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COMPLETE TITLE - Efficacy of Coulter Counter in Determining Low Sperm Concentrations

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One of the most useful parameters in the evaluation of seminal fluid is sperm concentration. Conventionally, this measurement has been performed by the hemocytometer method. However, there is considerable variation in the results obtained, whether applied to the counting of red blood cells[6] or sperm[1].

Several investigators have described the estimation of sperm concentration by means of the Coulter counter[2] [5] [7]. In the two reports which listed individual sperm counts the correlation between the results obtained by the Coulter counter and the hemocytometer methods was rather gross[5] [7]. By modifying the diluent as well as the technique for localizing the sperm population, we achieved a closer correlation between the two procedures[3].

In our earlier report, the ability of the Coulter counter to count oligospermic seminal fluid and the extinction point for this procedure were not adequately delineated. The present study compares the results of the two procedures when the sperm concentration is less than 10 million/ml. of seminal fluid. Furthermore, the lower limit for the electronic method is defined. While this report is an extension of our previous study, different seminal fluid specimens were used for each investigation.

Material and Methods

Seminal fluid specimens were collected from inmate volunteers at the Washington State Penitentiary as part of a study pertaining to the effects of radiation upon spermatogenesis. One hundred and ninety-four consecutive seminal fluid specimens with sperm concentrations of less than 10 million/ml. of seminal fluid were counted by both the electronic and hemocytometer methods.

Two tenths milliliter of well-mixed seminal fluid was diluted (1:500) with 100 ml. of a mixture of 10 ml. of 1% saponin in isotonic saline, 85 ml. of isotonic saline, and 5 ml. of glacial acetic acid. Counts were performed 60± 15 minutes after dilution.

A model B Coulter counter with a 50μ aperture tube and a 50λ mercury manometer was used. The settings for the aperture current, amplification, and internal gain trim were kept constant at 1/2, 1 and 65, respectively.

A size distribution pattern of particles was obtained by a plotter graph inserted into this system. The plotter measures the concentration of particles of progressively increasing size. With respect to seminal fluid specimens, the sperm population is usually observed separate from another population of smaller particles representing debris. The lowest point on the graph between these two curves, termed the "trough", is considered to be the lower size border of the sperm population[3]. The upper border is obtained by finding the point at which the sperm concentration most clearly matches the "trough" in height. These points are designated as windows on the plotter graph. Each window corresponds to four threshold divisions on the model B counter. Therefore, once the borders of the sperm population are delineated by the plotter graph, these points can be transposed to the counter and all particles within this size range can be counted.

A mean of five counts is obtained. Addition of a coincidence factor is necessary to correct for two or more particles passing through the aperture simultaneously and thus counted as a single particle. The frequency with which this occurs is directly proportional to the concentration of particles being counted[4] [6]. A solution of diluent without seminal fluid is counted at the same threshold settings as the sample. This result, which represents background, is subtracted from the coincidence corrected count. The difference is multiplied by 10,000* to obtain the sperm concentration/ml. of seminal fluid.

The hemocytometer count was performed using a standard white blood cell pipette (1:20 dilution) and the same diluent. Each sperm observed in two large squares of a Neubauer chamber, representing 0.1 cubic millimeter each, is equivalent to 100,000 sperm/ml. of seminal fluid. The results are expressed in millions of sperm/ml. of seminal fluid. Two to four hemocytometer counts were determined and the mean was accepted as the hemocytometer count. The two methods for estimating sperm concentration were performed independently by separate technicians.

Results

The values for the two methods of determining sperm counts are compared in Table 1. Note that there is a less precise correlation between the procedures when the sperm count was below 1.1 million. Furthermore, 17 sperm counts between 1.1 and 10 million/ml. of seminal fluid by the hemocytometer method were undeterminable by the electronic method. Forty-seven seminal fluid specimens contained sperm concentrations between 1.1 and 10 million/ml. of seminal fluid as determined by both techniques. These counts are plotted against each other in Figure 1. The calculated slope is 0.98843 and the correlation coefficient is 0.93269. An analysis of variance is depicted in Table 2.

