

Methods for Estimating Spermatozoal Numbers in Turkey Semen^{1,2}

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DURING THE course of an investigation in this laboratory of certain aspects of the physiology of turkey spermatozoa, it was found necessary to re-evaluate the currently most widely accepted method for estimating their concentration in a given volume of semen. This method, in essence, consists of applying the haemocytometer red-blood-cell-counting technique to the counting of spermatozoa. When applied to turkey semen, the technique merely has been an extension of the standard method for counting chicken spermatozoa in which the semen is diluted 1:200 in a r.b.c. pipette and the sperm counted in a regular haemocytometer chamber. This technique was used in this laboratory by Wakely (1949) and by Burrows and Kosin (1953). Their findings essentially have corroborated those of Parker (1946) in that the mean sperm count in the Broad Breasted Bronze turkey is about 8×10^6 per cubic millimeter, or almost twice the concentration recorded recently by Phillips and Wiegers (1952).

A pragmatic evaluation of the procedure outlined above raised the question about its intrinsic accuracy. This was based, principally, on the growing conviction in this laboratory that the 1:200 dilution of the average turkey semen leaves it too concentrated for accurate counting. In practice, it means counting about 800 individual spermatozoa packed in 80 small squares. Aside from difficulties of purely subjective nature presented by such a concentration of cells, there is the question of competition for space among the spermatozoa. This more fundamental difficulty, of course, directly affects the theoretical basis for accepting the Poisson type of distribution as the type describing the distribution of turkey spermatozoa within the counting chamber. This point has received considerable attention in the evolution of the haemocytometer method for counting the r.b.c. (Berkson, *et al.*, 1935, 1940; Magath, *et al.*, 1936).

Accordingly, a study was set up (1) to compare the variability of sperm counts in the turkey semen specimens diluted 1:200, 1:500, and 1:1000; and

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(2) to test the usefulness of absorptiometric technique for measuring turkey sperm concentration. Jones and Lamoreux (1942) used the latter in estimating chicken sperm concentration. Other workers have applied it to the semen of large farm animals: Salisbury, *et al.* (1946) in the bull; Nishikawa (1947) in the horse; Bangs and Underwood (1948) in the ram. Burbank (1935) studied the quantitative standardization of the opacity method for estimating sperm counts in the rabbit. In general these studies have shown that the optical density readings correspond closely to the estimates of sperm concentration obtained by the haemocytometer method.

Procedure

The semen was provided by five mature Broad Breasted Bronze males varying in age, at the beginning of the study, between 40 and 45 weeks. The study is based on eleven semen collections in April-May on an individual male basis. At least two 0.1 milliliter specimens of semen were secured from each ejaculate. These were diluted, in a volumetric flask, with avian Ringer's solution to 1:100. The flasks were then shaken mechanically for three minutes to assure, as far as possible, a homogenous suspension of the spermatozoa. To this 1:100-sperm suspension were then added 1-2 loopfulls of the 40-percent formaldehyde to immobilize the spermatozoa. It was found that the addition of such relatively minute amounts of formaldehyde had no discernible effect on sperm count. On the other hand, preliminary tests showed that the addition of formaldehyde prior to shaking led to a high incidence of lysis among the spermatozoa.

The final 1:200, 1:500, and 1:1000 dilutions were made from the original stock 1:100 suspension.

As a standard routine, the procedure called for two samples from each male's 1:200 dilution and three from each of the other two dilutions. The relatively small size of the turkey ejaculate rigidly limited the number of replicates of each dilution level. Each sample was then used both for the haemocytometer and the nephelometer readings.

In the former, duplicate counts were made of each dilution sample. In the latter, the optical density (O.D.) readings were made with the Coleman Universal Spectrophotometer. A pilot study showed that the optimal consistency of readings was approached at 650 millimicrons (Fig. 1). Both the 1:500 and the 1:100 dilutions were used in the study reported here with all the O.D. readings being taken at 650 millimicrons.

Results

The haemocytometer counts gave the following mean estimates: 1:200, 884×10^4 ; 1:500, 958×10^4 ; 1:1000, 981×10^4 . The only significant dif-

