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INTERRELATIONSHIPS AMONG THE CHARACTERISTICS OF HUMAN SEMEN AND FACTORS AFFECTING SEMEN-SPECIMEN QUALITY

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Summary. Significant among-donor differences in mean values for semen-specimen volume and sperm concentration, motility, forward progression, and morphology were demonstrated, but no correlation among these characteristics on a specimens-within-donor basis was demonstrable. When emission frequency was increased from 3·5 to 8·6 times per week, there was a decrease in specimen volume (−33%), in sperm concentration (−55%) and motility (−15%), but no effect on progression or morphology. At the high frequency, there was a decrease in total sperm number (−31%) and in motile sperm number (−41%) produced per week, but an increase in volume of semen per week (+39%). Specimens were rated at hourly intervals for 9 hr after collection; motility and progression declined at a linear rate with time, but no change in morphology was observed. No effect of seminal plasma on sperm motility or forward progression was noted in a complete factorial seminal-plasma-reversal study. No significant differences were observed in mean values for semen characteristics between masturbated and condom specimens.

INTRODUCTION

A single human semen specimen, as seen by or reported to the physician, remains the main (and, sometimes, sole) basis for an assessment of the male partner's role in an infertile marriage. Little is known about the specific effects of important factors, such as frequency of emission, age of the specimen, method of specimen collection, and influence of the seminal plasma, on the semen characteristics of a given specimen. Yet, a decision on fertility status must often be made on the basis of the semen characteristics determined in that given specimen.

The determination of mean values for semen characteristics in large groups of fertile men (actually, fathers) and of infertile men (actually, the male members in infertile marriages) and the comparison of the group means has yielded data which have been used to set up fertility 'standards,' in terms of the sperm concentration, motility, and morphology 'required' for conception. Extensive studies of this type have been carried out by Hotchkiss, Brunner & Grenley (1938), Harvey & Jackson (1945), MacLeod & Hotchkiss (1946), and

particularly by MacLeod (1951) and MacLeod & Gold (1951, 1952, 1956), and the data from many thousands of specimens have been reported. Estimates of the variation in semen characteristics among several specimens from the same donor were not made. Without an estimate of the variation among the specimens from each donor, no test of the significance of the differences in semen characteristics, either among donors or between fertile and infertile group means, is possible. The conditions of collection and methods of examination of the semen, as well as the definitions of male (and female) fertility and infertility, varied widely among the investigators. The result has been conflicting systems of classification in which varying levels of sperm concentration, motility and morphology have been cited as the minimum for normal male fertility. However, the investigators do agree that there are wide variations in semen characteristics among the fertility classes they have set up, among the men within each fertility class, and among the specimens from the same man. They also agree that many fertile men (fathers) have poor quality semen which falls well within the infertile classification, while many infertile men (childless men) have good quality semen which falls well within the fertile classification.

If we are to use the characteristics of the human semen specimen to estimate a man's fertility in the clinic or to study the physiological basis for semen production in the research laboratory, we must have data which will permit the quantitative assessment of each of the major factors (emission frequency, days to previous emission, age of the specimen, etc.) which affect semen characteristics. These data must be used to account for the influence of these inherent systemic and methodological variables so that the influence of the physiological variables (hormone status, tubule activity, etc.) may be measured.

The general plan of this investigation was to examine a number of semen specimens from each of a group of unselected donors, to determine the interrelationships among the semen characteristics, to compare the variability among specimens from the same donor and among the means of specimens by donors, to measure the factors affecting semen characteristics, and then to design studies so that these factors might be treated as the major fixed variables in planned experiments.

METHODS

The semen specimens used in this study were received from young white men who were students at this medical college. Their average age was 24·5 years, with a range of 21 to 31 years, at the time the study was begun. Specimens were received from each donor who agreed to bring them in on a regular twice-a-week schedule during the academic year (September to May) and to report all outside emissions during that time. No selection of donors or of specimens was made at any time during the course of these studies.

The specimens were collected at home by masturbation into clean dry sputum bottles. When the specimen was brought in, data on the time of collection and the dates of the last two or three emissions were recorded. The time elapsed from collection to examination ranged from 15 to 180 min and the mean values by donors are reported (Table 1). All specimens were already liquefied when received in the laboratory.

