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DECLINE IN SEMEN QUALITY AMONG FERTILE MEN IN PARIS DURING THE PAST 20 YEARS

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Abstract *Background.* Several studies have suggested a population-wide decline in the quality of semen over the past 50 years, but clear evidence of decreasing semen quality in recent decades is lacking.

Methods. From 1973 through 1992 we measured the volume of seminal fluid, the sperm concentration, and the percentages of motile and morphologically normal spermatozoa in 1351 healthy fertile men. The data on the semen samples were collected at one sperm bank in Paris. The data in each calendar year were analyzed as a function of the year of donation, the age of each patient, the year of birth, and the duration of sexual abstinence before semen collection.

Results. There was no change in semen volume during the study period. The mean concentration of sperm decreased by 2.1 percent per year, from 89×10^6 per milliliter in 1973 to 60×10^6 per milliliter in 1992 ($P<0.001$). During the same period the percentages of motile and normal spermatozoa decreased by 0.6 percent and 0.5 percent per year, respectively (both $P<0.001$). After adjustment in multiple regression analyses for age and the duration of sexual abstinence, each successive calendar year of birth accounted for 2.6 percent of the yearly decline in the sperm concentration and for 0.3 percent and 0.7 percent, respectively, of the yearly declines in the percentages of motile and normal spermatozoa (all $P<0.001$).

Conclusions. During the past 20 years, there has been a decline in the concentration and motility of sperm and in the percentage of morphologically normal spermatozoa in fertile men that is independent of the age of the men. (N Engl J Med 1995;332:281-5.)

DURING the past three decades, several reports have suggested that the quality of semen in normal men is declining.[1-4] Recently, in a meta-analysis of 61 studies worldwide, Carlsen et al. found a trend toward decreasing sperm count and volume of seminal fluid over the past 50 years.[5] The studies included in the meta-analysis were conducted in different countries at different times, and bias in the recruitment of men or in methods of semen analysis may have affected the findings.[6] It is important, therefore, to assess this finding and to determine whether there has been a parallel decline in male fertility.

The Centre d'Etude et de Conservation des Oeufs et du Sperme Humains is a sperm bank created in 1973 in a university hospital. All the donors are fathers, and the mode of recruitment of men and the method of semen analysis have remained the same during the past 20 years. In an analysis of data from this bank, we found that there have been significant declines in sperm concentration, the percentage of motile sperm, and the percentage of normal sperm over the past 20 years.

METHODS

Study Subjects

We analyzed the first ejaculate donated at the center between 1973 and 1992 by each of 1750 men to help infertile couples become parents. The donors were all healthy, unpaid volunteers who had previously fathered at least one child. Ninety-six percent were white, and 85 percent lived in the Paris area. One percent were farmers; 16 percent were manual workers; 40 percent were technicians, teachers, or tradesmen; 38 percent were executives; and the remaining 5 percent had other occupations. We divided the donors into three groups. One group was composed of 314 men requesting cryopreservation of their semen before vasectomy who agreed to provide additional specimens for use in artificial insemination. The second group was composed of 85 men who were brothers of infertile men who requested artificial insemination of their partners with donor semen. The third group was composed of 1351 men who were referred by unrelated infertile couples or by physicians or who appeared spontaneously.

The mean concentration of sperm, the percentage of motile spermatozoa, and the percentage of normal spermatozoa in the second group of fertile men (those whose brothers were infertile and requested artificial insemination of their partners) were significantly lower than those of the 1351 donors in the third group (Table 1). The values for mean concentration and motility were higher in the first group (the men studied before vasectomy) than in the third group (Table 1), since candidates for vasectomy were recruited for semen donation only if the motility of their sperm was relatively unaffected by freezing and thawing. As a result, the first two groups were excluded from the study. In the remaining group of 1351 men, the mean (±SD) age at the time of donation was 34±6 years (range, 19 to 59). Among these men, the mean age of those donating semen in a calendar year increased throughout the study from 32 years in 1973 to 36 years in 1992 ($P<0.001$) (Fig. 1).

