Within-subject variability of human semen in regard to sperm count, volume, total number of spermatozoa and length of abstinence

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Summary. The within-subject variability of the semen sperm count (n), volume (v) and the total number of spermatozoa (N) was studied on 220 ejaculates from 36 normal subjects after an abstinence of 7 days or less. For each of the three variables, the within-subject standard deviation , σ, was practically proportional to the mean, μ; the common value of the coefficient of variation σ/μ for all subjects was very high: 0·39 for n, 0·28 for v and 0·55 for N. The 95% confidence intervals based on a single ejaculate were asymmetrical and very large, the lower and upper limits being respectively 0·5 × n and 2·3 × n; 0·7 × v and 1·8 × v; 0·4 × N and 2·9 × N. The three semen characteristics for a given subject were highly correlated with the length of abstinence: for an increase in abstinence of 1 day there were mean increments of 13 × 10⁶/ml for n, 0·4 ml for v, and 87 × 10⁶ for N.

Introduction

Quantitative studies have already been made to investigate the great variability which exists for various semen characteristics in different ejaculates from the same individual (Hotchkiss, 1941; MacLeod & Gold, 1956; Freund, 1962; Read & Schnieden, 1978), as well as the influence of the length of abstinence (MacLeod & Gold, 1952, 1953; Freund, 1962; Van Duijn & Freund, 1971). However, the statistical analysis has often been limited and in no study are the results presented in such a way that direct comparisons can be made. Furthermore, the subjects of such studies have been either men consulting for sterility or volunteers with a high weekly ejaculation frequency.

The purpose of the present study was to evaluate within-subject variability for semen sperm count, volume and total number of spermatozoa as well as the effect of abstinence in normal subjects who maintained an approximately normal ejaculation frequency. The methods of statistical analysis used will permit direct comparisons with other studies.

Materials and Methods

Patients

The study was carried out at the sperm bank of Bicêtre Hospital near Paris with men who had requested cryopreservation of semen before undergoing vasectomy and had submitted several ejaculates for this purpose. No special instructions were given regarding sexual activity between visits to the sperm bank but a 3-day abstinence before each visit was advised. For this study, ejaculates preceded by an abstinence of over 7 days were excluded. All men seen in

consultation between 1 January 1974 and 31 December 1976 who provided at least 5 ejaculates with a mean sperm count between 20 and 200×10^6/ml were included in the study. This resulted in a population of 36 men who provided a total of 220 ejaculates with the following distribution:

Number of ejaculates	5	6	7	8	9
Number of subjects	17	5	9	3	2

The length of abstinence was found to vary considerably for most subjects, 25 (70%) reporting at least 3 different abstinence periods.

All subjects were fertile (having at least 1 child) and they had a mean ($\pm$ s.d.) age of $34 \cdot 4 \pm 5 \cdot 3$ years.

Techniques

All semen samples were collected at the sperm bank by masturbation. The sperm count (n) and volume (v) were determined for each, the total number of spermatozoa being calculated as $N = nv$. The sperm count was carried out by first diluting the semen to 1:20 with a 1% Ringer's formol solution and then placing a portion into the 2 chambers of a haemocytometer (Thoma cell), each $0 \cdot 1$ mm^3 in volume. A minimum of 400 spermatozoa was counted for each sample. Counting was carried out by the same person throughout the study period. Under these conditions, measurement errors according to Freund & Carol (1964) are principally due to semen sampling error resulting in a coefficient of variation $< 5\%$. The volume was measured with a pipette to the nearest $0 \cdot 1$ ml.

Statistical analysis

The statistical techniques utilized have been described by Snedecor & Cochran (1967). For the comparison of variances, distributions were compared to the χ^2 probability density. When variances were not found to differ significantly, their weighted mean value was calculated. For the study of regressions, correlation coefficients and slopes were calculated for each subject and a weighted mean determined. For the confidence intervals, the calculation was made by using log y ($y = $ n, v or N) since the variances of log y were found to be practically the same for all subjects (see 'Results'). Because there was not a normal within-subject distribution of log y, it was necessary to use the observed distribution of log y. All the above calculations have been detailed by Laplanche (1978).

Results

Mean values

The mean values calculated for the 220 semen samples were: $n = 99 \times 10^6$/ml, $v = 4 \cdot 1$ ml and nv (N) $= 401 \times 10^6$.