TABLE 1

MEANS OF SEMEN CHARACTERISTICS AND OF FACTORS AFFECTING THE SEMEN SPECIMEN (140 SPECIMENS)

Donor No.	*No. specimens*	*Emission frequency (per week)*	*Days to previous emission*	*Sperm conc. (10^6/ml)*	*Specimen volume (ml)*	*Sperm conc. (10^6/specimen)*	*Age of specimen (min)*	*Motility (%)*	*Rate of forward progression*	*Normal morphology (%)*
1	14	4·4	1·9	79·04	2·9	242·85	80	71	7·5	95
2	13	4·0	1·9	40·52	1·7	74·29	68	66	5·2	74
3	10	3·5	1·9	20·55	3·3	64·53	118	43	5·1	82
4	9	3·1	2·2	34·98	1·2	51·43	90	43	4·2	80
5	12	2·9	2·9	177·17	2·6	515·98	65	80	8·1	94
6	12	2·8	3·2	158·53	2·4	391·93	116	70	7·1	93
7	14	3·4	2·5	10·84	4·9	52·73	41	64	5·4	71
8	14	3·3	2·6	61·80	4·0	263·49	99	74	7·4	85
9	14	3·1	2·8	129·23	2·1	280·83	76	49	5·0	91
10	9	2·0	3·4	142·22	4·6	649·83	57	77	7·9	92
11	8	3·0	2·8	90·76	1·9	173·06	62	48	4·9	84
12	11	3·0	2.8	53·74	1·9	98·14	46	46	5·3	85
Mean* values		3·2	2·6	82·90	2·8	236·00	76	62	6·2	86

* Weighted means.

The methods used to determine semen characteristics followed closely the outline set up by the American Society for the Study of Sterility in the handbook, 'Evaluation of the Barren Marriage'. Specimen volume was measured to the nearest 0·1 ml in a recalibrated 5 ml graduate. A drop of well mixed raw semen was placed on a slide, covered with a cover slip, and placed in a slide warmer at 37° C. After 2 to 3 min, the semen was examined under both low ($\times 100$) and high ($\times 430$) power of the microscope. Motility was rated from 0% (no cells motile) to 100% (all cells motile) and estimated to the nearest 10%. Forward progression was rated from 0 (no forward progression) to 10 (all motile cells displaying vigorous forward progression) and every attempt was made to estimate forward progression only in terms of the progression of the motile cells, i.e. the non-motile cells were not considered in making this estimate. Sperm concentration was determined after dilution in a white blood cell pipette by counting in the haemocytometer. Semen smears were prepared, fixed in 10% formalin, and stained in Meyer's haematoxylin. Normal morphology was determined by counting 200 cells and was recorded to the nearest per cent.

Condom specimens were collected by the married donors during intercourse in condoms supplied through the courtesy of the Holland-Rantos Co, New York, and were brought into the laboratory the next morning. Motility and forward progression were not rated in these specimens.

Statistical analyses of the data followed the procedures outlined by Snedecor (1956).

RESULTS

VARIATION AMONG DONORS IN SEMEN CHARACTERISTICS

A basic assumption that is implicit in most of the clinical work with human semen is that there are significant 'among-donor' differences in semen characteristics, i.e. that there is less variability in semen characteristics among repeated specimens from the same donor than there is among specimens from different donors. Accordingly, a study was designed to test for the significance of the 'among-donors' differences in semen characteristics and 140 specimens were received from twelve donors (Table 1). In making such a comparison, one must consider the effects of frequency of emission and of age of the specimen on semen characteristics, since if the donors had markedly different frequencies of emission or if the time from collection to examination of the specimen varied widely among donors, these factors would have to be accounted for before direct comparisons could be made.

Inspection of the mean values for emission frequency and for days to previous emission (Table 1) indicates that there was a relatively small amount of variability in these donor means. Both of these figures include the two specimens which were brought in each week for the study. If one subtracts 2·0 (the two specimens collected each week for the study) from each figure in the emission-frequency column, it is apparent that, with the exception of Donor 10 who had no emissions outside of the specimens collected for this study, the frequency of emissions outside the study ranged only from 0·8 to 2·4 per week. Reference to the analysis of the data on days to previous emission (Table 2) indicates that, although there was a significant difference among donors, 91% of the total

variance was associated with the changes within donor in the emission frequency outside the study since the two specimens were brought in regularly during each week of the study and were constant for each donor. Therefore, the variance among donors in terms of days to previous emission was apparently a relatively minor factor in the differences among donors in this study.

Further examination of the influence of number of days to previous emission on semen characteristics may be made by correlation analysis (Table 3). Although there is an apparent correlation between days to previous emission and sperm concentration per millilitre of semen on total and among-donors

