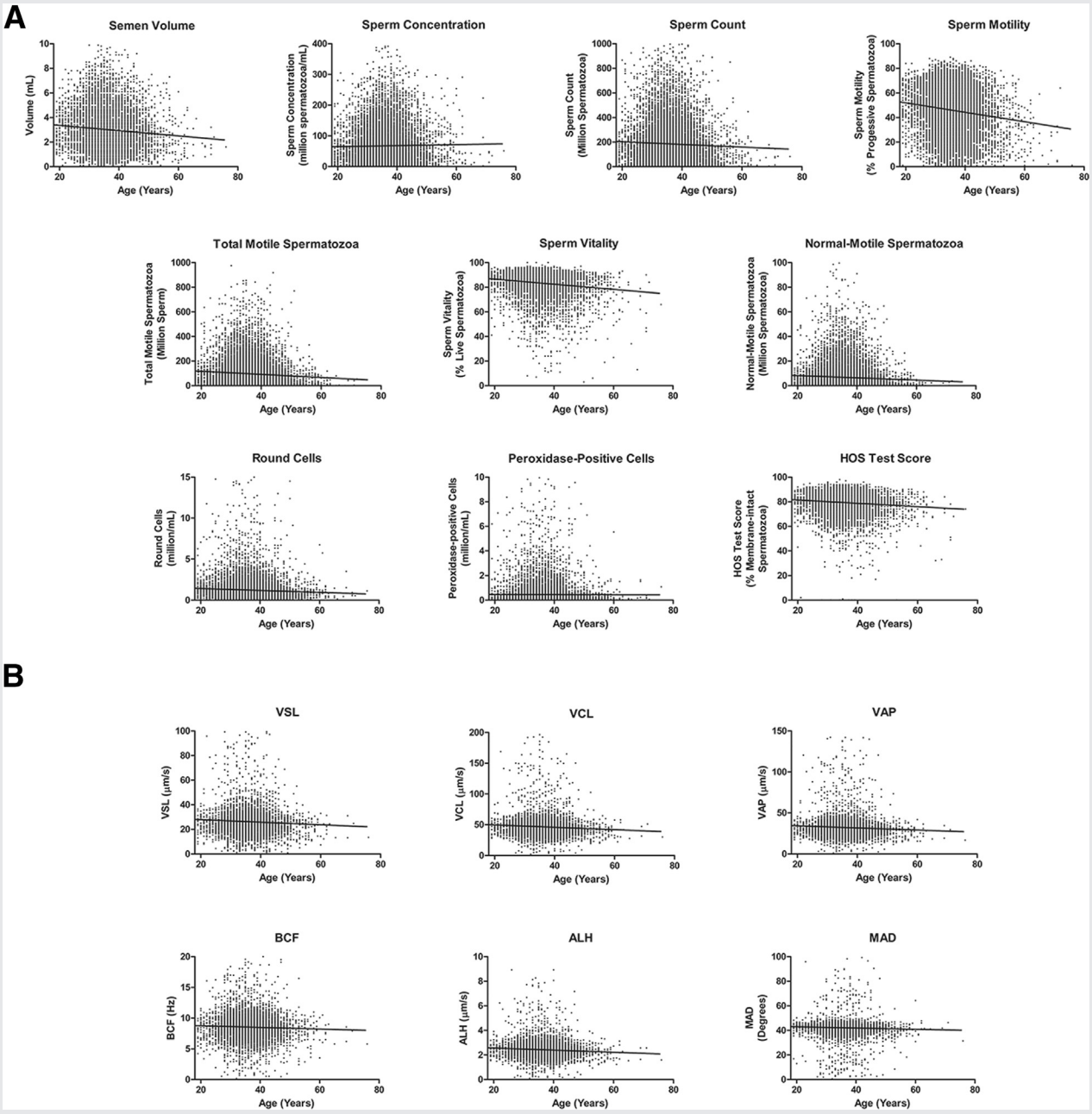
Intervención(es): Ninguna

Variables de resultado principal(es): Análisis del semen manual (volumen del eyaculado, concentración espermática y recuento total, motilidad, vitalidad, morfología, test hipo-osmótico, concentración de células redondas y células peroxidasa-positivas) y CASA: velocidad lineal, velocidad curvilínea, velocidad media, linearidad, rectitud, frecuencia de bateo, frecuencia de vibración de la cabeza, amplitud de desplazamiento lateral de la cabeza y desplazamiento angular medio; también se valoró el índice de masa corporal (IMC) de los pacientes.

