PRELIMINARY NOTES

FEULGEN-DNA CONTENT OF INDIVIDUAL BULL SPERMATOZOA

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Received April 1, 1966[1]

DURING Feulgen-DNA measurements on individual bull spermatozoa with a view on establishing a contingent correlation between the spread of the values found within a sample and the fertilizing capacity of the semen we noted, first, that values obtained with our method showed a considerably lower spread than had been found previously and, secondly, that the results are compatible with the presence within the samples of two populations, differing some 3 per cent in DNA content. The present paper deals with an analysis of two sperm samples with respect to these observations.

The cytophotometer used has been described previously [2]. It has been modified in that in the present apparatus the scanning is done in the image plane. It has been found empirically that this arrangement is less sensitive to focussing errors and allows of a slightly better resolution than that formerly employed. The standard deviation of repeated measurements on the same spermatozoon was found to amount to 1 per cent of its Feulgen-DNA content. This source of spread in measuring results will be referred to as the instrumental error.

The standard Feulgen technique was employed but hydrolysis was performed in 5.5 *N* HCl at 20°C ($\pm$ 0.1°C). Under these circumstances, and in our material, the staining intensity is relatively independent of the hydrolysis time within the range 45–60 min. In the present investigation hydrolysis was terminated after 50 min. All baths were symmetrically agitated and every detail of the technique was rigorously standardized; nevertheless we were unable to obtain identical staining over the entire surface of 2 × 3 cm smears of semen. Within an area of a few square milimeters the spread of the values obtained on 50 spermatozoa was the same as that found in a similar area elsewhere on the same slide or on a slide treated simultaneously, but the mean of the values found in one area could differ from zero to as much as 4 per cent from that found in another area. In order to pool the measuring results obtained on a large number of spermatozoa we measured closely adjacent spermatozoa in groups of 40 or 50 and corrected for the apparently unavoidable difference in staining effectiveness between the groups by converting every group mean into 100 arbitrary units.

The histograms for 760 spermatozoa from one ejaculate (P.C.), Fig. 1, and 450 spermatozoa from another bull (Joh. I.), Fig. 2, have been plotted in unit steps,

[1] Revised version received July 19, 1966.

the 100 units mark coinciding with the mean value. In both histograms one may discern a peak and a shoulder, to the left and the right of the mean value respectively. The overall standard deviation of the Feulgen-DNA values amounts to 2.4 units (P.C., Fig. 1) and 3.3 units (Joh. I, Fig. 2) respectively.

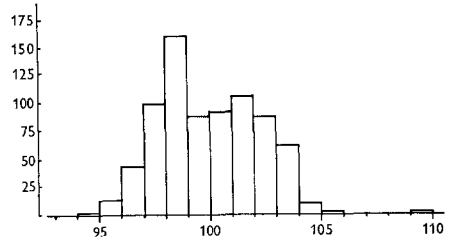

Fig. 1.

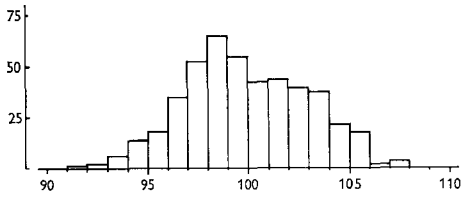

Fig. 2.

Fig. 1.—Histogram: Feulgen-DNA per sperm head in arbitrary units as determined in a sample of 760 randomly chosen spermatozoa from an ejaculate of bull P.C. Arithmetic mean of sample: 100 arbitrary units, standard deviation: 2.4 units.

Fig. 2.—Histogram: Feulgen-DNA per sperm head in arbitrary units as determined in a sample of 450 randomly chosen spermatozoa from an ejaculate of bull Joh. I. Arithmetic mean of sample: 100 arbitrary units, standard deviation: 3.3 units.

The spread of the Feulgen-DNA values in Fig. 2 is greater than that in Fig. 1, but both histograms show a much smaller spread than is suggested by earlier measurements, both on Feulgen-DNA and in ultraviolet light, e.g. [3, 4, 5, 6].

The most plausible distribution of DNA values in a sperm sample seems to be that of two populations, viz. of X- and Y-spermatozoa, whose DNA contents differ according to the difference in DNA contents of the X- and Y-sex chromosomes they carry. According to Baker *et al.* [1] this difference should amount to 3.1 per cent of the haploid DNA content in bull spermatozoa. The shape of the histograms is compatible with the existence of two populations whose means as judged from the distance of the peak and the shoulder differ some 3 per cent of the mean Feulgen-DNA content per spermatozoon.

A statistical analysis of our results by means of Fisher's test for *kurtosis* confirms the impression given by the shape of the histograms in that it shows at the 99 per cent confidence level that in both the P.C. and Joh. I samples there is an excess of values deviating moderately from the sample mean. This result is to be expected from an analysis of a sample containing two pooled populations with slightly different population means.

The probability that in spite of the apparent asymmetry of the histograms the results obtained represent pools of two normally distributed populations which are equal in every respect except for a 3 per cent difference in the population means, has been investigated by means of the chi-square adaption test. It was found to be less than 1 per cent. If the biologically plausible assumption that the X- and Y-spermatozoa in the sample are equal in number is retained, the asymmetry of the histograms should be ascribed to a greater spread in the X-spermatozoa values (the shoulder in the histogram) than in the Y-spermatozoa (the peak).

In Fig. 3 the data of the sample with the smaller overall spread (P.C.) have been plotted on normal frequency paper (solid line) along with a hypothetical curve (dotted line) calculated on the following assumptions: there are equal numbers of X- and Y-spermatozoa containing exactly 101.5 and 98.5 arbitrary units respectively of Feulgen-DNA and the spread in the measuring results is caused by the estimated

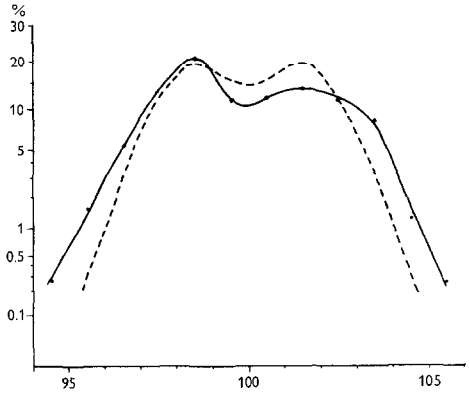

Fig. 3.—Frequency diagrams of the measurement on a sample of 760 spermatozoa from bull P.C. (*solid line*, see also Fig. 1) and of a hypothetical mixture of equal numbers of spermatozoa, means: 98.5 and 101.5 arbitrary units respectively, standard deviation for either category: 1 unit (equal to instrumental error). *Abscissa*: Feulgen-DNA content of individual spermatozoa in arbitrary units. *Ordinate*: relative frequencies in per cent.

instrumental error only (standard deviation within either category: 1 unit). A comparison of the left hand halves of the graphs shows that the frequency distribution of the experimental values almost coincides with that to be expected if the instrumental error is the sole source of spread. There is apparently little or no variation in the actual DNA content of the spermatozoa represented by this part of the diagram (presumably the Y-spermatozoa).

Even if the observations made in the foregoing paragraphs are valid there always remains an uncertainty because most probably an unknown number of spermatozoa with abnormal DNA contents tends to distort the distribution of the values.

The authors thank Mr. P. C. Sander, Dept. of Mathematical statistics, University of Utrecht, and Mr. C. van Duijn, jr., Research Institute for Animal Husbandry, Schoonoord, Zeist, for advice in preparing the manuscript.

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