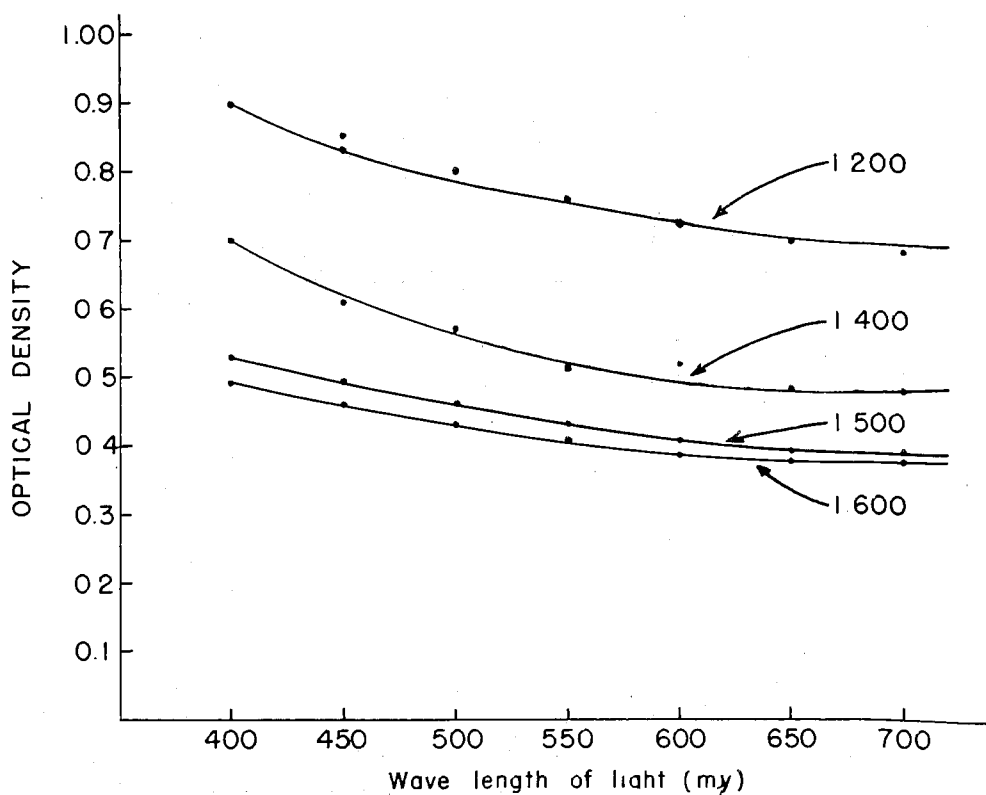


Figure 1. Comparison of the optical density readings according to semen dilution and wave length.

ference ($P < 0.01$) among these averages, as measured by an analysis of variance on the within-male basis, was that between 1:200 and 1:1000 counts. Differences in the observed variability were not statistically significant when, on the within-male basis, the sources of variability were: sides of the haemocytometer chamber and dilution samples from the same specimen. However, between the males' ejaculates, the variability was significantly different ($P < 0.05$) within dilutions, with the 1:500 level showing the highest degree of variability.

In general, the O.D. readings followed the pattern of variability established by the haemocytometer counts. However, there was less variability among the male's specimens and/or sample within either of the two dilutions studied.

The analysis of covariance, when applied to the corresponding haemocytometer and nephelometer data, demonstrated the existence of a considerable degree of correlation between the two sets of values. Thus, with d.f. of 59, the r for "total" and for "between means of males" was 0.55 and 0.36; 6.64