Within-subject variability

For each of the 3 characteristics studied, the within-subject standard deviation (σ), upon inspection, appeared to increase proportionally with the mean (μ) as is shown for n in Text-fig. 1 (for v and N the patterns were similar). In order to test the hypothesis σ/μ equals a constant, the within-subject variances of log y ($y = $ n, v or N) which are equal to σ^2/μ^2 (Lellouch & Lazar, 1974) were compared among the 36 subjects. As no significant difference was found, the hypothesis was retained. The common value for all subjects of the coefficient of variation (σ/μ) was $0 \cdot 39$ for n, $0 \cdot 28$ for v and $0 \cdot 55$ for N.
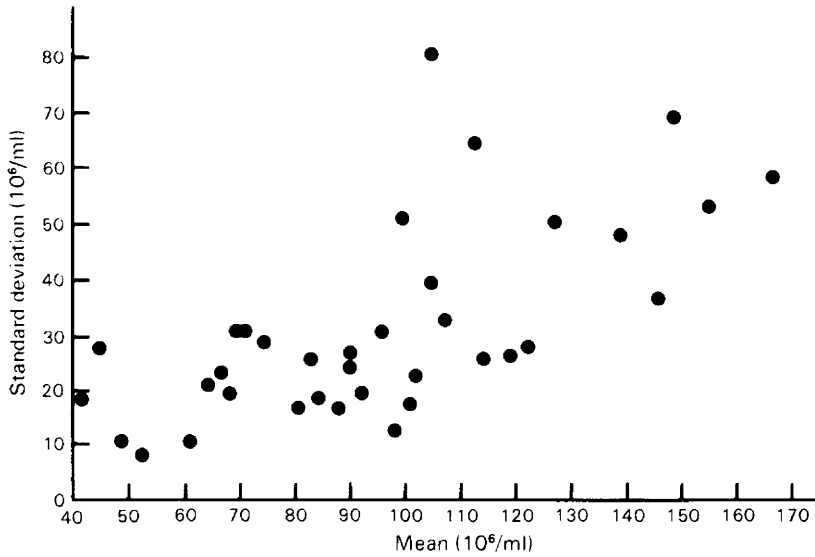
Text-fig. 1. Relationship between the within-subject standard deviation and the individual mean for the sperm count (n) in men.

For each of the 3 characteristics, the 95% confidence intervals for a given subject based on a single ejaculate (n_e, v_e, N_e) were obtained. The lower and upper limits, respectively, were found to be: $0.5 \times n_e$ and $2.3 \times n_e$; $0.7 \times v_e$ and $1.8 \times v_e$; $0.4 \times N_e$ and $2.9 \times N_e$.

Semen characteristics and length of abstinence

The relationship between each variable (n, v, N) and the length of abstinence for all 220 specimens appeared to be linear, at least between 1 and 5 days (Text-fig. 2).
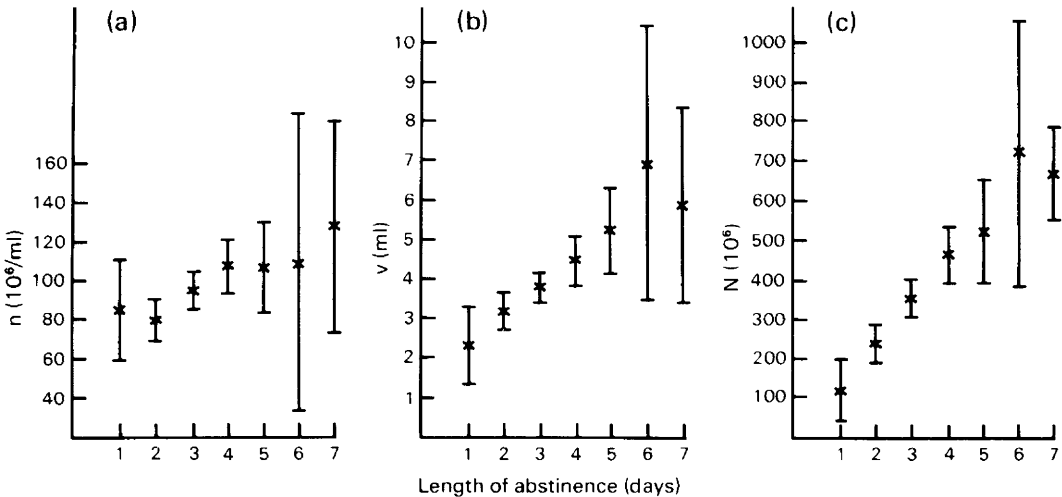
Text-fig. 2. Regression of (a) sperm count, n, (b) volume, v, and (c) total number of spermatozoa, N, on length of abstinence before ejaculation by men. The values represent the mean ± 2 s.e. for 8, 32, 92, 62, 20, 2 and 4 ejaculates after abstinence for 1, 2, 3, 4, 5, 6 and 7 days respectively.