Analysis of Semen Samples

All the semen samples were collected by masturbation at the laboratory after a recommended period of sexual abstinence of three to five days. Each sample was incubated at 37°C and analyzed within one hour. The volume of seminal fluid was determined by weighing, assuming that 1 g of semen is equivalent to a volume of 1 ml. The concentration of sperm per milliliter of sample was determined with

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a hemocytometer; the sperm were counted in the two chambers at a final magnification of 400, and the mean value was calculated.[7] The total sperm count was then calculated. To determine the percentage of motile sperm, a 20-μl drop of gently mixed semen was placed on a glass slide under a coverslip. The slide was placed on the heating stage of a microscope (37°C) and observed at magnifications of 100 and 400 with phase optics. The slide was scanned, and at least 100 spermatozoa in all were counted and classified in four to six fields chosen at random. The percentage of motile spermatozoa was calculated from the ratio of the number of rapidly and slowly moving sperm (grades a and b, according to the classification system of the World Health Organization[7]) to the total number of sperm counted. The percentage of morphologically normal spermatozoa was evaluated at a final magnification of 1000, after Shorr staining as described by David et al.[8]

During the 20 years of the study, 11 technicians worked in the laboratory, 4 for 1 to 4 years, 3 for 5 years, and 4 for 9 to 14 years. We determined coefficients of variation with respect to measurements of semen characteristics for the three technicians working in the laboratory at the conclusion of the study, who had been there for 14, 5, and 2 years. For each technician, coefficients of variation for sperm concentration and morphologic features were determined from triplicate analyses of three different semen samples. Coefficients of variation between technicians were determined from the first analysis of each of the three samples. For each technician, the coefficient of variation for sperm motility was determined from triplicate measurements of 4 semen samples; the coefficient of variation between technicians was determined from 15 samples.

The coefficients of variation for the three technicians with respect to the measurement of sperm concentrations were 1.3, 3.1, and 4.2 percent; the coefficient of variation between technicians was 4.6 percent. In the assessment of the morphologic features of sperm, the coefficients of variation for the three technicians were 2.0, 3.7, and 7.1 percent; the coefficient of variation between technicians was 9.9 percent. In the assessment of motility, the coefficients of variation for the three technicians were 0, 7.7, and 8.3 percent; the coefficient of variation between technicians was 7.3 percent.

Statistical Analysis

BMDP statistical software was used in all the statistical analyses.[9] Sperm concentrations and total sperm counts do not have normal distributions in large groups of fertile men,[2,10] and this was the case among the 1351 men studied (as determined by Wilk's test). Age at donation and the donor's year of birth had normal distributions, but the duration of sexual abstinence was skewed. The best transformation of the data that yielded normal distributions for each of the three variables without normal distributions was the logarithmic (base 10) transformation. The relations between each characteristic of the semen samples and the year of semen donation were studied by linear regression analysis. The variable for the year of semen donation was composite because it combined each man's age at the time of donation with his year of birth. The relation of each semen characteristic to these independent variables (age at donation and year of birth) was tested with multiple regression analysis. The duration of sexual abstinence before the collection of semen was also included, since it affects the semen characteristics,[11] had a wide range in this study, and increased significantly (P=0.02) with the advancing age of the men.

Table 1. Characteristics of Semen Samples from 1750 Fertile Donors.

Characteristic	All Donors (N = 1750)	Candidates for Vasectomy (N = 314)	Brothers of Infertile Men (N = 85)	Study Subjects (N = 1351)
	mean ±SD			
Volume of seminal fluid (ml)	3.8±1.8	3.8±1.8	4.1±1.9	3.8±1.8
Sperm concentration/ml ($\times 10^{-6}$)	102.6±74.5	123.9±75.5*	84.0±72.7†	98.8±73.5
Total sperm count ($\times 10^{-6}$)	372.2±320.4	440.5±353.1*	318.4±267.0	359.7±313.5
Motile sperm (%)	66±12	69±9‡	63±15§	66±12
Normal sperm (%)	61±13	62±12	55±15‡	61±13

*P<0.001. (This and the three following P values are for the comparison with the study subjects by the Mann–Whitney test.)

†P=0.01.

‡P=0.003.

§P=0.04.