Discussion

For many years the hemocytometer method has been used routinely to estimate sperm concentration, despite several disadvantages. The poor reproducibility of determinations by this method has been noted by others[1] and confirmed in our laboratory[3]. This is due to the variation in counting by the same or different technicians as well as unequal distribution of sperm in the counting chamber due to rapid chamber filling[1] [6]. The electronic counter has been demonstrated to be as consistent or more consistent than the hemocytometer method in the estimation of sperm concentration. This greater consistency is the main advantage of the electronic particle counter as a research tool. Where the effect of an agent upon sperm concentration may be small, reproducibility

of results of the counting procedure becomes particularly important. Factors such as technician fatigue and rapidity of counting also have to be considered. When large numbers of seminal fluid specimens have to be analyzed, the electronic particle counter provides a faster and less tiring technique for estimating sperm concentration. It should be stressed that microscopic examination is still necessary for sperm morphology and motility determinations.

Since the electronic method sizes particles according to their conductivity as they pass through an electric field, it does not count sperm, per se. However, in a seminal fluid sample treated with an appropriate diluent to destroy most crystals, white blood cells, and other debris, a population of particles is obtained on the plotter graph that is distinct by its size distribution pattern. These particles are counted as the sperm population. If one reduces the number of sperm present in a given sample, it becomes harder to distinguish these particles from the remaining debris. This does not become a serious problem, however, until the sperm concentration is below 1.1 million/ml. of seminal fluid (Table 1). Therefore, we chose 1.1 million/ml. of seminal fluid as the lower limits of sperm concentration detectable by the electronic method. Delineation of a sperm population was not achieved in 17 specimens in which the sperm concentration was determined to be between 1.1 and 10 million by the hemocytometer method. This was due to an unusually large amount of debris in these specimens which could not be completely destroyed; thus the sperm population was obscured.

Comparison of the 47 specimens that had hemocytometer and electronic counts between 1.1 and 10 million sperm/ml. of seminal fluid reveals a high degree of correlation between the two procedures (slope = 0.98843, correlation coefficient = 0.93269). Since the slope does not differ significantly from 1 ($t = -0.203$) and the intercept does not vary from zero ($t = -0.100$), the sperm counts performed by these two techniques are interchangeable. Furthermore, a comparison of regression coefficients of the slopes obtained when comparing

counts ranging from 1.4 to 471 million sperm/ml.[3] with the counts from 1.1 to 10 million sperm/ml. reveals no significant difference (t = 0.039).

Any electronic count determined to be 1.1 million sperm/ml. of seminal fluid or greater can be reliably accepted as a true estimation of sperm concentration. It is suggested that when a valid graph is unobtainable or an electronic count is less than 1.1 million sperm/ml. of seminal fluid, the sperm count should be re-estimated by the hemocytometer technique. The sample with an unusually large amount of debris that obscures the sperm population by the electronic method will then be readily detectable and a more realistic estimation of sperm concentration can be obtained.

Summary

One hundred ninety-four seminal fluid specimens from men with sperm counts of less than 10 million/mL were counted by both an electronic particle counter and by the routine hemocytometer technique. The results of the two procedures were interchangeable with sperm concentrations between 1.1 and 10 million sperm/ml. of the seminal fluid. Some samples with considerable debris could not be analyzed by the electronic method. There was a less precise correlation between the two procedures when the sperm concentration was below 1.1 million/ml. of seminal fluid.

It is recommended that any electronic count below 1.1 million be verified by the hemocytometer technique. An electronic count above this level can be accepted as an accurate determination of sperm concentration.

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FOOTNOTE TO PAGE 2

The number 10,000 is derived as follows:

Correction for dilution of sample	500
Correction for counting of 0.05 ml. of sample	x20
	10,000

REFERENCES

1. Freund, M., and Carol, B.: Factors affecting haemocytometer counts of sperm concentration in human semen. J. Reprod. Fertil., 8:149-155, 1964.
2. Glover, F. A., and Phipps, L. W.: Preliminary study of an electronic method of counting and sizing bull spermatozoa. J. Reprod. Fertil., 4:189-194, 1962.
3. Gordon, D. L., Moore, D. J., Thorslund, T., and Paulsen, C. A.: The determination of size and concentration of human sperm with an electronic particle counter. J. Lab. Clin. Med., 65:506-512, 1965.
4. Instruction Manual, Coulter Electronics Inc., Hialeah, Fla., p. 13.
5. Laurence, K. A., and Carpuk, O.: The counting and sizing of guinea pig spermatozoa. Fertil. and Steril., 14:451-455, 1963.
6. Mattern, C. F. T., Brackett, F. S., and Olson, B. J.: Determination of number and size of particles by electrical gating: blood cells. J. Appl. Physiol., 10:56-70, 1957.
7. Segal, S. J., and Laurence, K. A.: Automatic analysis of particulate matter in human sperm. Ann. N. Y. Acad. Sc., 99:271-279, 1962.