Resultado(s): Se encontró una correlación negativa entre edad y resultados de parámetros seminales tras análisis manual (volumen del eyaculado, recuento total, motilidad, vitalidad, recuento de espermatozoides móviles, motilidad de espermatozoides normales, concentración de células redondas y test hipo-osmótico. Varios de los parámetros analizados por CASA también se vieron negativamente afectados (velocidad lineal, velocidad curvilínea, velocidad media, frecuencia cruzada, amplitud de desplazamiento lateral de la cabeza y desplazamiento angular medio). Usando como punto de corte la edad de 40 años, diferencias significativas en la mayor parte de los parámetros se correlacionaron con la edad. En una subpoblación seleccionada de varones no expuestos a factores conocidos que puedan afectar a la fertilidad se valoraron los mismos parámetros, encontrándose alguno de ellos igualmente disminuidos. Aunque la obesidad ejerce un papel significativamente deletéreo en la calidad espermática de pacientes más añosos, el consumo de alcohol o el tabaquismo sólo la afectó de forma moderada.

Conclusión(es): La edad paterna junto con la contribución de otros hábitos de vida no saludables, ejercen un papel fundamental en el deterioro de los parámetros seminales.

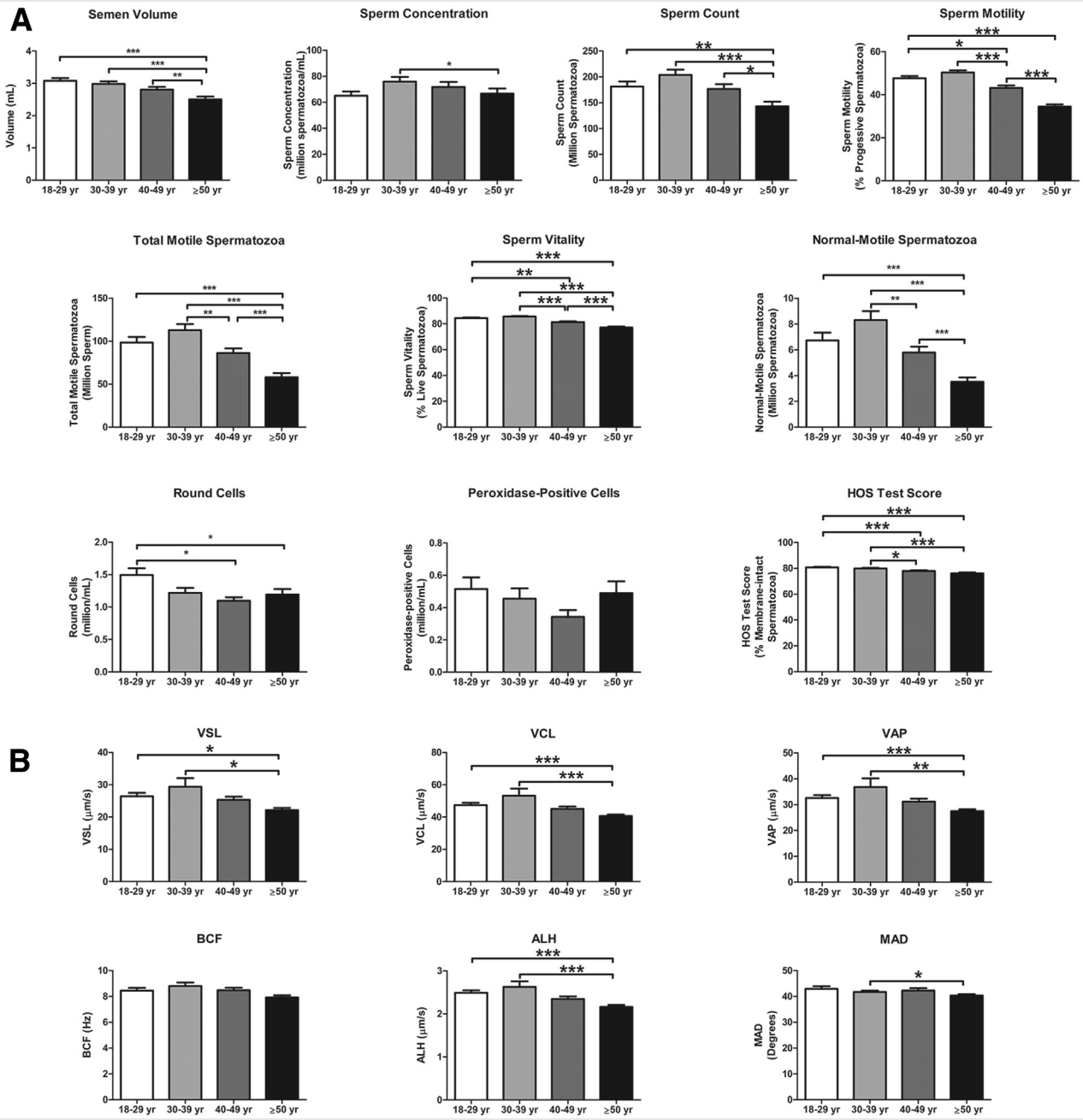
SUPPLEMENTAL FIGURE 1



Male age and semen parameters. Correlation analysis. (A) Routine semen analysis. Semen volume, sperm count, motility (%), vitality (%), sperm morphology (%), round cells ($\times 10^6$), and HOS test (%) were found to be negatively correlated to age. (B) CASA sperm kinematics. CASA parameters VSL, VCL, VAP, BCF, ALH, and MAD negatively correlated to age. No significant correlation was found for LIN, STR, and WOB (data not shown).