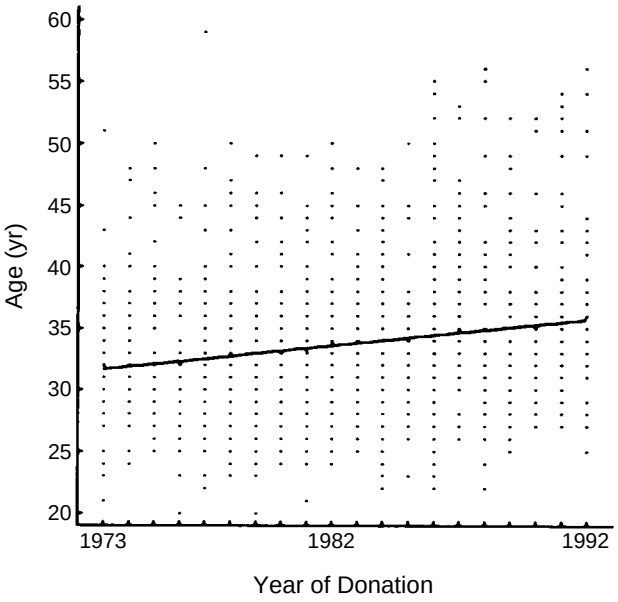

Figure 1. Change in the Mean Age of the Men Donating Semen in a Given Calendar Year, 1973–1992.

Linear regression analysis showed that the mean age of the donors increased significantly, from 32 years in 1973 to 36 years in 1992 (P<0.001). A total of 1351 men were studied.

Results

The mean volume of seminal fluid was 3.8 ml, and this value did not change during the study period. In contrast, the mean sperm concentration decreased by 2.1 percent per year (Fig. 2A), from 89×10^6 per milliliter in 1973 to 60×10^6 per milliliter in 1992. During the same period, the percentages of motile and normal spermatozoa decreased by 0.6 and 0.5 percent per year, respectively (P<0.001 for both) (Fig. 2B and 2C).

A man's age and the duration of his sexual abstinence before the collection of semen influence the characteristics of the semen.[11,12] We therefore assessed the contribution of these two factors to the declines measured. Age, duration of abstinence, and year of birth were included as independent variables in a multiple regression analysis of the data. Greater sexual abstinence was associated with an increase in the sperm concentration and a decrease in the percentage of motile spermatozoa (Table 2); it thus contributed to the observed decline in motility. Older age contributed significantly to the decreases in the sperm concentration, the percentage of motile sperm, and the percentage of normal spermatozoa. Multiple regression analyses after adjustment for age and the duration of sexual abstinence revealed that 2.6 percent of the yearly decline in the sperm concentration and 0.3 percent and 0.7