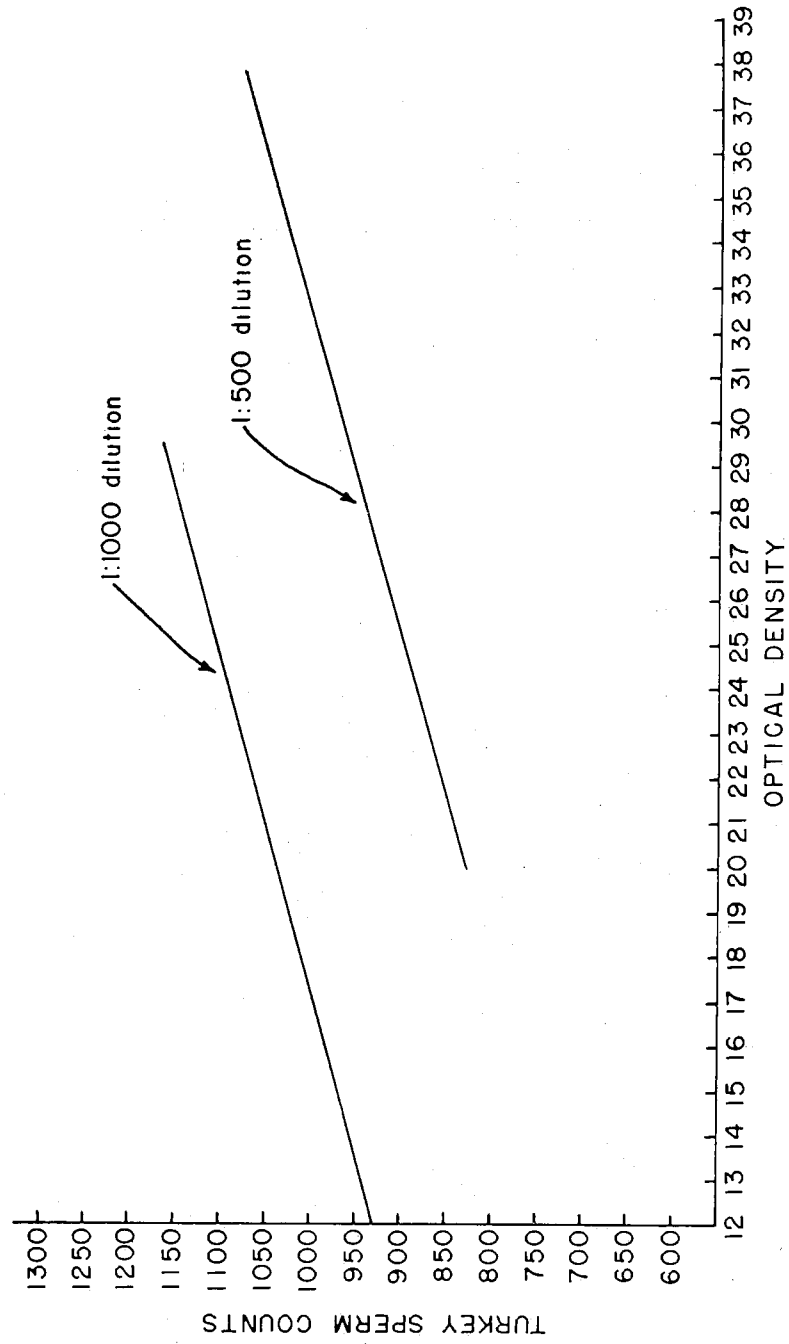


Figure 2. Regression lines for sperm counts on the basis of nephelometric readings (at 650 millimicrons).

and 0.41 at 1:500 and 1:100 dilutions, respectively. All of the above coefficients of correlation were positive. The first three of them were highly significant ($P < 0.01$). The scatter diagram of the corresponding readings obtained from the two estimates suggested that, within the range of values obtained, there is a linear type of relationship between the two. The two regression lines are shown in Figure 2.

Discussion and Conclusions

The fact that the haemocytometer counts were higher at the 1:500 and 1:1000 levels of dilution than those obtained from the 1:200 dilution is in line with the results obtained earlier in this laboratory (Kosin and Burrows, 1951). Even though the observed difference between the 1:200 and the 1:500 estimates was not significant, the evidence of the above-mentioned unpublished report as well as of the currently reported data (1:200 vs. 1:1000) suggests that an upward revision in sperm concentration may be in order. On the basis of the above, it appears that estimates near the 10×10^6 mark rather than the accepted general average of 8×10^6 are more likely to reflect the real concentration of the Broad Breasted Bronze sperm.

The higher sperm-count figures obtained with 1:500 and 1:1000 dilutions can be rationalized on the basis of a more even distribution of the turkey spermatozoa in the counting chamber. While the matter of settling of the individual spermatozoa within the individual counting "cell" can be taken care of by an adequate waiting period (Phillips and Wieggers, 1952), the problem of overcrowding can be solved only by suitable dilution. Stratification of the crowded spermatozoa within individual counting cells, leading to subjective errors of estimates, may also be a factor at the standard 1:200 dilution. Sampson and Warren (1939) had referred to this difficulty in their study on the methods of measuring the density of chicken sperm suspensions. Because of the higher concentration of spermatozoa in the turkey semen, this problem of error due to stratification can be expected to be aggravated. It is therefore suggested that either the 1:500 or the 1:1000 suspension is such a desirable level of dilution for more accurate estimates of turkey spermatozoa concentration with the haemocytometer.

The lower variability of the nephelometer readings plus the reasonably high correlation between the O.D. values and the sperm counts point to the potential usefulness of the absorptiometric method for estimating turkey sperm concentrations. The establishment of a line of regression for a specific situation with regard to dilution, instrument, and wave length should permit a more-rapid, less-variable procedure resulting in greater accuracy. As a less-desirable alternative to the above, the adoption of either 1:500 or 1:1000

dilution instead of the now generally accepted 1:200 can be considered as a means of securing more-accurate counts with haemocytometer.