TABLE 2

ANALYSIS OF VARIANCE OF AND ESTIMATED VARIANCES FOR SEMEN CHARACTERISTICS AND FACTORS AFFECTING THE SEMEN SPECIMEN

Semen characteristics	*Source of variation*	*d.f.*	*Mean square*	*Variance*‡	*Variance* (%)
Days to previous emission	Among donors	11	2·8189*	0·1285	9
	Specimens within donor	128	1·3245	1·3245	91
	Total	139		1·4530	100
Sperm concentration ($\times 10^6$/ml)	Among donors	11	37,172†	3098	73
	Specimens within donor	128	1139	1139	27
	Total	139		4237	100
Specimen volume	Among donors	11	15·9074†	1·3678	68
	Specimens within donor	128	0·6575	0·6575	32
	Total	139		2·0253	100
Sperm concentration ($\times 10^6$/specimen)	Among donors	11	405,881†	32,172	50
	Specimens within donor	128	31,722	31,722	50
	Total	139		63,894	100
Age of specimen	Among donors	11	7359†	518	28
	Specimens within donor	128	1340	1340	72
	Total	139		1858	100
Motility	Among donors	11	2175†	175	56
	Specimens within donor	128	140	140	44
	Total	139		315	100
Rate of forward progression	Among donors	11	21·4153†	1·6598	44
	Specimens within donor	128	2·1116	2·1116	56
	Total	139		3·7714	100
Normal morphology	Among donors	11	756†	61	59
	Specimens within donor	128	42	42	41
	Total	139		103	100

* Statistically significant ($P<0·05$).
† Statistically significant ($P<0·01$).
‡ Parameters estimated: Among donors, $\sigma^2 - 11·63\sigma^2$.
Specimens within donor, σ^2.

bases, there is no correlation on a within-donor basis (Table 3). Reference to the mean data (Table 1) supports this absence of within-donor or 'true' correlation, since it is evident that the four donors (Nos. 5, 6, 9, 10) with the highest mean sperm concentrations had values for days to previous emission which were well above the mean of the study (2·6 days) while the four donors (Nos. 2, 3, 4, 7) with the lowest mean sperm concentrations had values for days to previous emission which were well below the mean of the study. Thus the value of this type of correlation analysis is evident, since there was no demonstrable effect of frequency on sperm concentration within the range of 1·9 to 3·4 days to previous emission (an emission frequency of 2·0 to 4·4 times per week).

However, there was plainly an effect of number of days to previous emission on semen-specimen volume (Table 3). The significant correlation on a within-donor basis (r = 0·29) indicates that even in the limited range encountered in this study (1·9 to 3·4 days to previous emission), there was an increase in specimen volume with increase in days to previous emission. Furthermore, within the range of emission frequency encountered in this study, no relation-

TABLE 3

CORRELATIONS AMONG VARIOUS SEMEN CHARACTERISTICS AND FACTORS AFFECTING THE SEMEN SPECIMEN

Correlated factors	*Coefficients of correlation and d.f.*		
	Total	*Among donors*	*Specimens within donor*
d.f.	138	10	127
No. days to previous emission and:			
Sperm concentration per ml semen	0·28†	0·70*	0·09
Specimen volume	0·21*	0·17	0·29†
Sperm concentration per specimen	0·34†	0·71†	0·22*
Motility (%)	0·07	0·24	−0·01
Rate of forward progression	0·14	0·36	0·07
Normal morphology (%)	0·19*	0·43	0·10
Sperm concentration per ml semen and:			
Specimen volume	0·00	−0·16	0·38†
Motility (%)	0·30†	0·42	0·05
Rate of forward progression	0·38†	0·58*	0·11
Normal morphology (%)	0·54†	0·83†	−0·05
Sperm concentration per specimen and:			
Motility (%)	0·34†	0·64*	−0·02
Rate of forward progression	0·42†	0·79†	0·07
Normal morphology (%)	0·42†	0·76†	−0·02
Age of specimen and:			
Motility (%)	−0·08	−0·03	−0·12
Rate of forward progression	−0·08	0·13	−0·22*
Normal morphology (%)	0·18*	0·32	0·07
Motility (%) and:			
Rate of forward progression	0·76†	0·88†	0·63†
Normal morphology (%)	0·20*	0·32	0·02

* Statistically significant ($P<0·05$).
† Statistically significant ($P<0·01$).

ship between days to previous emission and motility, forward progression, or morphology was demonstrated.

Inspection of the mean values by donors for age of the specimen (Table 1) indicates that there was an appreciable difference in the mean number of minutes which elapsed between collection of the specimen by the different donors and its examination in the laboratory. These data are of special interest since it is not at all uncommon to receive clinical specimens 2 hr and more from the time of collection. An analysis of the data on age of the specimen (Table 2)

demonstrates that the greatest part (72%) of the variance associated with this factor was found among the repeated specimens from the same donor. This indicates that although, as was to be expected, there was a significant difference among donors in mean age of the specimen (Table 2), only 28% of the total variance of this factor is associated with among-donors differences in this study.

Age of the specimen, i.e. the time elapsed from collection to examination, within the range encountered in this study (Table 1), had no significant effect on motility, forward progression, or morphology (Table 3).

As might have been expected with a group of unselected donors, wide variations were apparent in the donor means for specimen volume and sperm concentration, motility, progression and morphology (Table 1). The analysis of variance (Table 2) demonstrates the significance of these 'among-donors' differences. These data provide an experimental and statistical basis for the clinical practice of estimating an individual's semen production and of assessing fertility status on the basis of the examination of one or two semen specimens.