The calculations were made on a within-subject basis. The within-subject correlation coefficients (r) were highly significant: 0·39 for n ($P < 10^{-5}$), 0·47 for v ($P < 10^{-6}$) and 0·54 for N ($P < 10^{-9}$). There were no departures from linearity for each subject for the 3 variables. The weighted mean value of individual slopes was such that each additional day of abstinence resulted in increases of n, v and N equal to 13×10^6/ml, 0·4 ml and 87×10^6 respectively. In order to determine if there were a relationship between individual slopes and means, correlation coefficients among the 36 subjects were calculated. For n, v and N the values were 0·21, 0·22 and 0·32 respectively. Even though only the last was significant, the results suggest that the slope and the mean are related in a positive sense.

Discussion

For the 3 semen characteristics studied, the within-subject standard deviation varies proportionally with the mean value and thus the within-subject coefficient of variation is constant. Four authors have provided sufficiently detailed data to permit us to calculate the coefficients of variation for their studies and a comparison of these with our own values reveals very good agreement (Table 1), even for the study by Read & Schnieden (1978) which was carried out with oligozoospermic and astheno-zoospermic subjects. The coefficients of variation for all studies are very high, being of the order of 40% for n, 30% for v and 55% for N.

Table 1. Within-subject coefficients of variation in different studies of human semen

	Hotchkiss (1941)	MacLeod & Gold (1956)	Freund (1962)	Read & Schnieden* (1978)	Present study
No. of subjects	23	12	12	16	36
No. of ejaculates	640	919	140	118	220
n	0·36	0·40	0·41	0·47	0·39
v	0·24	—	0·29	—	0·28
N	0·42	——	0·75	0·53	0·55

* Oligozoospermic or astheno-zoospermic subjects.

In view of this high within-subject variability, what information can be obtained from a single ejaculate? The present study has approached this question by determining the 95% confidence intervals for mean values of n, v and N which were wide and asymmetrical. For example, if a single sperm count of 100×10^6/ml were determined, the mean value for the subject could lie anywhere between 50 and 230×10^6/ml. Only a very small part of this variability can be attributed to measurement errors which were minimal as explained in 'Materials and Methods'. Thus, the variability must be essentially biological.

Certainly one influential factor is the period of abstinence. In our sample all 3 semen characteristics for a given subject are highly correlated with this period, the relationship being practically linear. In other studies the importance of the length of abstinence has been only mentioned (Belding & Boston, 1934; Hotchkiss, 1941; Hammen, 1944; MacLeod & Heim, 1945; Lampe & Masters, 1956; Zimmermann, Maude & Moldawer, 1965), described graphically (Raboch, 1957; Murphy, 1962) or subjected to more or less elaborate statistical tests (MacLeod & Gold, 1952, 1953; Freund, 1962; Van Duijn & Freund, 1971). In these studies, the slopes were not evaluated but it was possible to calculate one slope for each characteristic from the data of 2 studies: the increments per day of abstinence were 12×10^6/ml for n and 60×10^6 for N in the study of MacLeod & Gold (1952) and 0·3 ml for v in the study of Raboch (1957). These values agree well with the results of the present study which are respectively 13×10^6/ml,

87×10^6 and 0·4 ml. These increments are very large when compared to the respective mean value of each characteristic.

The part of within-subject variability attributable to length of abstinence (r^2) is 15, 22 and 29% for n, v and N respectively. Therefore, a very large part of the within-subject variability in semen characteristics remains unexplained and justifies further investigation. However, the results of this study indicate that it is no longer possible to ignore, from the clinical or the research point of view, the high variability which exists for semen characteristics or the relationship of these characteristics to length of abstinence.

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