TECHNICAL METHODS

A METHOD OF CONCENTRATING SPERMATOZOA IN HUMAN SEMEN

BY

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When carrying out artificial insemination it is inconvenient to use more than 1 ml. of semen in most cases. When the husband's semen is oligospermic, or has a low percentage of motile sperm, it is an advantage if a high proportion of the sperm content can be concentrated in a small volume of the fluid. Even when the number and motility of the spermatozoa are satisfactory, if some hours must elapse between ejaculation and insemination the viability of the sperm may be improved by the addition of suitable diluents, as is the usual practice in animal insemination. In human semen, however, the improved motility due to dilution is seldom enough to compensate for the reduction in density, unless the sperm are subsequently concentrated by centrifuging or some other means. The centrifugation of human semen, while it concentrates the sperm, frequently depresses the motility, inevitably concentrates also debris and mucus, and, in the case of very mucoid specimens, may be quite useless at speeds which do not injure the active cells. A much easier method of obtaining a high proportion of the sperm content of the ejaculate in a small volume is to ask the donor to collect the first part of the ejaculate in a separate container from the remainder.

The higher sperm content and greater motility in the first part of partitioned ejaculates have been reported by MacLeod and Hotchkiss (1942) and more recently by Oettle (1954). Lundquist (1949) has investigated the biochemistry of the different fractions, and we are employing his results in a study of the pattern of ejaculation and the activity of the accessory glands in husbands of sub-fertile marriages. However, the collection of semen in separate fractions has proved so useful for A.I.H. (homologous artificial insemination) that it seemed worth while to give a preliminary report on this application of the work.

The following report is based on 93 specimens from 21 donors, 15 of whom were sub-fertile with

a "fertility index" (Harvey, 1953) of less than 30, and six having potentially fertile semen. In each case the density and motility of the spermatozoa in both fractions of the ejaculate were determined by the usual methods (Harvey and Jackson, 1945) and the corresponding values which would have been found had the whole specimen been collected in one container were calculated. In what follows statements about the whole specimen refer to these calculated values.

Method

The relative concentration of sperm in the first fraction is conveniently measured by the ratio of the density of this fraction to that of the whole ejaculate. Thus a semen having a sperm density of 10m./ml. in the complete ejaculate but of 35m./ml. in the first fraction would give a ratio of 3.5, which means that it would have been necessary to use 3.5 ml. of the undivided ejaculate for insemination in order to introduce as many sperm as are present in 1 ml. of the first fraction. If the volume of the first fraction is less than 1 ml. the total number of sperm in the first fraction must be substituted for its density. For instance, if in the above example only 0.6 ml. of fluid was collected in the first container, the total sperm content of this fraction would be $0.6 \times 35m.$, and only 21m. sperm would be introduced into the cervix if the whole of the 0.6 ml. was used for insemination. For practical purposes therefore the

TABLE I
FREQUENCY OF VARIOUS DEGREES OF CONCENTRATION OF SPERM IN FIRST FRACTION

	No. of Ejaculates	Ratio of Sperm in 1 ml. of First Fraction of Ejaculate (or Whole Fraction if Less than 1 ml.) to Sperm in 1 ml. of Complete Ejaculate				Highest Value
		0.8 First Fraction Inferior	0.8-1.2 No Significant Difference	1.21-1.99 Fair Concentration	2.0 and Over Good Concentration	
All sperm	93	8 (8.6%)	16 (17.2%)	48 (51.6%)	21 (22.6%)	3.9
Motile sperm	87	3 (3.5%)	4 (4.6%)	41 (47.1%)	39 (44.8%)	4.6

FIG. 1.—Relationship of concentration of sperm to proportion of ejaculate collected in first fraction.

improvement in this case, compared with 1 ml. of the complete ejaculate, would be only 2.1, not 3.5.

The density of motile sperm in the first fraction may be compared with the number in the whole ejaculate in the same way, the actual number of motile sperm in 1 ml. of fluid being of more interest than the percentage motility.

The sperm content of the first fraction may be regarded as significantly higher than that of the whole specimen if the ratio obtained in this way is greater than 1.2, and significantly less if the ratio is less than 0.8. The degree of concentration obtained in this series is shown in Table I.

Table II and Fig. 1 show the variation in the mean value of the "first fraction/whole specimen" ratio with different proportions of the ejaculate in the first fraction. The superior motility of the earlier part of the ejaculate, which is one important reason for using this fraction for insemination, is clearly shown in the graph.

Differences in the efficiency of concentration occur with different donors, and with the same donor on different occasions. It might be expected that men

TABLE II
RELATION OF SPERM CONCENTRATION IN FIRST FRACTION TO PROPORTION OF EJACULATE COLLECTED

% of Ejaculate Collected in First Fraction	Mean Volume of First Fraction (ml.)	Concentration of Sperm in First Fraction			
		All Sperm		Motile Sperm	
		No. of Ejaculates	Mean Relative Concentration	Ejaculates	Mean Relative Concentration
0-9	0.35	2	0.42	2	0.58
10-19	0.52	5	1.52	5	2.36
20-29	1.14	18	1.63	17	2.34
30-39	1.47	15	1.89	14	2.45
40-49	1.48	17	1.98	15	2.15
50-59	2.45	18	1.51	17	1.7
60-69	2.01	9	1.35	9	1.54
70-79	2.31	8	1.23	7	1.35
80-89	—	—	—	—	—
90-99	4.5	1	1.07	1	1.12

usually producing exceptionally large volumes of semen would give the best results; this is not necessarily the case. As shown in Table III, semen with a total volume of 3 to 4 ml. may concentrate as well as specimens with larger volumes. It is obviously worth while asking an A.I.H. donor to collect his semen in two parts if the volume of his complete ejaculate is usually over 2 ml.

It does not necessarily follow that the chances of conception following insemination are appreciably improved by using the first fraction of the ejaculate. Where the total sperm count of the whole ejaculate is only 1 or 2 million, or where the motility is very low, putting all the sperm available into 1 ml. of fluid does not greatly increase the chance of success. If two

TABLE III
RELATION OF CONCENTRATION OF SPERM IN FIRST FRACTION TO VOLUME OF EJACULATE

Vol. of Whole Ejaculate (ml.)	Concentration of Sperm in First Fraction			
	All Sperm		Motile Sperm	
	Ejaculates	Mean Relative Concentration	Ejaculates	Mean Relative Concentration
1-1.9	7	1.3	7	1.43
2-2.9	26	1.55	23	1.9
3-3.9	21	1.72	20	2.09
4-4.9	13	1.6	12	2.05
5-5.9	7	1.85	7	2.35
6-6.9	6	1.52	6	2.04
7-7.9	7	1.28	7	2.2
8-9.9	6	2.04	6	2.5

"fertility indices" are calculated for each divided ejaculate, one based on the first fraction and the other on the complete specimen, in some cases the former is appreciably higher than the latter, while in other cases there is very little difference between the two. Successes have so far occurred only with semen that has given a higher value for the "fertility index" when based on the first fraction, but it is too early to say whether such a difference in the index is a reliable criterion of the usefulness of dividing the ejaculate in the case of any given donor. There is, however, nothing to be lost by collecting semen in this way. Pregnancies resulting from use of the first fraction have shown that the absence of the plasma of the second part of the ejaculate does not impair the fertility of the sperm in the first part. In the rare cases where the first fraction is inferior, or insufficient in amount, the second fraction will be similar to the undivided ejaculate, and may be used for insemination instead of, or mixed with, the first fraction.

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