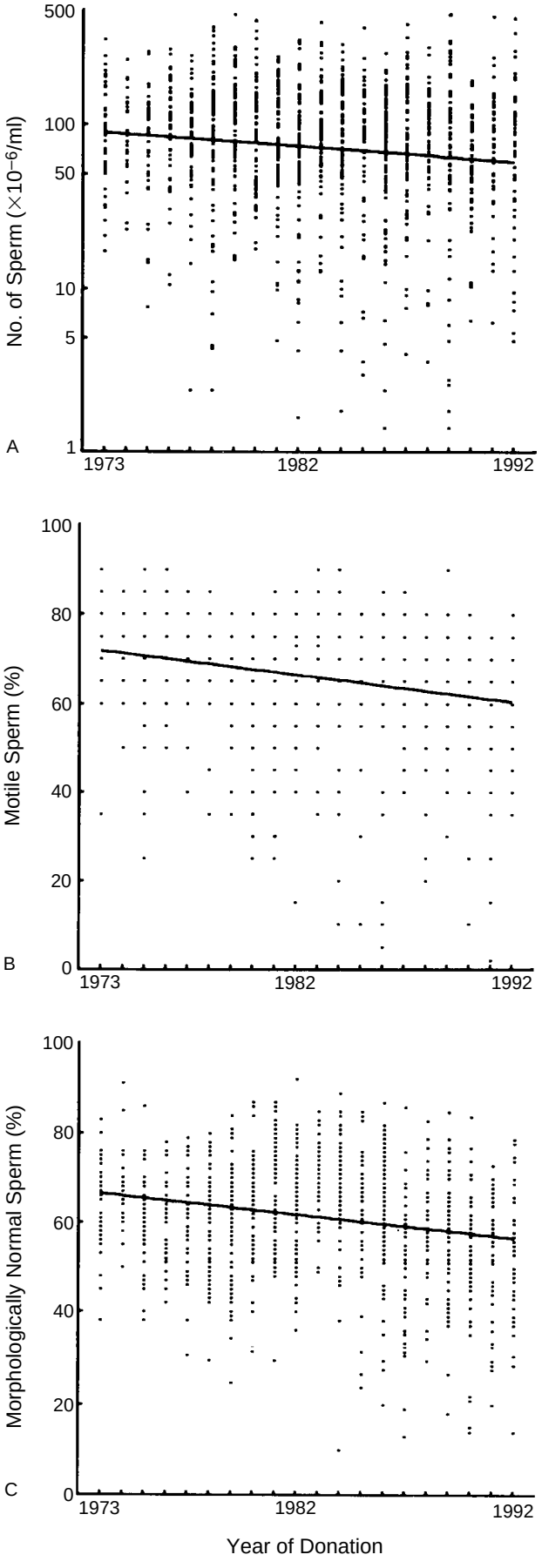

Figure 2. Changes in the Sperm Concentration (Panel A), the Percentage of Motile Sperm (Panel B), and the Percentage of Morphologically Normal Sperm (Panel C) in 1351 Fertile Men, 1973–1992.

Linear regression analysis revealed a decrease of 2.1 percent per year in the mean sperm concentration, from 89×10^6 per milliliter in 1973 to 60×10^6 per milliliter in 1992. The concomitant decreases in the mean percentages of motile and normal spermatozoa were 0.6 and 0.5 percent per year, respectively.

percent of the yearly decline in the percentages of motile and normal spermatozoa, respectively, were associated with each successive calendar year of birth (all $P<0.001$) (Table 2).

A preliminary analysis (data not shown) indicated that to eliminate the confounding effects of the duration of sexual abstinence, a subgroup of men with a narrower age range (28 to 37 years) and comparatively similar durations of abstinence (three or four days) should be studied. In this restricted group of 382 men, age and the duration of sexual abstinence were not significantly correlated. Linear regression analysis of the data for this subgroup revealed that the mean sperm concentration decreased by 3.7 percent per year, from 101×10^6 per milliliter in 1973 to 50×10^6 per milliliter in 1992 ($P<0.001$), whereas the percentage of normal spermatozoa declined by 0.7 percent per year ($P<0.001$). After adjustment for age, the yearly decline in the sperm concentration with each successive year of birth was more pronounced in this subgroup than in the entire group (Table 3). For example, the predicted sperm concentration of men 30 years old who were born in 1945 was 102×10^6 per milliliter, as compared with 51×10^6 per milliliter for 30-year-olds born in 1962.

Discussion

We found a decline in the concentration and motility of sperm and in the percentage of morphologically normal sperm in fertile men studied in Paris over a 20-year period, but unlike Carlsen et al.,[5] we found no decline in the volume of semen. We doubt that the decline in the quality of semen between 1973 and 1992 could be attributed to changes in personnel, techniques, or equipment. During the 20 years of the study, there were few changes in staff. All the technicians had the same training, the method of study did not change and was regularly verified, and no new equipment was introduced. In our laboratory, the coefficients of variation for each technician and between technicians in the assessment of the percentage of motile sperm were less than 10 percent in the 1970s,[13] and the results were similar for the staff members working at the end of the study. Although the coefficients of variation in the assessment of semen characteristics were less than 10 percent, the evaluations by technicians may have differed with regard to characteristics assessed subjectively, particularly those pertaining to morphologic features of sperm.[14] However, there was no change in the procedure used to evaluate these features over the 20-year period.

Our study confirms that both the duration of sexu-

Table 2. Effects of Age, Year of Birth, and Duration of Sexual Abstinence before the Collection of Semen on Changes in Characteristics of Semen Samples from 1351 Fertile Men Studied by Multiple Regression Analysis.*

Characteristic and Variable Studied	Regression Coefficient (95% CI)	P Value
	percent change	
Sperm concentration		
Older age (by 1 yr)	−3.3 (−1.8 to −4.7)†	<0.001
Later birth (by 1 yr)	−2.6 (−1.3 to −3.9)†	<0.001
Longer abstinence (by 1 day)	+2.2 (+0.7 to +3.8)†	0.004
Percentage of motile sperm		
Older age (by 1 yr)	−0.6 (−0.4 to −0.8)	<0.001
Later birth (by 1 yr)	−0.3 (−0.2 to −0.5)	<0.001
Longer abstinence (by 1 day)	−0.3 (−0.1 to −0.5)	0.009
Percentage of normal sperm‡		
Older age (by 1 yr)	−0.9 (−0.7 to −1.1)	<0.001
Later birth (by 1 yr)	−0.7 (−0.5 to −0.9)	<0.001

*Year of birth had no significant effect on seminal volume in this analysis. CI denotes confidence interval.