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Table 1 Comparison of 194 Sperm Counts Between 0 and 10 Million Determined by the Hemocytometer and Electronic Methods.

Table 2 Analysis of Variance for Slope Plotted in Figure 1.

Figure 1 Comparison of 47 Sperm Counts Between 1.1 and 10 Million Determined by the Hemocytometer and Electronic Methods.

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Hemocytometer Count (million sperm/ml of seminal fluid)	Electronic Count (million sperm/ml of seminal fluid)										
	No Sperm Population Delineated	0.1-1.0	1.1-2.0	2.1-3.0	3.1-4.0	4.1-5.0	5.1-6.0	6.1-7.0	7.1-8.0	8.1-9.1	9.1-10.0
No Sperm Count	65	4	1	0	0	0	0	0	0	0	0
0.1-1.0	50	9	0	0	0	0	0	0	0	0	0
1.1-2.0	5	1	8	0	0	1	0	0	0	0	0
2.1-3.0	4	0	1	3	1	0	0	0	0	0	0
3.1-4.0	3	0	0	2	1	3	0	0	0	0	0
4.1-5.0	0	0	0	0	0	2	3	0	1	0	0
5.1-6.0	1	0	0	0	0	0	0	1	0	1	0
6.1-7.0	2	0	0	0	0	1	1	2	1	0	1
7.1-8.0	0	0	0	0	0	0	0	2	0	1	0
8.1-9.0	1	0	0	0	1	0	0	0	1	1	1
9.1-10.0	1	0	0	0	0	0	0	0	0	3	3

TABLE 1

Source	Degrees of Freedom	Sum of Squares	Mean Square
Slope (b)	1	3.32741	3.32741
Error	45	0.40963	0.00910
Total	46	3.73704	

TABLE 2

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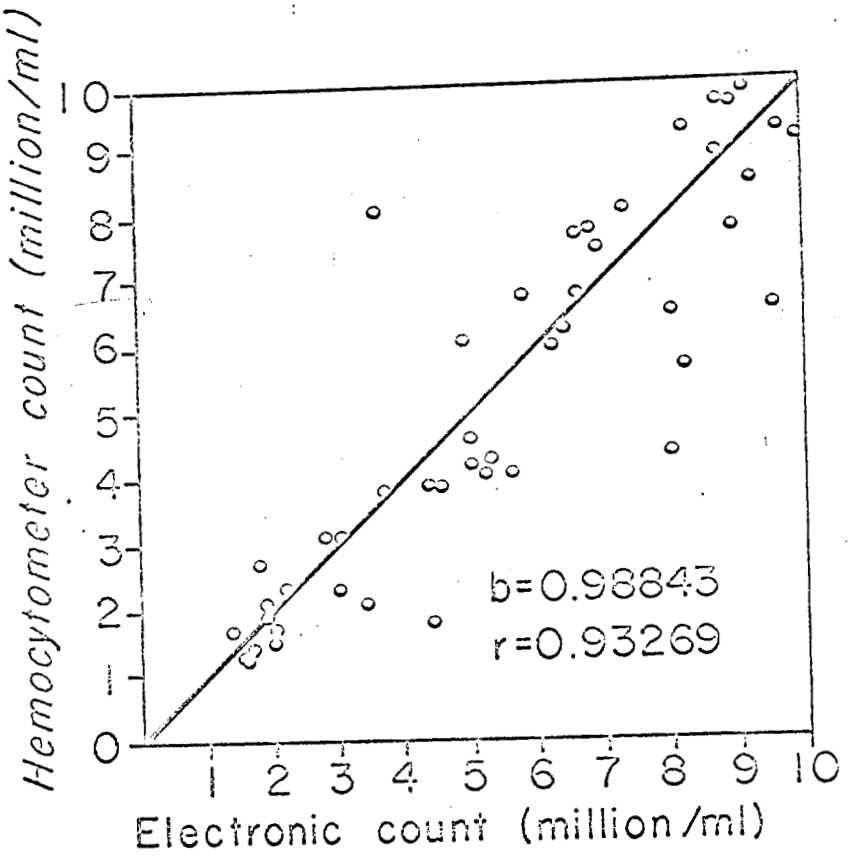

FIGURE I

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