It is of interest to note (Table 1) that the data confirm the common clinical observation that donors of good quality specimens are usually high in three semen characteristics (sperm concentration, motility and morphology), while donors of specimens of poor quality are usually low in all three semen characteristics. On total and among-donors bases, there was a significant degree of correlation among sperm concentration and motility, progression and morphology (Table 3). However, there was no correlation among these characteristics on a specimens-within-donor basis. This indicates that the tendency of semen characteristics to be either all high or all low reflects the influence of the donor since this relationship does not exist among the specimens from the same donor. Furthermore, it is evident that per cent motility and rate of forward progression are so closely related ($r = 0{\cdot}63$, Table 3) that, for all practical purposes, they are estimates of the same thing.

Examination of the analysis of estimated variances for semen characteristics (Table 2) reveals that a very large part of the total variance was associated with the percentage variance for specimens-within-donor in the cases of motility (44%), forward progression (56%), and morphology (41%). The specimens-within-donor variance term includes the error associated with the method of estimating these values, and the rating of motility or of forward progression remains a subjective process based on an informed estimate by the investigator. Nevertheless, in a further analysis of these data (Table 4), it becomes apparent that most of the specimens-within-donor variance for motility and forward progression was due to the high degree of variability in these characteristics among repeated specimens from the same donor. At least seven of the donors in this study (Nos, 3, 4, 6, 7, 9, 11 and 12) had motility ratings that ranged from very poor to very good, and at least five of the donors (Nos. 2, 3, 4, 9, 11) had forward progression ratings which covered almost the entire range of values (Table 4). The great variability in morphology (Table 4) among repeated specimens from the same donor was unexpected and cannot be ascribed to the technique of rating. Although there are marked differences of opinion among the workers in the field as to what constitutes normal morphology, this should not affect the morphology ratings within a single study by a single investigator.

These data do not support the concept (MacLeod & Gold, 1951) that morphology is one of the more stable of semen characteristics.

EFFECT OF FREQUENCY OF EMISSION ON SEMEN CHARACTERISTICS

In view of the unexpected finding in the previous study that there was no demonstrable effect of number of days to previous emission (within the range 1·9 to 3·4 days) or of emission frequency (within the range 2·0 to 4·4 emissions per week) on sperm concentration, motility or morphology, a study was set up to directly vary the emission frequency and to examine the effect on semen characteristics.

The effect of changing from a 'low' frequency of 3·5 emissions per week to a 'high' frequency of 8·6 emissions per week, was striking (Table 5). It is of interest to note that the outside emission frequency, exclusive of the specimens for the study, did not change, i.e. the outside emission frequency was 1·5 (3·5 — 2·0) at

TABLE 4

MEANS OF AND RANGES FOR SELECTED SEMEN CHARACTERISTICS

Donor No.	*Motility* (%)		*Rate of forward progression*		*Normal morphology* (%)	
	Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*
1	71	50 to 90	7·5	5 to 9	95	88 to 98
2	66	50 to 90	5·2	3 to 8	74	64 to 84
3	43	30 to 60	5·1	2 to 8	82	72 to 96
4	43	10 to 60	4·2	1 to 7	80	68 to 94
5	80	60 to 90	8·1	7 to 9	94	88 to 98
6	70	40 to 90	7·1	5 to 9	93	87 to 98
7	64	40 to 80	5·4	4 to 7	71	52 to 87
8	74	60 to 90	7·4	6 to 9	85	71 to 97
9	49	30 to 70	5·0	2 to 8	91	78 to 97
10	77	70 to 80	7·9	7 to 9	92	84 to 96
11	48	30 to 70	4·9	2 to 7	84	64 to 95
12	46	30 to 60	5·3	3 to 7	85	71 to 96

the low frequency and 1·6 (8·6 — 7·0) at the high frequency, so that this did not affect the results.

Increase in emission frequency resulted in a marked and uniform decrease (Table 5) in sperm concentration (−55%), specimen volume (−33%) and motility (−15%). The increase in emission frequency had no effect on morphology and no significant effect on forward progression (Table 5).