Summary

A study of Broad Breasted Bronze semen was conducted with the objective of measuring the comparative variability of estimates of sperm concentration. It was found that when the haemocytometer method was the basis of estimates, there was no difference in the variability of sperm counts of semen diluted 1:200, 1:500, or 1:1000. However, the counts at the two last levels gave estimates about 2×10^6 per cubic millimeter higher than those of the 1:200 dilution. The difference in the mean count between the 1:200 and the 1:1000 sperm suspensions was highly significant ($P < 0.01$). When optical density of the 1:500 and 1:1000 semen specimens was obtained at 650 millimicrons, it was found that these O.D. values were correlated with the corresponding haemocytometer counts. The absorptiometric method showed lower variability.

It is suggested, therefore, that the average concentration of the turkey spermatozoa is of the order of 10×10^6 per cubic millimeter rather than of the heretofore generally accepted 8×10^6 per cubic millimeter. Moreover, either the 1:500 or the 1:1000 dilutions should be adopted for routine counts by the haemocytometer method. The optical-density measurement of sperm concentration appears to be preferable to that of haemocytometer—both from the standpoint of accuracy and speed, especially when large numbers of specimens are involved.

Literature Cited

- Bangs, C. L., and E. J. Underwood. 1948. A comparison of methods of estimating sperm concentration in ram's semen. *Australian Vet. Jour.*, 24: 89-93.
- Berkson, J., T. B. Magath, and M. Hurn. 1935. Laboratory standards in relation to chance fluctuations of the erythrocyte count as estimated with the haemocytometer. *Jour. Amer. Stat. Assoc.*, 30: 414-426.
- . 1940. The error of estimate of the blood cell count as made with the haemocytometer. *Amer. Jour. Physiol.*, 128: 309-323.
- Burbank, R. C. 1935. The quantitative standardization of sperm suspensions by means of opacity. *Quart. Jour. Expt. Physiol.*, 25: 393-397.
- Burrows, W. T., and I. L. Kosin. 1953. The effects of ambient temperature on production and fertilizing capacity of turkey spermatozoa. *Physiol. Zool.*, 26: 131-146.
- Jones, D. G., and W. F. Lamoreux. 1942. Semen production of White Leghorn males from strains selected for high and low fecundity. *Poultry Sci.*, 21: 173-184.
- Kosin, I. L., and W. T. Burrows. 1951. Progress Reports of the Poultry Council of the State College of Washington, October. pp. 134-137 (mimeo).
- Magath, T. B., J. Berkson, and M. Hurn. 1936. The error of determination of the erythrocyte count. *Amer. Jour. Clinical Path.*, 6: 568-579.

- Nishikawa, Y. 1947. Nephelometric determination of spermatozoa concentration in horse semen. Research Reports Inst. Horse Breed. Hyg., 1: 111-113 (in Japanese). From an abstract in *Animal Breed. Abstracts*, 19(1).
- Parker, J. E. 1946. Semen production in Broad Breasted Bronze Turkeys. *Poultry Sci.*, 26: 118-121.
- Phillips, H. J. and H. L. Wiegers. 1952. Settling rate of turkey sperm on a haemocytometer. *Poultry Sci.*, 31: 681-684.
- Sampson, F. R., and D. C. Warren. 1939. Density of suspension and morphology of sperm in relation to fertility in the fowl. *Poultry Sci.*, 18: 301-307.
- Salisbury, G. W., G. H. Beck, I. Elliott, and E. L. Willett, 1943. Rapid methods for estimating the number of spermatozoa in bull semen. *Jour. Dairy Sci.*, 26: 69-77.
- Wakely, W. J. 1949. Seasonal variations in the morphology of turkey spermatozoa. Unpublished M.S. thesis, State College of Washington. 37 pp.

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Regarding Corticium hiemalis Laurila

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Recently the combination *Aleurodiscus hiemalis* (Laurila) Erikss. was made.¹ A personal letter from Harold St. John pointed out that the combination was invalid because no reference had been made to *Corticium hiemale* Laurila, nor at that time had Cooke's paper been published in which Cooke mentioned correspondence from John Eriksson. Eriksson had written Cooke as follows: "This is a typical *Aleurodiscus*. The material is not good but seems to agree with Laurila's species." Cooke mentioned that the combination had not been made. Cooke's paper has now been published.² It is clear that Eriksson considered this an *Aleurodiscus*. We prefer to credit this to the late John Eriksson: *Aleurodiscus hiemalis* (Laurila) Erikss. comb. no. Syn. *Corticium hiemale* Laurila, *Ann. Bot. Soc. Zool. Bot. Fenn. Vanamo* 10: extra p. 4, 1939.

¹ Sprague, R. A check list of fungi of Glacier Bay, Alaska. *Research Studies of the State College of Washington*, 23 (3): 202-224 (cf. p. 215). September, 1955.

² Cooke, W. B. Some fungi from Alaska. *Northwest Science*, 29 (4): 127-138 (cf. p. 131). November, 1955.