Semen Quality Assessment in Fertile Men in Madrid During the Last 3 Decades

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OBJECTIVE To evaluate semen quality of men with proven fertility in Spain over the last 3 decades.

METHODS We conduct a retrospective analysis of ejaculate samples of 992 men between 1985 and 2009. All patients had proven fertility as demonstrated by fathering at least 2 children and a semen analysis performed before they underwent vasectomy. A standardized procedure was used for the semen analysis. Semen volume, total sperm count, sperm concentration, motility, and percentage of morphologically normal spermatozoa were assessed. Mean values were calculated by examining microscopic fields of 100 spermatozoa.

RESULTS Statistically significant differences were found in all semen parameters analyzed. For the periods 1985-1990, 1990-2000, and 2000-2009, the mean (standard deviation [SD]) sperm concentration was 27.7 (22.97), 20.73 (14.79), and 20.18 (20.79) $\times$ 10^6 (P <.0001). The mean (SD) progressive motility for each period was 53.19 (20.35), 47.22 (15.84), and 40.57 (15.15; P <.0001). The mean (SD) normal-shaped spermatozoa for each period was 67.69 (10.24), 58.87 (14.67), and 51.02 (15.76; P <.0001). Multivariate analysis using a logistic regression model showed that age had no significant effect in the variation of semen parameters at the cut-points analyzed, except for normal forms at percentile 25 (P = .001). Multivariate analysis revealed a trend for decline of semen in sperm concentration, progressive and nonprogressive motility, and the percentage of morphologically normal spermatozoa (P = .001-.002).

CONCLUSION Over the last 3 decades, a decline in semen quality was found in all the parameters analyzed in Spanish men with proven fertility. UROLOGY 85: 1333–1338, 2015. © 2015 Elsevier Inc.

Several reports have suggested that the quality of semen has declined during last decades.[1-3] However, there are reports stating that semen quality has remained stable.[4] Moreover, if semen quality has declined, there is no consensus on what parameters are affected and if the presence of alterations in sperm concentration or in sperm morphology necessarily translate into subfertility. Although, a bias in the selection of patients may have some influence on the result of the series published, poor semen quality seems to be a widespread phenomenon nowadays. Recent studies carried out in Europe have showed that only 23% of men had the optimal semen quality from a fecundity perspective.[2,5] It has been also suggested that low semen quality may be a potential contributing factor to lower fertility rates and the increasing number of children who are born after assisted reproductive technology.[6,7] Since the publication of World Health Organization (WHO) guidelines in 1980 on the reference values for human semen,[8] subsequent editions have decreased the normal values of these parameters.[9-12] In the 2010 edition, to select a population of fertile men, the reference values were calculated using men who had fathered a child within 1 year of trying to induce pregnancy.[13]

The aim of the present study was to analyze the changes to semen quality of men referred to the Department of Urology in a tertiary care University Hospital in Madrid, Spain, over the last 3 decades.

METHODS

Participants

This is a retrospective analysis of the ejaculate of 992 men presenting for a vasectomy procedure to the clinic in Madrid, Spain, during the years 1985-2009. The patients included in had proven fertility capacity as they had fathered at least 2 children as was required by Spanish law before vasectomy in the Public Health System. The medical records of the patients were reviewed. All patients were required to undergo a spermiogram before the vasectomy procedure for investigation purposes. All patients consented to this research.

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Semen Collection and Analysis

The semen analysis was performed in our laboratory of andrology and was carried out by just only 1 nurse and a specialist, andrologist. Moreover, there was no variation in the procedure used to perform the semen analysis during the years included in the study. Semen samples were assessed in each period according to the standard approved methods according to the *WHO Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction* (WHO, 1980, 1987, 1992, 1999, 2010).[8-13] Semen samples were collected by the patient after a recommended period of sexual abstinence of 3-5 days and delivered to the hospital unit within 1 hour of collection. Semen volume was estimated by specimen weight, assuming a semen density of 1 g/mL. The concentration of sperm per milliliter was determined manually using a hemocytometer with a magnification of ×400. The mean (standard deviation [SD]) value was calculated. To determine the percentage of motile sperm, a semen sample was placed on a microscope slide and observed under magnifications of ×100 and ×400 with phase optics. The slide was scanned, and a minimum of 100 spermatozoa were counted and classified in 4-6 fields chosen at random. Progressive motility was defined as sperm that had slow or rapid forward movement. Nonprogressive motility was defined as no forward movement of the sperm. Mean (SD) values were also determined.