In a further analysis of this study, the total number of spermatozoa per week and the number of motile spermatozoa per week, were compared at low (3·5 per week) and high (8·6 per week) emission frequencies (Table 6). There was a marked and uniform decrease in both total number of spermatozoa (−31%) and number of motile spermatozoa (−41%). Apparently, the high frequency of emission caused a depletion of sperm reserves with the result that the number of spermatozoa ejaculated in each specimen was reduced to such a degree (−72%, Table 5) that the total number of spermatozoa per week declined even though

TABLE 5

EFFECT OF LOW AND HIGH FREQUENCIES OF EMISSION ON SEMEN CHARACTERISTICS

Donor No.	*No. specimens*		*Emission frequency (per week)*		*Sperm conc. (10^6/ml)*		*Specimen volume (ml)*		*Sperm conc. (10^6/specimen)*		*Motility (%)*		*Rate of forward progression*		*Normal morphology (%)*	
	Low	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*
1	14	22	4·4	9·0	79·04	49·09	2·90	2·12	242·85	106·56	71·4	66·4	7·5	8·1	94·6	91·1
2	13	22	4·0	7·0	40·52	13·06	1·74	1·55	74·28	19·84	66·2	44·6	5·2	3·8	74·5	83·6
3	10	22	3·5	9·0	20·55	3·77	3·26	2·53	64·53	8·73	43·0	37·9	5·1	3·3	81·8	82·6
4	9	22	3·1	8·3	34·98	14·34	1·21	0·97	51·43	14·64	43·3	47·9	4·2	4·6	80·4	81·0
5	12	22	2·9	7·0	177·17	94·44	2·60	1·55	515·98	147·67	80·0	75·0	8·1	8·1	94·3	93·0
6	12	22	2·8	9·3	158·53	58·82	2·42	1·82	391·93	109·62	70·0	63·6	7·1	7·1	93·2	90·3
7	14	22	3·4	10·0	10·84	1·93	4·90	3·10	52·73	6·42	63·6	50·7	5·4	4·4	71·1	81·6
8	14	22	3·3	9·3	61·80	23·03	4·01	2·50	263·49	59·42	73·6	62·1	7·4	6·7	85·1	85·4
Mean* values			3·5	8·6	73·46	32·51	2·98	2·00	212·20	59·98	65·3	55·5	6·4	5·7	84·4	85·1
Mean % change						−55		−33		−72		−15		−11		0

*Weighted means.

emission frequency was more than doubled. This analysis is supported by a comparison of the mean of the total number of spermatozoa ejaculated per week during the whole of the high emission frequency period, $515{\cdot}83 \times 10^6$ (Table 6), with the 3-weekly means for total number of spermatozoa ejaculated per week — 1st week, $547{\cdot}80 \times 10^6$; 2nd week, $484{\cdot}78 \times 10^6$; 3rd week, $437{\cdot}49 \times 10^6$. There was an evident decline in total number of spermatozoa per week with time at the high emission frequency, which probably reflected depletion of the sperm reserves.

The increase in emission frequency resulted in a decrease in individual specimen volume (−33%, Table 5), but in an increase in the total volume of semen per week (+39%, Table 6).

TABLE 6

EFFECT OF LOW AND HIGH FREQUENCIES OF EMISSION ON SEMEN PRODUCTION

Donor No.	*Emission frequency per week*		*Total No. sperms* (10^6/*week*)		*No. motile sperms* (10^6/*week*)		*Volume of semen* (*ml*/*week*)	
	Low	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*
1	4·4	9·0	1068·54	959·04	762·94	636·80	12·76	19·08
2	4·0	7·0	297·12	138·80	196·69	61·90	6·96	10·85
3	3·5	9·0	225·86	78·57	97·12	29·78	11·41	22·77
4	3·1	8·3	159·43	121·51	69·03	58·20	3·75	8·05
5	2·9	7·0	1496·34	1033·69	1197·07	775·27	7·54	10·85
6	2·8	9·3	1097·40	1019·47	768·18	648·38	6·78	16·93
7	3·4	10·0	179·28	64·20	114·02	32·55	16·66	31·00
8	3·3	9·3	869·52	552·61	639·96	343·17	13·23	23·25
Mean* values	3·5	8·6	742·70	515·83	484·98	286·28	10·43	17·20
Mean % change				−31		−41		+39

* Weighted means.

EFFECT OF TIME FROM COLLECTION ON SEMEN CHARACTERISTICS

A study was set up to examine the effect of time from collection on those semen characteristics which might change with time (motility, forward progression and morphology). Thirty semen specimens were received from nine donors and each specimen was received within an hour of collection. The specimens were kept at room temperature for 9 hr and the motility, progression, and morphology of each specimen was rated at hourly intervals. The data indicated that motility and forward progression declined at a linear rate with time under the conditions of this study (Table 7). No effect of time from collection on morphology was demonstrated in this study.

The linear decline in motility with time, over a period of 9 hr, may be represented graphically (Text-fig. 1). For the sake of clarity, only the mean values for the study, the mean values for the two donors (A and B) with the best motility, and the mean values for the two donors (C and D) with the poorest motility, have been included in the graph. The other five donors in

this study were intermediate in terms of quality of motility and displayed the same type of linear decline. These slopes (Text-fig. 1) are quite linear in nature although there is some suggestion of a levelling off after the 8th hr.

