Nuclear Size of Live and Dead Bovine Spermatozoa

THE size distributions of spermatozoan nuclei are of interest from two points of view. On one hand there is the question of the identification and separation of *X* and *Y* chromosome bearing spermatozoa, and on the other hand the relation of nuclear size to total content of chromatophilic substances measured by the plug or core method of microspectroscopy. We do not wish to become involved in the former question; but are interested in the latter in connexion with work on the DNA content of bovine spermatozoa being examined in this laboratory.

We have adopted the van Duijn[1] formula for determining nuclear areas. This method has been shown to yield a high correlation with areas determined by planimetry[2]. The regression of optical density of Feulgen-stained bovine spermatozoa on nuclear area is negative and highly significant[3]. Under some experimental conditions 51 per cent of the variation in optical density could be explained by this regression. Suspecting that dead cells might be an important factor in this regression, an experiment was designed to determine the size and Feulgen staining characteristics of live and dead spermatozoa. This communication reports the nuclear size relationships.

Live and dead spermatozoa were differentiated by the eosin method of Lasley, Easley and McKenzie[4] but omitting the background stain. Areas on each slide containing 10 or more live (eosin-negative) and 10 or more dead (eosin-positive) cells were mapped so that the individual cells could be relocated. The slides were then fixed, hydrolysed and stained by the Feulgen method following normal procedure[5]. The process of fixation in acetic alcohol and hydrolysis in 1 N hydrochloric acid effectively removes all the colour from the eosin-stained spermatozoa as determined by visual inspection, but there is an effect of eosin treatment on the apparent DNA content of the cell. After relocating the fields, length, maximum width and base width of the Feulgen-stained, mapped sperm heads were measured with an ocular micrometer. Twenty-eight slides were examined, representing three different semen samples, with sub-samples ranging in age from fresh to 13 days old (stored in yolk-citrate at 5° C).

The dead cells were found to be larger than the live cells in all the dimensions studied. These differences were significant at the 0·001 level of probability, except for base width which was significant only at the 0·05 level. The means and standard deviations are given in Table 1. With the exception of the standard deviations for maximum width, all the means, standard deviations and ranges were larger for the dead cells than for the live ones. Nuclear areas were likewise larger for the dead than for the live sperm. The difference in area amounted to 9·78 per cent of the area of the live cells, and was significant at the 0·001 level of probability.

The distributions of live and dead cell nuclear areas are depicted in the histograms in Fig. 1. While the modes are distinctly different, there is a large overlap of areas in the two arrays. The sampling method used does not permit any exact statements with regard to bimodality of the sperm populations studied, but if the two distributions are pooled on the assumption of a single population containing 53 per cent live cells, no evidence of bimodality can be shown.

Thus it can be seen that nuclear area of a spermatozoon cannot be taken as *prima facie* evidence that the cell was

Table 1. MEANS AND STANDARD DEVIATIONS OF NUCLEAR DIMENSIONS AND AREAS OF LIVE AND DEAD BOVINE SPERMATOZOA

	Live	Dead	Difference (%)	Significance
No. of cells	300	279		
Length	8·53 ± 0·50μ	8·93 ± 0·55μ	4·69	***
Maximum width	3·98 ± 0·32μ	4·19 ± 0·28μ	5·28	***
Base width	1·80 ± 0·24μ	1·84 ± 0·27μ	2·22	*
Area	27·51 ± 2·05μ^2	30·20 ± 2·78μ^2	9·78	***

* Indicates significance at the 0·05 level of probability. *** Indicates significance at the 0·001 level of probability.

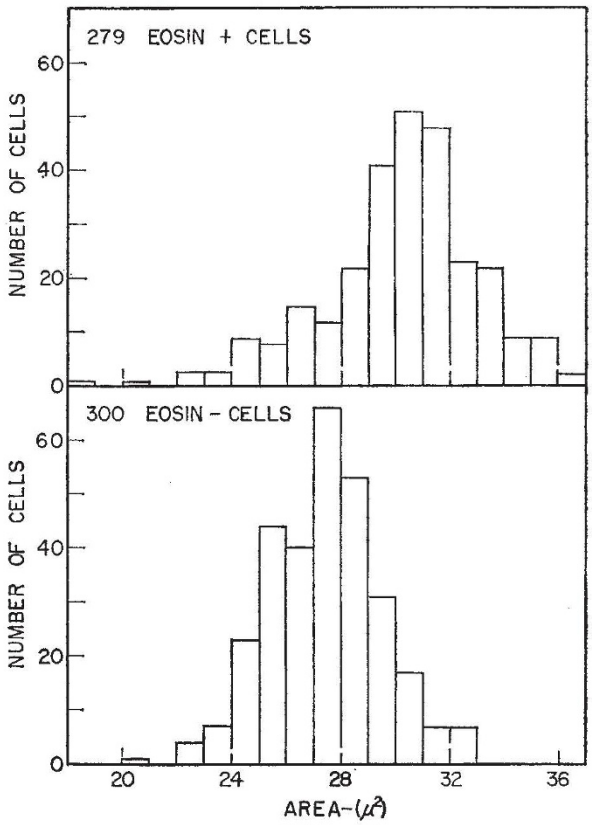

Fig. 1. Distribution of nuclear areas of dead (eosin-positive) and live (eosin-negative) sperm cells

live (or dead) at time of fixation. However, the large differences between mean areas and the larger standard deviations of dead cells may account for some of the differences in DNA and the greater variability in DNA content reported in the spermatozoa of infertile as contrasted to fertile males[6,7]. Certainly such studies in the future should include some estimate of the live cells present in the sample at fixation, for such differences in fertility may be due entirely to the paucity of live spermatozoa in the semen of infertile males and not to intrinsic differences in DNA content of those live cells which are capable of penetrating the vitelline membrane.

We wish to acknowledge the support provided through a grant, *RG*–8753, to one of us (G. W. S.) from the National Institutes of Health, U.S. Public Health Service.

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VIROLOGY

Measles Virus

COOPER[1] placed measles virus in the deoxyvirus group. There is now considerable evidence which is contrary to this view. The appearance of acridine-orange stained inclusions in measles virus infected cells under ultraviolet illumination suggests the presence of a ribovirus[2].