Data were analyzed according to the specific year in which samples were taken and the decade: 1985-1990, 1990-2000, and 2000-2009. The homogeneity of the 3 periods was evaluated with regard to patient characteristics, comorbidities, and risk factors for impaired spermatogenesis. Comorbidities were identified using chart review history.

Statistical Analysis

Quantitative data are reported as mean ± SD or median with interquartile ranges (Q1-Q3). Qualitative variables are expressed as absolute and relative frequencies. The normality of the main outcome variables (total sperm count, semen volume, sperm concentration, percentage of motile spermatozoa progressive and nonprogressive motility, and the percentage of morphologically normal spermatozoa) was evaluated using the Shapiro-Wilk test. In all cases, the normality distributions of either outcome were rejected. The variables total sperm count per ejaculate, semen volume, and sperm concentration have extreme value in the upper tail, whereas the variables regarding sperm motility and the percentage of morphologically normal spermatozoa are affected by the preference digits phenomenon. To normalize the variables, several methods are used, namely logarithm transformations and square root and inverse square root (suitable when there are extreme values). We also try to normalize the variables by elimination of extreme values (those >95% and/or <5%) and subsequent transformation with logarithm (x + 1) and the square of the variable. In all cases, the normalization of the variables was rejected (P value <.001). Therefore, comparisons of the distribution of continuous measurements with nonparametric tests or relation between continuous variables with the Spearman correlation were used, as appropriate. The outcome variables were categorized using the distribution of data with different cut points (median and interquartile [Q1-Q3]). A logistic regression model adjusted by surgery year and patient age was generated. Associations are given as odds ratios with 95% confidence intervals. Statistical analysis was performed using SAS software, version 8.0 (SAS Institute Inc., Cary, NC).

RESULTS

Patient characteristics including sperm analyses conducted are shown in Table 1. No significant differences were found in terms of comorbidities and risk factors for impaired spermatogenesis such as cryptorchidism, hypospadias, hypogonadism, smoking, body mass index, and diabetes mellitus. Patients' age in the period 2000-2009 was significantly higher than the previous 2 decades (P <.0001).

Statistically significant differences were found among the different decades in all the semen parameters analyzed (Table 1; P <.0001). The most important differences indicating the decline in semen quality were found in the sperm concentration, sperm motility, and number of normal forms. Figure 1 showed sperm concentrations, the percentage of morphologic normal spermatozoa, and sperm with progressive motility for each year of examination. The percentage of normal forms appeared to decline year on year, whereas the progressive motility revealed sporadic declines for certain years.

Multivariate analysis using a logistic regression model showed that age did not have a significant effect in the variation of semen parameters at the cut-point analyzed (percentiles 25, 50, and 75), except for the percentage of normal forms at percentile 25 (P = .001). Multivariate analysis did reveal a trend with year of semen analysis for decline in sperm concentration, progressive and nonprogressive motility, and the percentage of morphologically normal spermatozoa (P <.001-.002; Table 2).

COMMENT

Several studies have suggested a decline in semen quality over the time. Carlsen et al reported a worldwide decline in sperm counts between 1938 and 1990 in a meta-analysis of 14,947 men from 61 studies.[1,3] The present study supports this finding in terms of a decline in sperm concentration, sperm motility, and percentage of normal spermatozoa. Several suggestions have been made as to the cause of the decline in semen quality, such as environmental toxins and increased rates of genitourinary congenital abnormalities, for example, cryptorchidism and hypospadias.[2,14] It has also been proposed that the quality of semen may also be influenced by geographic and ethnic factors,[15] although a decline in sperm quality has been demonstrated in studies from Europe, the United States, and New Zealand.[16-19] A higher decline in sperm concentrations has been suggested in European studies, especially those carried out in Northern Europe.[20] However, it should be mentioned that some reports that have not demonstrated a decline in semen quality, such as those carried out in the United States by Fisch et al and Paulsen et al,[4,21] showed a statistically significant increase in sperm concentration over a period of time of 25 years. Although we did not evaluate the reason for the decline in sperm parameters because it was not the purpose of our study, 2 studies from Spain suggested a decline in sperm quality in the last decade with a proposal that there was