The linear decline in rate of forward progression with time, over a period of 9 hr, has been similarly graphed (Text-fig. 2). The slopes for decline in the rate of forward progression are quite linear in nature and are very similar to the slopes for motility. This confirms the previous observation that per cent motility and rate of forward progression are so closely related ($r = 0{\cdot}63$, Table 3), that, for all practical purposes, they are estimates of the same thing.

EFFECT OF SEMINAL PLASMA ON SPERM MOTILITY

The large degree of variability among donor means for motility and forward progression was apparent in this study (Table 1), and has been previously noted by other investigators (Hotchkiss, 1941; MacLeod & Heim, 1945; MacLeod &

TABLE 7

EFFECT OF TIME FROM COLLECTION ON VARIOUS SEMEN CHARACTERISTICS*

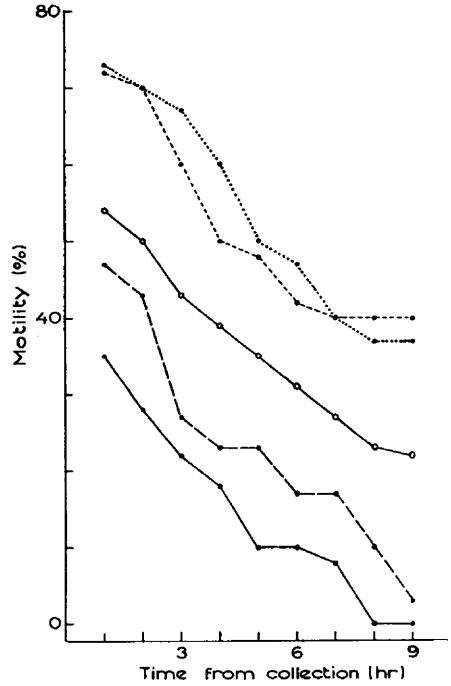
Time from collection (hr)	*Motility* (%)	*Rate of forward progression*	*Normal morphology* (%)
1	54	5·4	87
2	50	5·1	84
3	43	4·4	85
4	39	3·9	84
5	35	3·4	83
6	31	2·8	83
7	27	2·3	83
8	23	1·9	84
9	22	1·8	84

* Thirty specimens from nine donors.

Gold, 1956). However, this study has also demonstrated that the variability among the motility and forward progression ratings in repeated specimens from the same donor (Table 4) makes up a large percentage of the total variance associated with these terms (Table 2). The influence of the seminal plasma on sperm motility has not been definitively studied, although MacLeod & Freund (1958) found no effect of the seminal plasma on sperm motility in a series of seminal plasma reversal experiments. Recently, Rozin (1960) has suggested that the seminal plasma may play a role in sperm motility and has reported that when spermatozoa from oligospermic specimens with low motility were suspended in seminal plasma from normal specimens, there was a marked and sustained increase in motile activity.

The availability of repeated specimens from donors with a known semen quality made possible a study in which two donors (F and J) of semen with good motility and two donors (L and A) of semen with poor motility were requested to bring in specimens on each of 4 days. Each donor's spermatozoa were

separated from the seminal plasma by centrifugation and suspended in the donor's seminal plasma and in each of the seminal plasmas of the other three donors. The initial centrifugation was at 600 *g* for 10 min in order to pack the sperm cells in each specimen without damaging them. The seminal plasma in each specimen was decanted off and centrifuged at 1500 *g* for 30 min in order to remove all remaining sperm cells. Four trials were made on different days and each donor's cells were suspended in all four seminal plasmas during each trial, in a complete factorial seminal plasma reversal design (Table 8). No effect of seminal plasma on sperm motility or on rate of forward progression



TEXT-FIG. 1. *Effect of time from collection on the motility of spermatozoa.* (Mean values from Table 7.)

· . . . . . · Donor A (four specimens)
· – – – – – – · Donor B (three specimens)
○———————○ Mean of thirty specimens
·—— —— ——· Donor C (three specimens)
·———————· Donor D (four specimens)

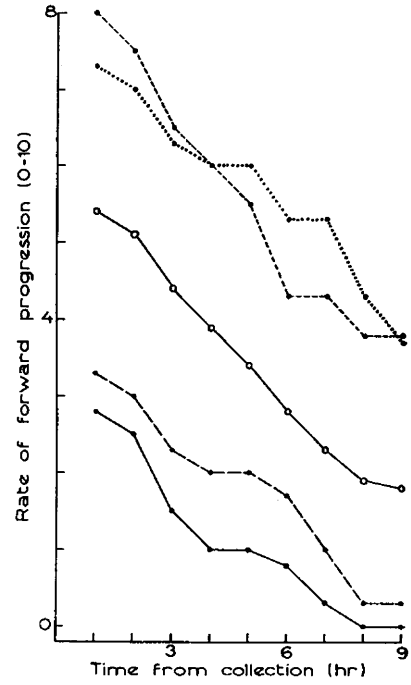
was apparent either immediately after reversal or after 3 hr of incubation at 25° C.

