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EFFICACY OF COULTER COUNTER IN DETERMINING LOW SPERM CONCENTRATIONS

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Several investigators have described the estimation of sperm concentration by means of the Coulter counter.[1, 3, 5, 7] In these, as well as in our earlier report,[4] the ability of the Coulter counter to analyze oligospermic seminal fluid and the extinction point for this procedure were not determined. The present study compares the results obtained by the Coulter counter and the hemocytometer methods when the sperm concentration was less than 10 million per ml. Although 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 ninety-four consecutive seminal fluid specimens with sperm concentrations of less than 10 million per ml. were counted by means of both the electronic and hemocytometer methods, as described previously.[4]

For each specimen a mean of five counts was obtained and corrected for coincidence. A solution of diluent without seminal fluid was counted at the same threshold settings as the sample. This background was subtracted from the corrected coincidence count. The difference then was multiplied by 10,000 to obtain the sperm concentration.

The hemocytometer count was performed with the same diluent and a standard white blood cell pipette (1:20 dilution). Two to four hemocytometer counts were determined and the mean was calculated. The two methods for estimating sperm concentration were performed independently by separate technologists.

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RESULTS

The two methods for determining sperm counts are compared in Figure 1. There was a good correlation between the Coulter counter and hemocytometer results when the sperm counts were between 1.1 and 10 million per ml. by both procedures. The calculated slope for these determinations was 0.99 and the correlation coefficient was 0.93. A less precise correlation existed when the sperm count was below 1.1 million. Furthermore, there were 17 seminal fluid specimens where the sperm counts by the hemocytometer method were between 1.1 and 10 million per ml., but by the electronic method no sperm population could be differentiated from the background debris.

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[2] and confirmed in our laboratory.[4] It is due to the variation in counting by the same or different technologists, as well as to unequal distribution of sperm in the counting chamber resulting from rapid chamber filling.[2, 6] In two samples counted repeatedly, the electronic counter was demonstrated to be more consistent than the hemocytometer method in the estimation of sperm concentration.[4] This greater consistency is the chief advantage of the electronic particle counter as a research tool. Where the effect of an agent upon sperm concentration is small, reproducibility of results of the

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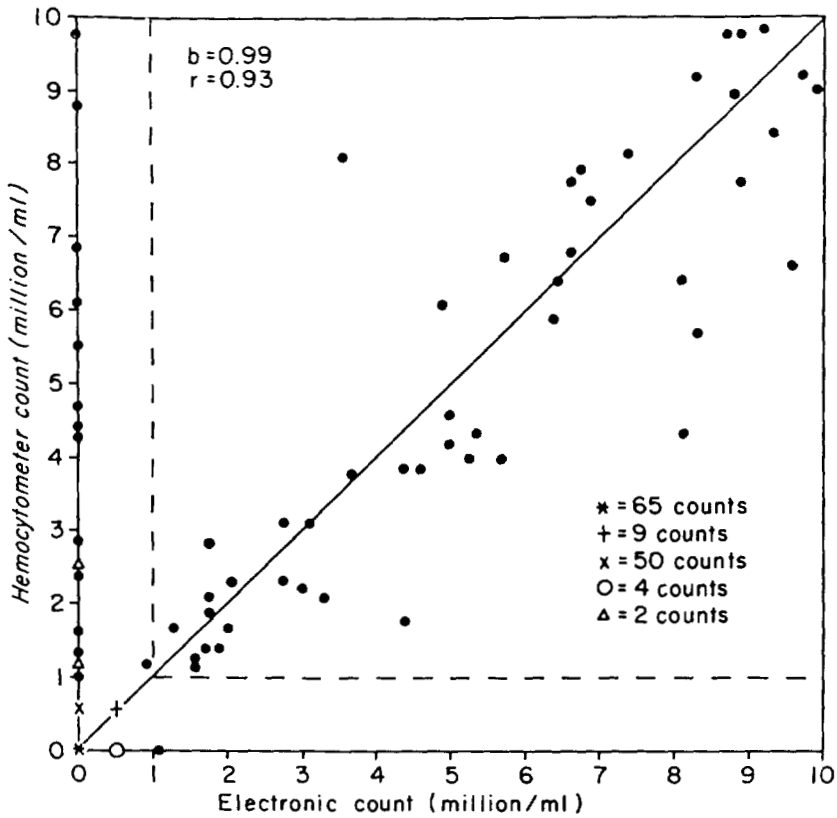

FIG. 1. Comparison of 194 sperm counts between 0 and 10 million per ml., as determined by the hemocytometer and electronic methods. The slope (*b*) and correlation coefficient (*r*) are calculated from the 47 points between 1.1 and 10 million.

counting procedures becomes particularly important. The electronic particle counter also provides a faster and less tiring technic 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, 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 on account of its size distribution pattern. These particles are counted as the sperm population. If sperm concentration is low, it becomes more difficult 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 per ml. Therefore, we chose 1.1 million per ml. as the lower limit of sperm concentration for which the electronic method is useful. Nevertheless, in 17 specimens where the concentration was estimated to be between 1.1 and 10 million by the hemocytometer method, it was not possible to delineate the sperm population electronically. In these specimens, an unusually large amount of debris which could not be completely destroyed obscured the sperm population. Therefore, it is suggested that when a valid graph is unobtainable or an electronic count is less than 1.1 million sperm per ml. of seminal fluid, the sperm count should be reestimated by the hemocytometer technic. 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 specimens of seminal fluid from men with sperm counts of

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less than 10 million per ml. were counted by an electronic particle counter and by the routine hemocytometer technic. The results of the two procedures were interchangeable with sperm concentrations between 1.1 and 10 million sperm per ml. of 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 per ml. of seminal fluid. It is recommended that any electronic count below 1.1 million be verified by the hemocytometer technic. An electronic count above this level can be accepted as an accurate determination of sperm concentration.

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1001756