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.