COMPARISON OF SEMEN CHARACTERISTICS FROM SEMEN SPECIMENS PRODUCED BY MASTURBATION OR AT INTERCOURSE

An assumption that is implicit in the accepted clinical procedure for assessing male fertility (the examination of a semen specimen which has been collected by masturbation) is that the masturbated specimen is representative of the specimen produced at intercourse. There are, however, no published data to

support this assumption. Hotchkiss *et al.* (1938) reported differences between the condom specimen and the masturbated specimen, but based the work on single specimens from 200 men, with each of thirty-three men submitting one condom specimen and each of 167 different men submitting one masturbated specimen.

The opportunity arose to examine the regular twice-a-week specimens collected by masturbation and also to receive and examine the condom specimens collected at intercourse, during the same period of time, by five of the married



TEXT-FIG. 2. *Effect of time from collection on the forward progression of spermatozoa.* (Mean values from Table 7.)

Donor A (four specimens)
Donor B (three specimens)
Mean of thirty specimens
Donor C (three specimens)
Donor D (four specimens)

donors in this series. Since the condom specimens were collected at home on the preceding evening, it was not possible to rate them for motility and forward progression.

There were no marked or uniform differences apparent among the means for semen characteristics from specimens collected by masturbation or at intercourse, although some large individual donor variations were evident (Table 9). Certainly in the case of Donor 2 (Table 9), examination of the masturbated specimen did not give an accurate estimate of the specimen produced at intercourse.

TABLE 8

EFFECT OF SEMINAL PLASMA ON SPERM MOTILITY AND FORWARD PROGRESSION

Sperm donor	*F plasma*				*J plasma*				*L plasma*				*A plasma*			
	Treatment	*Motility and progression*			Treatment	*Motility and progression*			Treatment	*Motility and progression*			Treatment	*Motility and progression*		
		Initial		3-*hr*		*Initial*		3-*hr*		*Initial*		3-*hr*		*Initial*		3-*hr*
F	F/F*	52	6·0†	38 3·5	F/J	50	5·2	40 3·5	F/L	52	5·5	38 3·2	F/A	50	6·0	38 3·8
J	J/F	65	7·5	48 6·2	J/J	68	7·8	52 6·0	J/L	65	7·5	50 5·8	J/A	65	6·8	52 5·2
L	L/F	18	2·2	12 1·5	L/J	20	2·2	12 1·2	L/L	18	2·0	12 1·0	L/A	18	1·8	12 1·0
A	A/F	32	3·8	22 1·8	A/J	35	3·5	22 2·0	A/L	32	2·8	20 1·2	A/A	32	2·8	20 1·8

* The numerator identifies the sperm donor and the denominator the seminal plasma donor F/F = F spermatozoa suspended in F seminal plasma and F/J = F spermatozoa suspended in J plasma.

† Motility (%) and rate of forward progression — each value is the mean of four trials made on different days.

DISCUSSION

Under the conditions of this study, there was no demonstrable effect of frequency of emission on sperm concentration, motility, forward progression, or morphology, although there was an increase in semen specimen volume with increase in days to previous emission (Table 3). The donors had a mean frequency of 3·2 emissions per week (range, 2·0 to 4·4 emissions), of which two emissions were collected as specimens for the study, and a mean of 2·6 days to previous emission (range, 1·9 to 3·4 days). Evidently, within this range of frequency of emission, the spermatozoa are replaced about as rapidly as they are ejaculated and no effects of variation in frequency within this range can be demonstrated. MacLeod & Heim (1945) and MacLeod & Gold (1952) have reported that periods of continence of 6 and 10 days in men producing good quality semen resulted in an increase in the number of spermatozoa per millilitre and per specimen and in a decrease in motility.

TABLE 9

COMPARISON OF SEMEN CHARACTERISTICS FROM SPECIMENS COLLECTED BY MASTURBATION OR AT INTERCOURSE

Donor No.	*No. specimens*		*Sperm conc.* (10^6/*ml*)		*Specimen volume* (*ml*)		*Sperm conc.* (10^6/*specimen*)		*Normal morphology*	
	*M**	*C*†	*M*	*C*	*M*	*C*	*M*	*C*	*M*	*C*
1	3	3	125·10	133·00	1·77	1·23	262·84	167·44	65	77
2	5	5	38·52	68·76	1·68	2·00	87·42	139·16	61	66
3	4	4	15·82	8·39	2·28	1·65	38·19	14·11	19	13
4	5	5	72·44	75·77	1·40	1·10	105·98	85·08	59	67
5	5	5	6·15	8·37	3·80	4·28	22·55	36·34	15	21
Mean‡ values			46·55	54·41	2·22	2·14	91·86	84·62	43	48

* Specimens collected by masturbation.
† Specimens collected in a condom at intercourse.
‡ Weighted means.