Verón. Aging, semen analysis, CASA, BMI. Fertil Steril 2018.

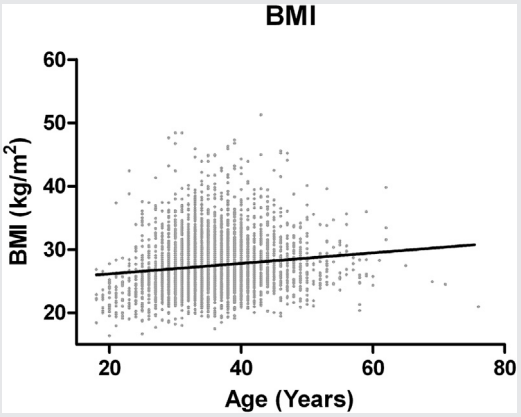
SUPPLEMENTAL FIGURE 2



Male age and semen parameters. Analysis by decades. (A) Routine semen parameters. A significant decrease was found between samples of groups aged <40 years and those of older groups. (B) CASA sperm kinematics. A significant decrease was found for VSL, VCL, VAP, BCF, ALH, and MAD between samples from groups aged <40 years and ≥40 years.

Verón. Aging, semen analysis, CASA, BMI. Fertil Steril 2018.

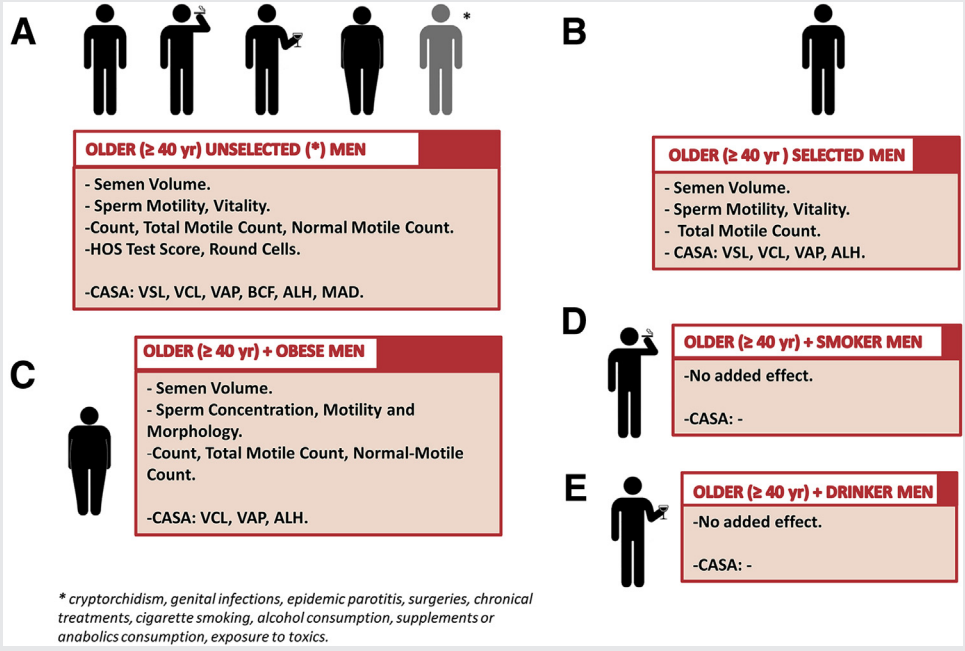
SUPPLEMENTAL FIGURE 3



Correlation analysis between male age and BMI. Age positively correlated to BMI. Spearman $r = 0.13$; $P<.0001$.

Verón. Aging, semen analysis, CASA, BMI. Fertil Steril 2018.

SUPPLEMENTAL FIGURE 4



Age, obesity, and lifestyle impact on sperm quality. Aging was found to negatively affect semen quality in several parameters when the population was exposed to sperm-damaging conditions (A), and only some of those were directly linked to aging per se in a selected population (B). Obesity was an added deleterious factor that further deteriorated semen quality (C). Cigarette smoking (D) and alcohol consumption (E) depicted a moderate effect.

Verón. Aging, semen analysis, CASA, BMI. Fertil Steril 2018.