†As converted from the antilog of the logarithmic value of the regression coefficients.

‡Only two factors were used in the multiple regression analysis of this variable, because the duration of sexual abstinence had no significant effect on the percentage of normal spermatozoa.

al abstinence before the collection of semen and the age of the donor influence the characteristics of semen.[11,12,15] Thus, these factors should be considered and recorded accurately in all studies of the characteristics of semen in fertile men. In most studies of these characteristics, the subjects are asked to remain abstinent for three to five days before donating the sample. We made the same request, but only 66 percent of the men adhered to it. After the duration of abstinence was taken into account, there were still significant declines in the concentration of sperm and in the percentages of motile and normal spermatozoa with each successive year of birth. Thus, we conclude that there has been a true decline in the quality of semen during the past 20 years, since the characteristics of semen from a fertile man of a given age in 1992 were significantly poorer than those of a fertile man of the same age in 1973. This decline is unexplained. If this trend concerns not only the population of fertile men we studied but also all the men in the population, the proportion of men with fertility problems will increase.

Table 3. Effect of Age and Year of Birth on Changes in Characteristics of Semen Samples from 382 Fertile Men 28 to 37 Years of Age Who Were Sexually Abstinent for Three to Four Days before the Collection of Semen.*

Characteristic and Variable Studied	Regression Coefficient (95% CI)	P Value
	percent change	
Sperm concentration		
Older age (by 1 yr)	−5.8 (+1.9 to −9.6)†	0.004
Later birth (by 1 yr)	−3.6 (−1.4 to −5.8)†	0.003
Percentage of normal sperm		
Older age (by 1 yr)	−0.9 (−0.3 to −1.5)	0.002
Later birth (by 1 yr)	−0.8 (−0.4 to −1.1)	<0.001

*The effect of the independent variables on semen volume and the percentage of motile sperm was not significant. CI denotes confidence interval.

†As converted from the antilog of the logarithmic value of the regression coefficients.

The decline in the sperm concentration may reflect impaired spermatogenesis and may be linked to a decrease in the number of Sertoli cells.[16,17] The fact that not only the concentration of sperm but also the percentage of normal spermatozoa declined indicates a qualitative impairment of spermatogenesis and perhaps of the Sertoli cells. Such modifications have been reported in experiments involving heat-induced inhibition of spermatogenesis and Sertoli-cell function.[18,19] The decline in the quality of semen coincides with an increasing incidence of abnormalities of the male genital tract, including testicular cancer and cryptorchidism, in various countries.[20,21] In some regions of France, the incidence of testicular cancer increased from 1975 to 1992,[22] but no data are available for the Paris area. Whether there has been an increase in cryptorchidism in France, as in the United Kingdom,[23] is unknown, but the incidence of postpubertal cryptorchidism may have increased.[24,25]

The decline in semen quality and the increasing incidence of genital abnormalities in a geographic area may have a common origin.[5] Estrogens or compounds with estrogen-like activity taken by pregnant women have been suggested to affect the testicular function of male offspring adversely.[16,17] Diethylstilbestrol is thought to be responsible for an increase in abnormalities of the reproductive tract and for reductions in the output and fertilizing potential of sperm of male offspring.[26,27] However, the number of donors at our center who may have been exposed in utero to diethylstilbestrol is probably very low. Other routes of estrogen exposure may be involved if the hypothesis of an estrogen effect is true.[17]

If the finding of a decline in semen quality with the advancing year of a donor's birth suggests prenatal alterations of testicular function, it may also be related to changes in diet or lifestyle after birth or puberty. However, the significant decline in the concentration of sperm and the quality of semen during the past 20 years in the Paris area may be related to an interaction of the age of the donors and the chronologic period that in turn could implicate factors affecting all the inhabitants of an area, such as the water supply[28] or environmental pollution.[29]

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