Increase in emission frequency from 3·5 per week to 8·6 per week resulted in a marked and uniform decrease in sperm concentration per millilitre and per specimen and in specimen volume. The total number of spermatozoa per week declined at the higher frequency of emission, while the total volume of semen per week increased (Table 6). Thus, the situation in man is different from that in the bull where Dukelow, Frederick & Graham (1960) found in long-term studies with monozygotic twin and triplet bulls that increasing the collection frequency from two ejaculates per week to seven ejaculates per week resulted in a marked increase in total number of spermatozoa so that 2·1 times as many spermatozoa were ejaculated per week at the higher frequency as at the lower frequency. The increase in total number of spermatozoa per week occurred in the bull because sperm concentration per millilitre did not decline with increase in emission frequency as it did in this study in man. Volume of semen per ejaculate decreased and volume of semen per week increased with increase in frequency of ejaculation in the bull study, just as it did in this study in man.

Evidently, in both man and bull, the accessory organs of reproduction can markedly increase their rate of secretion in response to more frequent ejaculation. The result, in both man and bull, is a marked increase in the volume produced per week (bull, 4·61 ml increased to 10·51 ml and man, 10·43 ml increased to 17·20 ml). At a high frequency of emission (seven ejaculates per week), the bull can apparently maintain a constant level of sperm reserves so that there is no decline in sperm concentration per millilitre and, therefore, there is an increase in total spermatozoa per week. However, at a high frequency of emission (8·6 emissions per week), man cannot maintain sperm reserves so that there is a marked decline in sperm concentration per millilitre and, therefore, a decrease in total number of spermatozoa per week. These data suggest that in man the accessory glands can replace the volume of seminal plasma emitted in the ejaculate in somewhat more than 24 hr, while about 2 to 3 days are required for the testes to completely replace that part of the sperm reserve lost during ejaculation. Thus, daily (or more frequent) ejaculation in man results in a steady depletion of sperm reserves.

The effect of time from collection of the semen specimen to its examination in the laboratory is of great importance and must be taken into account in the estimation of sperm motility and forward progression. This has been well demonstrated in this study (Table 7) by examining each of thirty semen specimens every hour for 9 hr. The outstanding characteristic of the decline in motility and forward progression with time is the linearity of the mean and individual slopes (Text-figs. 1 and 2). This suggests that reasonable estimates of initial motility might be made from 4 to 5 hr-old specimens by estimating three points on the slope, e.g. motility ratings, at 4, 5 and 6 hr after emission, and by extrapolating back up the slope to the origin. If data could be collected on the rate of decline in motility and in forward progression from a large number of repeated specimens from fertile and infertile men, a nomograph could be constructed that would permit extrapolation from per cent motility at any time up to 8 hr after emission to per cent motility at 'zero time' (immediately after emission).

Data have been collected and analyses made in this study (Tables 1, 2 and 3) to provide an experimental basis for the clinical assumption that a single specimen is representative of a man's semen production. This is the case when the specimen is received and examined under controlled conditions of emission frequency, days to previous emission, and age of the specimen. It has been shown, however, that a very large part of the variance for the motility, forward progression, and morphology ratings is associated with the variability among repeated specimens from the same donor (Table 2), and that motility and forward progression ratings on repeated specimens from several of the donors in this study covered almost the entire range of values (Table 4).

The clinical observation that three semen characteristics, sperm concentration, motility and morphology, are usually all high in semen specimens from some donors and all low in semen specimens from other donors, has been confirmed by demonstrating significant correlation among these three characteristics on an among-donors basis (Table 3). This was shown to be true only among donors since no such correlation exists among these three characteristics in

repeated specimens from the same donor. In view of this high degree of correlation among the three semen characteristics and the evidence that this is due to donor differences, one must question the use of fertility indices based on the multiplication of the ratings for the three semen characteristics (MacLeod, 1951). There would seem to be no physiological rationale for this practice and the net result of comparing the product derived from multiplying three significantly correlated high ratings with the product derived from multiplying three significantly correlated low ratings would be the creation of a larger mathematical difference between them. The construction of fertility indices is complicated by the fact that we do not know whether each of the three characteristics, sperm concentration, motility and morphology, is directly related to fertility in a cause and effect relationship or whether only one of the three characteristics is directly related to fertility and the others are only correlated with it and bear no direct relationship to fertility. Finally, there is no definitive evidence that any of the commonly measured semen characteristics is directly related to fertility in a cause and effect relationship.

It is suggested that a useful approach to the problem of relating semen characteristics to each other and to fertility would be the use of multiple correlation technique to assess the direct relationship of each semen characteristic to fertility and the use of analysis of covariance to determine the significance of the relationships.

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